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By Edgar DUSACRE

Devenir et effets des microplastiques, des nanoplastiques et des additifs résultant de la dégradation des engins de pêche au cours de leur cycle de vie. Étude dans le golfe de Gascogne

Fate and effects of microplastics, nanoplastics and additives resulting from the degradation of fishing gear during their life cycle. Study in the Bay of Biscay

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Abstracts

Devenir et effets des microplastiques, des nanoplastiques et des additifs résultant de la dégradation des engins de pêche au cours de leur cycle de vie. Étude dans le golfe de Gascogne

L'industrie de la pêche, qui est un secteur socio-économique essentiel, peut contribuer à la pollution marine en raison de l'utilisation d'engins de pêche (EP) en plastique. Cependant, les études scientifiques sur la façon dont les EP en plastique contribuent à la pollution océanique par les microplastiques (MP), les nanoplastiques (NP) et les produits chimiques issus des plastiques dans les océans étaient rares. Dans ce contexte, les quantités de filets de pêche (FP) opérés et perdus dans le sud-est du Golfe de Gascogne (SE GG) ont été estimées. Les engins de pêche abandonnés, perdus ou autrement rejetés (ALDFG) échoués sur les plages ont également été quantifiés. Ensuite, six FP pétrosourcés (un en polyéthylène-polypropylène (PE-PP) et cinq en polyamide (PA)) et deux FP partiellement biosourcés/biodégradables (polybutylène succinate-polybutylène adipate téréphtalate (PBS-PBAT)) ont été sélectionnés pour étudier leur dégradation (vieillissement artificiel, VA) et leur toxicité. Les effets des lixiviats de FP ont été étudiés grâce à des essais de toxicité aiguë et subchronique sur quatre espèces aquatiques : *Aliivibrio fischeri*, *Tisochrysis lutea*, *Acartia tonsa* et *Oryzias latipes*. De plus, une étude comparative de la toxicité des extraits organiques de FP neufs et en fin de vie (EOL) sur *A. fischeri*, la lignée cellulaire RTL-W1, et *O. latipes* a été menée. Il a été estimé que la flotte de pêche du SE GG a opéré 211 tonnes d'EP en 2023 et en a perdu 6 tonnes. Au cours de la même période, il a été estimé que 3 tonnes d'ALDFG se sont échoués. La plupart des EP échoués étaient des pièces de ramendage de chaluts et de sennes de fond. Le VA des FP a révélé une production de MP et de produits chimiques plastiques. La production de ces produits de dégradation a eu tendance à augmenter avec le VA, bien que non linéairement. Trois des sept FP étudiés ont produit un nombre significatif de MP après 25 jours de VA ($1{,}1x10^5$ MP/g de FP à $6{,}8x10^5$ MP/g de FP) avec une taille médiane de 21,3 µm (min-max : 5 – 430 µm). Des NPs (< 1 µm) ont également été identifiés dans les lixiviats de tous les FP. Des concentrations significatives de six métaux traces (Cu, Fe, Pb, Zn, Ni, Ag) ont été quantifiées dans les lixiviats. De plus, nous avons identifié 27 composés organiques. Concernant la toxicité des extraits DMSO de FP neufs et EOL, les trois espèces étudiées ont été affectées par la plupart des extraits de FP. L'inhibition de la bioluminescence d'*A. fischeri* a été reliée aux métaux traces. Chacune des 4 espèces exposées aux lixiviats de FP vieillis artificiellement a subi des degrés d'effets variables. La bioluminescence d'*A. fischeri* a été significativement impactée par la plupart des FPs, mais plus particulièrement par ceux en PBS-PBAT. La concentration de microalgues (*T. lutea*) n'a pas été significativement affectée après 72 h d'exposition aux lixiviats de FP. La plupart des FP testés ont significativement perturbé le rythme cardiaque et le comportement des larves d'*O. latipes*. Les altérations du comportement étaient principalement liées à la libération de composés organiques. La survie des copépodes a été significativement diminuée par la plupart des FP. La CL_{50} la plus faible a été attribuée à un FP en PA (1,32±1,14 g/L) et un FP en PBS-PBAT (1,55±0,82 g/L). L'exposition subchronique des copépodes a induit des effets significatifs sur la production de pelotes

fécales et d'œufs et sur le succès d'éclosion. Globalement, ces résultats ont mis en évidence que les FP contribuent à la pollution des océans aux MP (1,48 MPs/m^3 (95% IC: 0-3,5 MP/m^3) to 2,2 MPs/m^3 (95% IC: 0-14,8 MP/m^3)), NP et composés chimiques des plastiques. Les FP biosourcés/biodégradables pourraient offrir une solution pour mieux gérer les FP EOL et réduire la pêche fantôme, mais leur composition actuelle doit être améliorée pour réduire leur toxicité. L'évaluation du risque associé aux produits de dégradation des filets maillants opérés et rejetés dans le SE GG a été estimée comme posant un risque environmental annuel allant d'absent à léger. Cependant, l'accumulation potentielle des produits de dégradation des filets de pêche au fil des années dans le SE GG pourrait causer un danger léger à modéré.

Mots clés : Écotoxicologie, filets de pêche, microplastiques, nanoplastiques, produits chimiques des plastiques, évaluation des risques.

Fate and effects of microplastics, nanoplastics and additives resulting from the degradation of fishing gear during their life cycle. Study in the Bay of Biscay

The fishing industry, which is an essential socio-economic sector, may contribute to marine pollution due to the use of plastic fishing gear (FG). However, scientific studies on how plastic FG contribute to the global oceanic pollution of microplastics (MPs), nanoplastics (NPs) and plastic chemicals were scarce. In this context, used and lost fishing nets (FNs) in the Southeastern Bay of Biscay (SE BOB) were estimated and beached abandoned, lost or otherwise discarded fishing gear (ALDFG) were quantified. Then 6 oil-based FNs (1 made of polyethylene-polypropylene (PE-PP) and 5 made of polyamide (PA)) and 2 partially biobased/biodegradable FNs (polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT)) were selected to investigate their degradation and toxicity. The degradation of FNs was analyzed through artificial aging. Then, the effects of leachates of FNs were investigated through acute, and subchronic toxicity assays with lethal and sub-lethal endpoints on 4 aquatic species, *Aliivibrio fischeri, Tisochrysis lutea, Acartia tonsa and Oryzias latipes*. In addition, a comparative study of the toxicity of organic extracts from new FNs and end-of-life (EOL) ones on *A. fischeri*, RTL-W1 cell line, and *O. latipes* was conducted. It was estimated through models that the fishing fleet of the SE BoB used 211 tons of FG in 2023 and lost 6 tons of them. In the same period, it was also estimated that 3 tons of ALDFG were beached. Most of the beached FG were mending pieces coming from trawl and bottom seine FG repairs. The artificial aging (AA) of FNs revealed FN-dependent production of MPs and plastic chemicals. Production of these degradation products tended to increase with AA, although not linearly. Three of the seven FNs studied produced a significant number of fragment-shaped MPs after 25 days of AA ($1.1x10^5$ MP/g of FN to $6.8x10^5$ MP/g of FN) with a median size of 23.6 µm (min-max: 5 – 430 µm). NPs (< 1 µm) were also identified in the leachates of all FNs. Significant amounts of six trace metals (Cu, Fe, Pb, Zn, Ni, Ag) were quantified in the leachates. Furthermore, we identified 27 organic compounds. Concerning the comparative toxicity of DMSO extracts of new and EOL FNs, the three species studied were affected by most of the products leached from the FNs. The bioluminescence inhibition of *A. fischeri* was linked to the occurrence of trace metals. Each of the 4 species exposed to leachates of artificially aged FNs experienced varying degrees of effects. The bioluminescence of *A. fischeri* was significantly impacted by most FNs, but more prominently by the PBS-PBAT ones. The bioluminescence inhibition was mainly related to the release of trace metals. The concentration of microalgae cells (*T. lutea*) was not significantly affected after 72 h of exposure to FN's leachates. Most tested FNs significantly disrupted the heart rate and behavior of *O. latipes* larvae. Alterations in behavior generally increased with FN aging time and were mainly related to the release of organic chemicals. The survival of adult and juvenile copepods was significantly decreased by most of the FNs. The lowest LC50 estimated was found for one PA FN (1.32±1.14 g/L) and one PBS-PBAT FN (1.55±0.82 g/L). Subchronic exposure of copepods caused significant effects on egg production and hatching success. Globally, these results highlighted that FNs contribute to MP (1.48±1.34 to 2.2 MPs/m^3 (95% CI: 0-14.8 MP/m^3)), NP and plastic chemicals pollution of the oceans. Biobased/biodegradable

FNs could offer a solution to better manage EOL FG and to reduce ghost fishing when lost, but their current composition needs to be improved to reduce their toxicity. The assessment of the risk associated with the degradation products from both operated and discarded gillnets in the SE BoB has been determined to pose an annual hazard ranging from absent to slight. However, potential accumulation of the degradation products of FNs across the years in the SE BoB could cause a slight to moderate hazard.

Keywords: Ecotoxicology, fishing nets, microplastic, nanoplastics, plastic chemicals, risk assessment

Destino y efectos de los microplásticos, nanoplásticos y aditivos derivados de la degradación de las artes de pesca durante su ciclo de vida. Estudio en el Golfo de Vizcaya

La industria pesquera, que constituye un sector socioeconómico esencial, puede contribuir a la contaminación marina debido al uso de artes de pesca (AP) de plástico. Sin embargo, los estudios científicos sobre la contribución de las AP de plásticoa la contaminación global oceánica por microplásticos (MPs), nanoplásticos (NPs) y productos químicos asociados al plástico han sido escasos. En este contexto, se estimaron las redes de pesca (RP) utilizadas y perdidas en el sureste del Golfo de Vizcaya (SE GV) y se cuantificaron las artes de pesca abandonadas, perdidas o descartadas en playas (ALDFG, por sus siglas en inglés). Posteriormente, se seleccionaron seis RP basadas en derivados del petróleo (una de polietileno-polipropileno (PE-PP) y cinco de poliamida (PA)) y dos RP parcialmente biobasadas/biodegradables (de succinato de polibutileno-adipato-tereftalato de polibutileno (PBS-PBAT)) para investigar su degradación y toxicidad. La degradación de las RP se analizó mediante envejecimiento artificial. Posteriormente, los efectos de los lixiviados de las RP se investigaron mediante ensayos de toxicidad aguda y subcrónica en los que se determinaron parámetros letales y subletales en cuatro especies acuáticas: *Aliivibrio fischeri*, *Tisochrysis lutea*, *Acartia tonsa* y *Oryzias latipes*. Además, se llevó a cabo un estudio comparativo de la toxicidad de extractos orgánicos de RP nuevas y tras su uso (EOL) sobre *A. fischeri*, la línea celular RTL-W1 y *O. latipes*. Se estimó mediante modelos que la flota pesquera del SE GV utilizó 211 toneladas de AP en 2023, de las cuales perdió 6 toneladas. En el mismo período, también se estimó que 3 toneladas de ALDFG llegaron a las playas, siendo la mayoría restos de reparación de RP de arrastre y cerco de fondo. El envejecimiento artificial (AA) de las RP reveló la producción tanto de MPs como de productos químicos asociados al plástico en función del tipo de red. La producción de estos productos de degradación tendió a incrementarse con el AA, aunque no de forma lineal. Tres de las siete RP estudiadas produjeron una cantidad significativa de MPs en forma de fragmentos tras 25 días de AA (de 1.1x10^5 MP/g de RP a 6.8x10^5 MP/g de RP) con un tamaño medio de 23.6 µm (mín-máx: 5 – 430 µm). También se identificaron NPs (< 1 µm) en los lixiviados de todas las RP estudiadas. Se cuantificaron cantidades significativas de seis metales traza (Cu, Fe, Pb, Zn, Ni, Ag) en los lixiviados. Además, se identificaron 27 compuestos orgánicos. En lo relativo a la toxicidad comparativa de los extractos de DMSO de RP nuevas y EOL, la mayoría de los productos lixiviados de las RP afectaron a las tres especies estudiadas . La inhibición de la bioluminiscencia de *A. fischeri* se vinculó a la presencia de metales traza. Cada una de las cuatro especies expuestas a lixiviados de RP envejecidas artificialmente experimentó efectos de distintos grados. La bioluminiscencia de *A. fischeri* fue impactada significativamente por la mayoría de las RP, pero especialmente por las compuestas de PBS-PBAT, y la inhibición de la bioluminiscencia se relacionó principalmente con la liberación de metales traza. La concentración de células de microalgas (*T. lutea*) no se vio significativamente afectada tras 72 h de exposición a los lixiviados de RP. La mayoría de las RP ensayadas alteraron significativamente la frecuencia cardíaca y el comportamiento de larvas de *O. latipes*, con

alteraciones en el comportamiento que tendieron a incrementarse con el tiempo de envejecimiento de las RP, y que se relacionaron principalmente con la liberación de compuestos orgánicos. La mayor parte de las RP disminuyeron significativamente la supervivencia de copépodos adultos y juveniles , encontrándose las concentraciones letales 50 (LC50) más bajas para una RP de PA (1.32±1.14 g/L) y para una de PBS-PBAT (1.55±0.82 g/L). La exposición subcrónica de copépodos a los lixiviados causó efectos significativos sobre la producción de huevos y el éxito de eclosión. Globalmente, estos resultados destacan que las RP contribuyen a la contaminación de los océanos por MPs (1.48±1.34 a 2.2 MPs/m^3, IC 95%: 0-14.8 MP/m^3), NPs y productos químicos asociados al plástico. Las RP biobasadas/biodegradables podrían ofrecer una solución para facilitar la gestión al final de la vida útil de las AP y reducir la “pesca fantasma” en caso de pérdida, pero su composición actual necesita ser mejorada para reducir su toxicidad. La evaluación del riesgo asociado a los productos de degradación tanto de las redes utilizadas como descartadas en el SE GV indica un peligro anual que varía de ausente a leve. Sin embargo, la acumulación potencial de productos de degradación de las RP a lo largo de los años en el SE GV podría conducir a un peligro de leve a moderado.

Palabras clave: Ecotoxicología, redes de pesca, microplásticos, nanoplásticos, productos químicos asociados al plástico, evaluación del riesgo

Arrantza-tresnek bizi-zikloan jasaten duten degradazioaren ondorioz sortzen diren mikroplastikoen, nanoplastikoen eta plastiko gehigarrien patua eta eraginak. Azterketa Bizkaiko Golkoan

Gizarte eta ekonomiarentzat garrantzitsua den arrantza-industrian plastikozko arrantza-tresnen (AT) erabilerak itsas kutsadura eragin dezake. Hala ere, plastikozko ATek mikroplastikoen (MP), nanoplastikoen (NP) eta plastikoari asoziaturiko konposatu kimikoen ozeano mailako kutsaduran duten ekarpena ikertu duten azterketa zientifikoak urriak dira. Testuinguru horretan eta ekarpen horien gaineko informazioa jasotze aldera, Bizkaiko Golkoko hego-ekialdean (BG HEan) erabilitako eta galdutako arrantza-sareak (AS) estimatu ziren; eta hondartzaratutako abandonatutako, galdutako edo baztertutako arrantza-tresnak (ALDFG ingelesez) ere kuantifikatu ziren. Ondoren, petrolio-jatorridun 6 AS (1 polietileno-polipropilenozkoa (PE-PP) eta 5 poliamidazkoak (PA)), eta partzialki jatorri biologikodun 2 AS (polibutileno-sukzinato-polibutileno-adipato-tereftalatozkoak (PBS-PBAT)) hautatu ziren euren degradazioa eta toxikotasuna aztertzeko. ASen degradazioa, zahartze artifizialaren bidez aztertu zen. Ondoren, ASen lixibiatuen eragina toxikotasun akutu eta azpi-kronikozko saioetan ikertu zen, parametro letal eta azpiletalak aztertuz 4 espezie urtarretan: *Aliivibrio fischeri*, *Tisochrysis lutea*, *Acartia tonsa* eta *Oryzias latipes*. Horrez gain, AS berrien eta erabilitako (EOL) estraktu organikoen toxikotasuna alderatu zen *A. fischeri*-n, RTL-W1 zelula-lerroan eta *O. latipes*-en. Ereduen bidez BG HEko arrantza-flotak 2023an 211 tona AT erabili zituela estimatu zen, zeintzuetatik 6 galdu egin ziren. Aldi berean, hondartzetan 3 tona ALDFG estimatu zen; hondartzatutako AT gehienak arrastre- eta hondo-arrantzarako ASen konpontze-puskak izanik. ASen zahartze artifizialak (ZA) AS-motaren araberako MP eta plastikoari asoziaturiko konposatu kimikoen sorkuntza erakutsi zuen. Degradazio-produktu hauen sorrerak ZArekin handitzeko joera zuen, baina ez modu lineal batean. Aztertutako 7 ASetatik hiruk MP ugari sortu zituzten 25 eguneko ZA eta gero ($1.1x10^5$ MP/g-tik $6.8x10^5$ MP/g-ra). Sortutako MPen tamainaren batazbestekoa 23.6 µm-takoa izan zen (min-max: 5 – 430 µm). NPak (< 1 µm) ere AS guztien lixibiatuetan identifikatu ziren. Lixibiatuetan sei metal-trazen (Cu, Fe, Pb, Zn, Ni, Ag) kopuru esanguratsuak kuantifikatu ziren. Are gehiago, 27 konposatu organiko identifikatu ziren. AS berrien eta EOLen DMSO estraktuak alderatzerakoan, ASetatik askatutako lixibiatuen konposatu gehienek aztertutako hiru espezieak eragin zituzten. *A. fischeri*-ren bioluminezentziaren inhibizioa metalen presentziarekin lotu zeen. Lau espezieek AS zahartuen lixibiatuenpean eragin desberdinak jasan zituzten. AS gehienek eragin zuten *A. fischeri*-ren bioluminezentzian, bereziki PBS-PBAT motakoek. Bioluminezentziaren inhibizioa nagusiki metalen askatzearekin lotuta zegoen. Mikroalgen (*T. lutea*) zelulen kontzentrazioan ez zen aldaketa esanguratsurik izan ASen lixibiatuenpean 72 orduko esposizioaren ostean. Aztertutako AS gehienek *O. latipes* larben bihotz-taupadak eta portaera esangarriki kaltetu zituzten. Orokorrean, aldaketak ASen zahartzearekin handitu ziren, gehien bat konposatu organikoen askatzearekin lotuzirelarik. AS gehienek *Acartia tonsa* kopepodo heldu eta gazteen biziraupena esangarriki murriztu zuten. Estimatutako LC50 txikienak PA baten pean (1.32±1.14 g/L) eta PBS-PBAT baten pean (1.55±0.82 g/L) neurtu ziren. Kopepodoen esposizio azpikronikoak efektu esangarriak eragin

zituen arrautzen ekoizpenean eta eklosio arrakastan. Orohar, emaitzek erakutsi zuten ASek MP (1.48±1.34 MP/m^3tik 2.2 MP/m^3ra (95% KT: 0-14.8 MP/m^3)), NP eta plastikoari asoziaturiko konposatu kimikoen bidezko itsas kutsadurari ekarpena egiten diotela . Jatorri biologikoa duten edo bidegradagarriak diren ASak konponbide bat izan daitezke bizi-ziklo bukaerako ATaren kudeaketa hobetzeko eta, galdutakoan, "*mamu-arrantza*" murrizteko; baina gaur egun duten osaera hobatu beharra dago beraien toxikotasuna murrizteko. Urte batean BG HEan bai erabilitako eta bai baztertutako enmalle sareen degradazio produktuen arrisku mailaren azterketaren arabera, ez dago arriskurik edo arriskua txikia da. Hala ere, BG HEan urteetan zehar ASen degradazio-produktuak akumulatuko balira arriskua txikitik moderatua izatera heldu daiteke.

Gako-hitzak: Ekotoxikologia, arrantza-sareak, mikroplastikoak, nanoplastikoak, plastikoari asoziaturiko konposatu kimikoak, arriskuaren ebaluazioa

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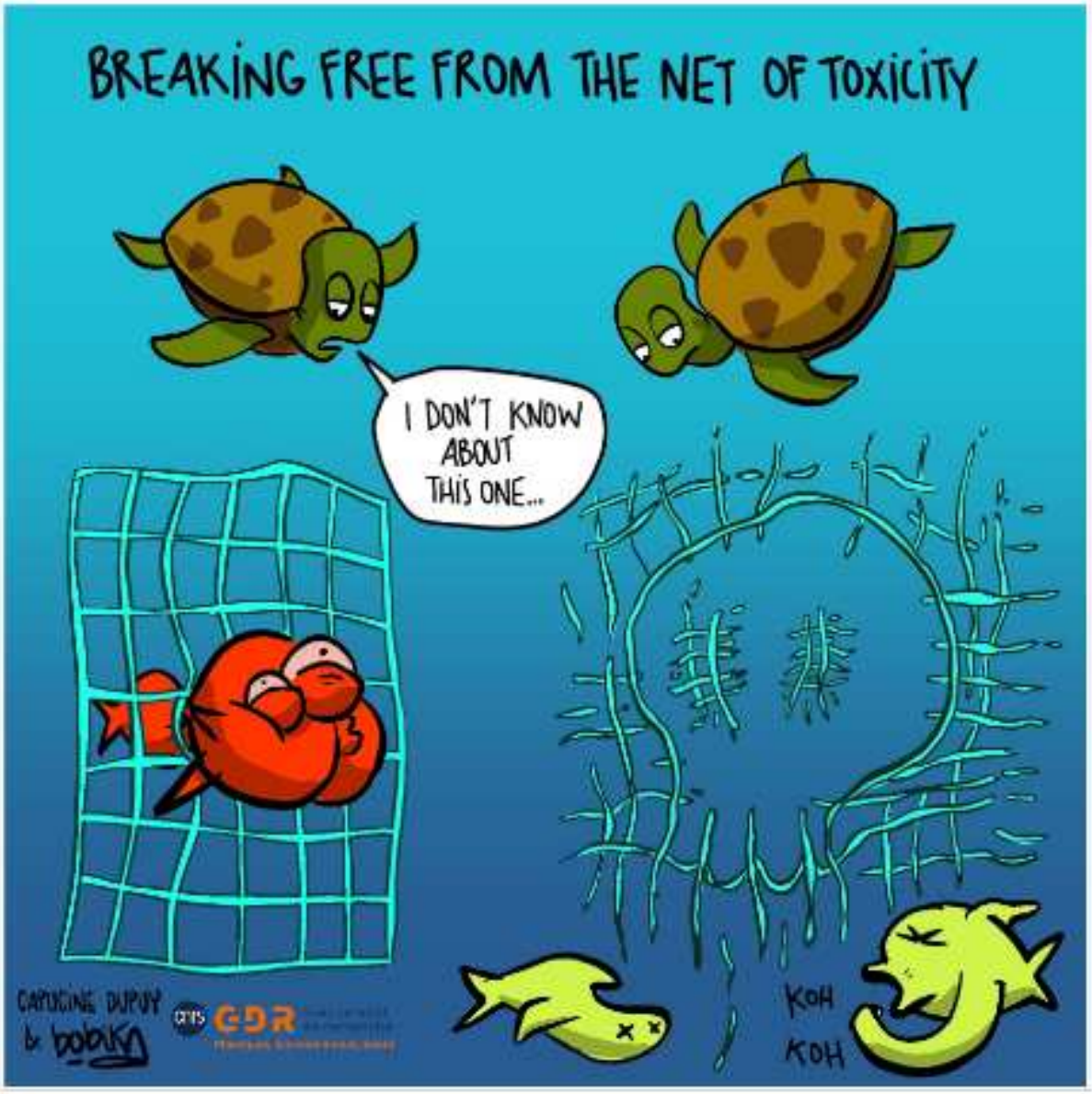

Are biodegradable plastics really less toxic than conventional ones?? For industries like fishing, aquaculture, agriculture and cosmetics, whose nets, ropes, mulch film, microbeads and other plastic components inevitably end up in the environment, that would be a big win. After all, biodegradable plastics break down faster.

But research tells a different story. Scientists exposed fish larvae and marine bacteria to a "net juice" (a leachate released as fishing nets degrade) . The result? The biodegradable, bio-based plastics tested were just as toxic, if not more so, than their fossil-based counterparts. As they break down, they release additives and fillers that are harmful to certain organisms.

That doesn't mean that all biodegradables should be written off. In some cases, like fishing nets, they could help reduce ghost fishing. And for certain applications, they might serve as viable alternatives to fossil-based plastics. But here's the catch: they need to be designed with the bigger picture in mind. That means rethinking their chemical composition, being transparent about their impacts, and carefully considering the environments they will end up in. For now, we are left navigating murky waters.

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PLASFITO
RESEARCH PROJECT

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Un arbre qui tombe, fait plus de bruit qu’une forêt qui pousse.

A falling tree makes more noise than a growing forest.

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List of abbreviations

AA: Artificial aging
AChE: acetylcholinesterase
AIS: Automatic Identification System
ALDFG: Abandoned, lost or otherwise Discarded Fishing Gear
ASD: Acclimatization to Sudden Darkness
ATR-FTIR: Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy
BLA: Basal Locomoto Activity
BoB: Bay of Biscay
BSI: Behavioral Stress Index
CI: Confidence Interval
DCSMM: Descriptor Criteria and Standards for Marine Monitoring
df: degree of freedom
DMSO: Dimethyl sulfoxide
dpf: days post fertilization
EPDM: Ethylene Propylene Diene Monomer
EPS: Extracellular Polymeric Substances
EOL: End-of-Life
EU: European Union
EVA: Ethylene-vinyl acetate
FG: Fishing gear
FN: Fishing net
GC/MS: Gas Chromatography/Mass Spectrometry
HDPE: High-density polyethylene
HPLC: High-Performance Liquid Chromatography
HQ: Hazard Quotient
ICP-OES: Inductively Coupled Plasma–Optical Emission Spectrometry
ID-K: Identification Key
LDIR: Laser Direct Infrared
LDPE: Low-density polyethylene
LOA: Length Overall
LOE: Line of Evidence
LOFF: Light Off
LON: Light On
LQ: Limit of quantification
MNPs: Microplastics and Nanoplastics
MPs: Microplastics
NIAS: Non-Intentionally Added Substances
NPs: Nanoplastics
OSPAR: Oslo-Paris Convention for the Protection of the Marine Environment of the North-East Atlantic
PA: Polyamide
PAH: Polycyclic Aromatic Hydrocarbons
PBAT: Polybutylene adipate terephthalate
PBEAS: Poly(butylene adipate-co-butylene succinate-co-ethylene adipate-co-ethylene succinate)
PBS: Polybutylene succinate
PBS-PBAT: Polybutylene succinate–polybutylene adipate terephthalate
PBSAT: Poly(butylene succinate-co-adipate-co-terephthalate)
PC: Polycarbonate
PE: Polyethylene
PE-PP: Polyethylene-Polypropylene
PERI: Potential Ecological Risk Index
PET: Polyethylene terephthalate
PHA: Polyhydroxyalkanoate
PHB: Polyhydroxybutyrate
PHBV: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PHI: Potential Hazard Index
PLA: Polylactic acid
PLI: Pollution Load Index
PP: Polypropylene
PS: Polystyrene
PTFE: Polytetrafluoroethylene
PU: Polyurethane
PVC: Polyvinyl chloride
RQ: Risk Quotient
ROS: Reactive oxygen species
SE BoB: Southeastern Bay of Biscay
SEM: Scanning Electron Microscopy
TPZ: Time in Peripheral Zone
UV: Ultraviolet light
VLMR: Vibrational and Light Motor Response
VLSR: Vibrational and Light Startle Response
VMR: Visual Motor Response
VSR: Visual Startle Response
WOE: Weight of Evidence
WWII: Wolrd War II

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GENERAL INTRODUCTION, HYPPOTHESIS AND OBJECTIVES

General introduction

Around you or even on your skin, whether you are in the mountains, in the middle of the ocean or in front of your desk, there are high probabilities that you will see plastic. And in case no macroplastic is within your reach, many objects within your proximity may include plastic components, even if you cannot see them. Furthermore, there are very likely microplastics (MPs) (particles < 1 mm) and nanoplastics (NPs) (particles < 1 µm) around you that you cannot discern (Thompson et al. 2024; Yang et al. 2025). This ubiquitous presence of plastic objects or particles is due to the continuous growth of plastic production, of their use, non-adequate disposal and recycling, and of their loss in the environment and fragmentation since 1950 (Barnes et al. 2009; Geyer 2020; Andrady 2022). Plastic was a revolutionary material that substantially contributed to the building of the society as we currently know it (Chalmin 2019). However, as a secondary effect, plastic became a susbstantial pollutant that threathens living beings, including humans (Landrigan et al. 2023; Scientists' Coalition for an Effective Plastics Treaty 2024). Progressively since the beginning of the century, publications have confirmed that plastic pollution is ubiquitous and has toxic effects on numerous species. However, given the huge diversity and quantity of plastics (Carrega & Verney 2017; Geyer 2020; Wagner et al. 2024), there are still knowledge gaps to fill in about plastic pollution amounts, sources and risks, particularly with regard to MPs, NPs and plastic chemicals. Evaluating the hazard and risk posed by plastics is particularly complex as it depends on many factors such as the characteristics of MPs, NPs and plastic chemicals, their behavior in the environment or exposure media, the sensitivity of the different organisms tested and the different biological levels studied (Botterell et al. 2019; Landrigan et al. 2023). Therefore, one of the main challenges in plastic pollution research is to independently study the different sectors that use plastics to better understand their contribution to the MP, NP and plastic chemical pollution and to establish their environmental risk.

In this context, while packagings, pellets and tyres have been extensively investigated (Hale et al. 2020), very few studies have been carried out on the hazards of plastic fishing gear (FG) degradation products such as MPs, NPs and plastic chemicals. This presents a significant knowledge gap, as FG are in direct daily contact with aquatic environments. Although the fishing gear account for only a small proportion of global plastic production (Schwarz et al. 2023), it is still a question of several millions of tonnes of plastic produced and used every year, from which a substantial amount is lost into the aquatic environment (Kuczenski et al. 2021). Moreover, even for plastic FG not lost during their operation, their end-of-life is poorly managed, since very few of them are recycled, and most are buried or incinerated, thus contributing to plastic pollution (Bertling & Nühlen 2019; Basurko et al. 2023). Therefore, in recent years, to mitigate the consequences of mismanagment of end-of-life FG and the known impact of lost ones (ghost fishing), biobased and biodegradable FNs were developed (Kim et al. 2016; Deroiné et al. 2019; Cerbule et al. 2023). However, there is very little knowledge about their compostability, degradation and environmental hazards (Paul-Pont et al. 2023).

It is currently recognized that plastics, whether oil-based, biobased or biodegradable, could be degraded when conventionally used or when lost or abandoned in the environment. It has been proven for many plastics that their degradation is accentuated by exposure to ultraviolet

(UV) irradiation, high temperature, biocolonization and strong mechanical forces (abrasion or elongation) (Masry et al. 2021; Andrady 2022; Dimassi et al. 2022). All these factors are known to affect FG during their operation in the environment, as they are subjected to mechanical forces during their use in the water column and sea bottom (Deschamps 2004, 2009; Le Gué 2024) and are often stored on boat decks and port docks, with varying degrees of UV irradiation and temperature changes (Al-Oufi et al. 2004). This continuous degradation during their operation can potentially lead to the production of MPs, NPs and plastic chemicals (Hahladakis et al. 2018). These degradation products were recognized as a toxic mixture of pollutants for most of the living organisms studied, particularly aquatic ones (Parsai et al. 2022; Ferreira et al. 2023; Ricardo et al. 2024; Zhao et al. 2024). Furthermore, increasing evidence highlighted that the observed toxic effects were significantly associated with the chemical compounds released during the plastic degradation process (Zimmermann et al. 2020; Da Costa et al. 2023; Savva et al. 2023). However, in the case of fishing nets (FNs), their chemical composition is rarely provided and there is no regulation that limits the use of hazardous compounds in the manufacture of FNs. The toxic hazard of FNs is further complicated because plastics are capable of adsorbing and absorbing hazardous environmental pollutants such as metals, pesticides or hydrocarbons and can later release them, specially when ingested by organisms (Hartmann et al. 2017; Hermabessiere et al. 2017; Le Bihanic et al. 2020; Wu et al. 2021; Al Harraq et al. 2022; Cormier et al. 2022; Martínez-Álvarez et al. 2022, 2024). FNs, whether conventional or biobased/biodegradable, are in direct contact with the environmental pollutants which could enhance their toxic effects to aquatic organisms (Lima et al. 2023; Almeida et al. 2024).

Thus, considering the amount of FNs produced and used annually, the proportion of these nets that are abandoned, lost, or discarded, and their potential degradation into toxic MPs, NPs, and plastic chemicals, it is essential to investigate their fate and effects in detail. To do this, in this Ph.D. thesis, FNs were studied as new products, after use by fishermen, and after aging under controlled conditions. Five different FNs used by different fishing *métier* and made of different polymers with distinct chemical compositions were studied. Among these five FNs, one was a trawl cod end made of a blend of polyethylene and polypropylene, another one was a straight gillnet made of polyamide 6 (PA) and two others were trammel nets made of PA but produced by different manufacturers. This work also included a partially biobased and biodegradable trammel net made of polybutylene succinate-polybutylene adipate terephthalate. This latter FN was selected to compare its degradation and toxicity with those of the conventional oil-based and non-biodegradable FNs described above. Trawl and gillnets were chosen for this study because they are the primary fishing métiers in terms of hours of operation in the Southeastern Bay of Biscay (SE BoB) (GFW 2024). The SE BoB was an ideal area to conduct this study for three main reasons. First, because the fishing fleets and the FNs are very variable from one region of the world to another, and it was more relevant to focus the investigation on a smaller area and a smaller fishing fleet. Second, there is an intense fishing activity and the area is known as an accumulation zone of marine plastic litter, with a high percentage of abandoned, lost or otherwise discarded fishing gear (ALDFG) (Borja et al. 2019; Mendoza et al. 2020; Ruiz et al. 2020). Third, the SE BoB is known for its rich and vulnerable biodiversity with several marine protected areas where fishing is authorized (Ruiz

et al. 2022; Victorero et al. 2025). In order to assess the toxicity of the degradation products of FNs, several aquatic species with different sensitivities can serve as models. Since there are heterogeneous patterns of toxic effects of MPs, NPs and plastic chemicals depending on the species characteristics, such as feeding strategy among others, it was chosen to study the hazard of plastic FNs on different aquatic species of different trophic levels. In this context, the marine bacterium *Allivibrio fischeri*, the microalga *Tisochrysis lutea*, the copepod *Acartia tonsa*, the rainbow trout liver cell line RTL-W1 and the larva fish *Oryzias latipes* were selected. To properly assess the environmental risk of the degradation products of FNs, several lethal and sublethal biological responses at different biological levels were studied.

Hypotheses & Objectives

The **hypotheses** of this Ph.D. thesis are: first, that fishing nets degrade and produce a significant amount of microplastics, nanoplastics and plastic chemicals during their operation or when they are abandoned, lost, or discarded, in the Southeastern Bay of Biscay. Second, the mixture of degradation products (MPs, NPs and plastic chemicals) is toxic to several aquatic species at different trophic levels such as marine bacteria, microalgae, copepods and euryhaline fishes, thus posing a risk to the aquatic environment.

The **general objective** of this doctoral thesis is to evaluate the production and ecotoxicological impacts of the mixture of microplastics, nanoplastics and plastic chemicals, coming from the degradation of plastic fishing nets on four different species (marine bacterium *Allivibrio fischeri*, microalga *Tisochrysis lutea*, copepod *Acartia tonsa*, fish larva *Oryzias latipes*), with a specific focus on the nets predominantly used in the Southeastern Bay of Biscay.

The general objective has been subdivided into **six specific objectives**. The specific objectives, as outlined below, will be addressed within the chapters of the **Results and Discussion** section of this Ph.D. thesis.

1- To understand the historical evolution of the fishing sector and the fishing gear used in the Southeastern Bay of Biscay, as well as to revise the knowledge available about plastic pollution and its toxicity, from a general point of view and focusing on fishing gear.

2- To characterize the efficient fishing activity of the Southeastern Bay of Biscay, to be able to document the main fishing nets used and to determine their polymer composition, and to estimate the quantity of fishing nets used, lost and washed up on selected beaches of the Southeastern Bay of Biscay.

3- To determine the toxicity of extracts of dimethyl sulfoxide containing plastic chemicals and environmental pollutants of several new and end-of-life oil-based fishing nets used in the Southeastern Bay of Biscay, as well as biobased and biodegradable fishing nets under development.

4- To investigate the degradation kinetics of three fishing nets of different polymer composition and different fishing *métiers* in terms of the release of microplastics, nanoplastics and plastic chemicals and to examine their effects on two aquatic species.

5- To establish a first risk assessment of the degradation products (MPs, NPs and plastic chemicals) of three new fishing nets made of polyamide but with different chemical composition and one biobased and biodegradable fishing net, based on their toxicity to four aquatic species, of which three are present in the Southeastern Bay of Byscay.

6- To assess the sub-chronic toxic effects on the reproductive outcomes of the copepod species *Acartia tonsa*, resulting from exposure to degradation products derived from an oil-based fishing net, as well as a partially biobased and biodegradable alternative.

The **General Discussion** will then synthesize the findings and conclusions from each chapter by critically relating them to the existing academic knowledge to provide a comprehensive overview of the environmental risk of the degradation products of fishing nets.

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RESULTS AND DISCUSSIONS

Chapter 1 State of the art

Context:

Millions of inhabitants around the world depend on fishing activities for their livelihood and therefore rely directly on plastic fishing gear. While this fishing gear is an essential plastic products, it has negative effects on the aquatic environment. Ghost fishing is one of the best-known impacts of fishing nets (FNs), and numerous studies have been conducted on the sources, effects, and ways of mitigating abandoned, lost, or otherwise discarded fishing gear in the marine environment, as well as reducing their abandonment and loss. It is increasingly recognized in the scientific community that, in addition to macroplastic impacts, plastic degradation products, including microplastics, nanoplastics, and plastic chemicals, have negative effects on aquatic species. However, little knowledge is available about plastic FNs. Therefore, to better characterize the degradation of the FNs used in the Southeastern Bay of Biscay, it was first necessary to understand the fishing sector more comprehensively. Secondly, it was essential to quantify the use of plastic in the manufacture of FNs, as well as to identify the specific polymers that constitute these nets. Thirdly, conducting an analysis of the existing body of knowledge regarding plastic degradation processes would facilitate a deeper understanding of the degradation processes specific to FNs. Finally, a review of the toxicity of degradation products originating from commonly used plastic products to various aquatic species will provide initial insights into the potential toxicity associated with FNs.

Objective:

To understand the historical evolution of the fishing sector and the fishing gear used in the Southeastern Bay of Biscay, as well as to revise the knowledge available about plastic pollution and its toxicity, from a general point of view and focusing on fishing gear.

1.1 The fishing sector in the Bay of Biscay

1.1.1 Description of the Bay of Biscay

The Bay of Biscay is delineated by "Cape Finisterre" (43°N, Galicia, Spain) to the south and "La Pointe du Raz" (48°N, France) to the north, encompassing approximately 175,000 km². Its abyssal basin constitutes approximately 50% of the total surface area, reaching a mean depth of 4,800 meters (Lavín et al. 2006). The Bay comprises three distinct margins: the Armorican margin in the north with a broad continental shelf (up to 200 km of width), a steep canyon-dominated slope in the Aquitaine margin in the southeast with a narrower shelf (between 70 to 150 km) and the Cantabrian shelf in North of Spain which is thinner (between 10 to 40 km) (Figure 1.1) (Lavín et al. 2006; Almazán-Gómez et al. 2018). A notable bathymetric characteristic is the extensive presence of submarine canyons, with approximately 135 canyons recorded along the continental margin (Figure 1.1) (Bourillet et al. 2006; Van den Beld et al. 2017). Water circulation patterns within the Bay are complex, influenced by bathymetry, tides, density-driven currents, and wind regimes. The oceanic region is characterized by a relatively weak (<2 cm s^{-1}) anticyclonic circulation (Figure 1.1) (Assassi 2015). A significant current is the poleward current along the Atlantic coast of the Iberian Peninsula during

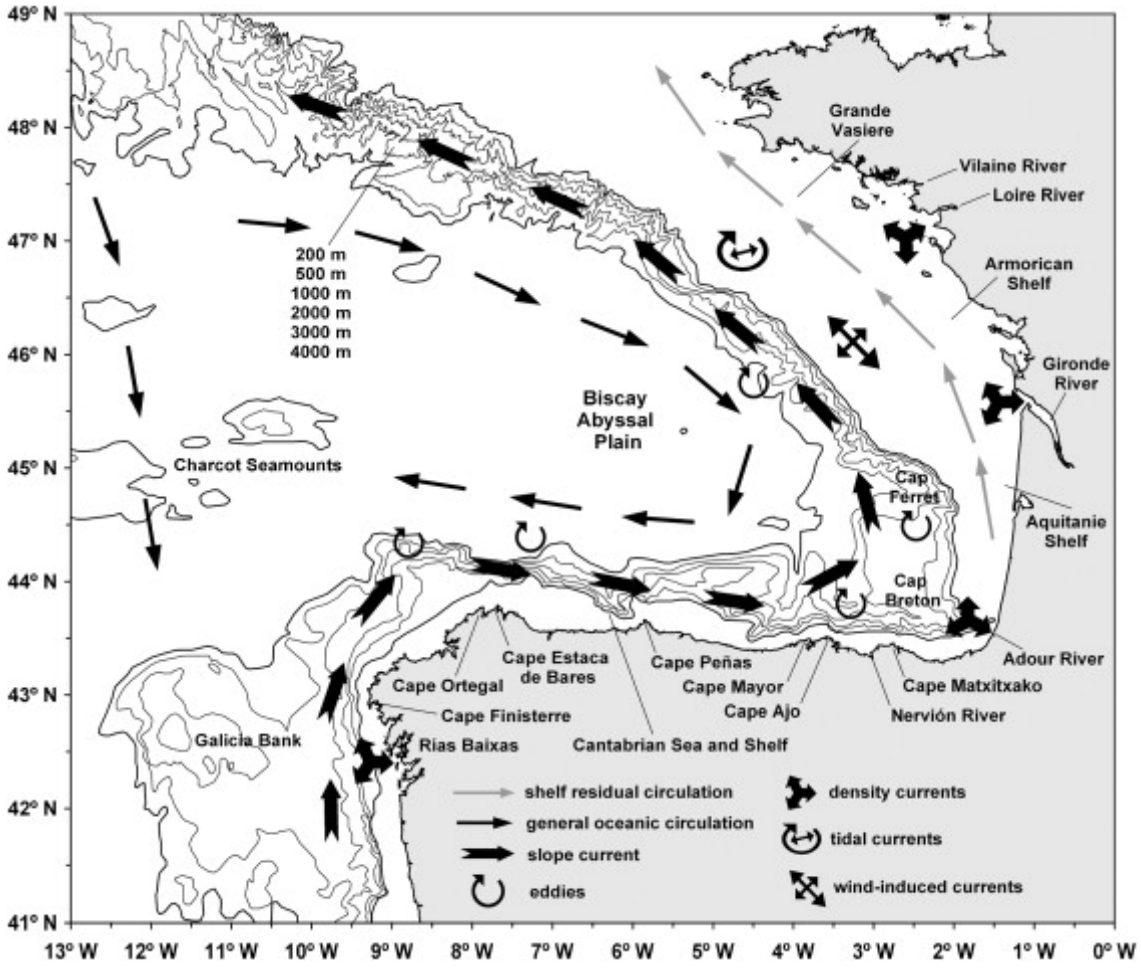

Figure 1.1: General water circulation in the Bay of Biscay (taken from Ferrer et al., (2009) and modified from Koutsikopoulos and Cann (1996)).

autumn and winter, transporting warm, saline water masses along the continental slope of the Bay of Biscay at velocities of approximately 25 cm s^{-1} (Figure 1.1) (Pingree & Le Cann 1992). The Bay exhibits significant spatial variation in biological productivity. The southwestern region (Galician waters) shows the highest primary productivity levels, with annual averages of 43.44 mg Chl_a/m^2 and 138.07 mg C/m^2/day, attributable to the influence of the Galician upwelling system (Borja et al. 2019). Coastal upwellings are also identifiable along the Cantabrian Sea and the French continental shelf, particularly during summer months (Puillat et al. 2004). Additionally, water discharge from four the major French rivers, Vilaine, Loire, Gironde, and Adour, contributes approximately to 80% of the freshwater input to the Bay, significantly influencing nutrient cycling and productivity (Borja et al. 2019). The Bay of Biscay encompasses 42 distinct benthic habitats out of the 62 identified in the Northeast Atlantic, according to the European Union Nature Information System classification (Galparsoro et al. 2014; Vasquez et al. 2015). These habitats range from estuarine and coastal environments to continental shelf, slope, and deep-sea ecosystems. The submarine canyons represent particularly essential habitats, hosting vulnerable marine ecosystems constructed by scleractinian corals, sponges, and deep-sea oysters (Van den Beld et al. 2017; Borja et al. 2019). These canyon systems serve also as conduits for sediment transport and organic matter transfer from the coast, creating unique ecological niches. The Bay's ecosystem is primarily pelagic-dominated, especially at lower trophic levels, with strong benthic-pelagic coupling (Corrales et al. 2022).

The Bay of Biscay supports remarkable biodiversity due to its transitional biogeographical position between subtropical and boreal regions (Almazán-Gómez et al. 2018). This transition zone results in many species reaching either their southern or northern distribution limits within this region. Phytoplankton constitutes approximately 17.5% of the total biomass and exhibit pronounced seasonal patterns, with spring blooms dominated by diatoms, subsequently replaced by dinoflagellates during summer months (Casas et al. 1999; Varela et al. 2001). Zooplankton communities, which represent 26.0% of the biomass, are predominantly composed of copepods (71-81% of total zooplankton abundance), with 95 species identified in the region (Valdés Santurio 1993; Albaina & Irigoien 2007). The Bay hosts also significant cold-water coral ecosystems which create complex three-dimensional structures enhancing local biodiversity, particularly along the continental slope and within submarine canyon systems (Costello et al. 2005; Huvenne et al. 2011). Benthic ecosystems are dominated by invertebrate groups (21%) including decapods (7.3%). Fish communities are diverse, with pelagic fishes representing 22.5% of the Bay’s biomass and demersal fish groups accounting for 4.9% of it (Corrales et al. 2022). Nearly 700 fish species have been documented, including commercially important species such as sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*), Atlantic mackerel (*Scomber scombrus*), European hake (*Merluccius merluccius*) and Albacore tuna (*Thunnus albacares*) (Corrales et al. 2022). These forage fish play a crucial role in transferring energy from the pelagic compartment to higher trophic levels, fostering benthic-pelagic coupling. There is also a remarkable marine mammal diversity, with 42 species reported, representing approximately 35% of global marine mammal species (Jefferson et al. 2011).

To protect this rich biodiversity, a Marine Protected Areas (MPAs) network was developed, driven by the OSPAR Commission (OSPAR Commission 2021). In the Bay of Biscay, Spain created 8 MPAs (26,747 km²) and France 103 (39 OSPAR MPAs covering 22,102 km², along with 64 other overlapping areas, which prevents the calculation of total protected area) (Figure 1.2) (OSPAR Commission 2021). Conservation efforts target multiple ecological components including 5 invertebrates, 9 birds, 22 fishes, 4 marine mammals, and crucial habitats including intertidal mudflats, deep-sea corals colonies, deep-sea sponge aggregations and various species of birds (Alloncle et al. 2019; OSPAR Commission 2021). The network is coupled by other area-based conservation measures including Galician's critical area for Orcas with seasonal restrictions and various areas with temporary or permanent fishing restrictions (OSPAR Commission 2021).

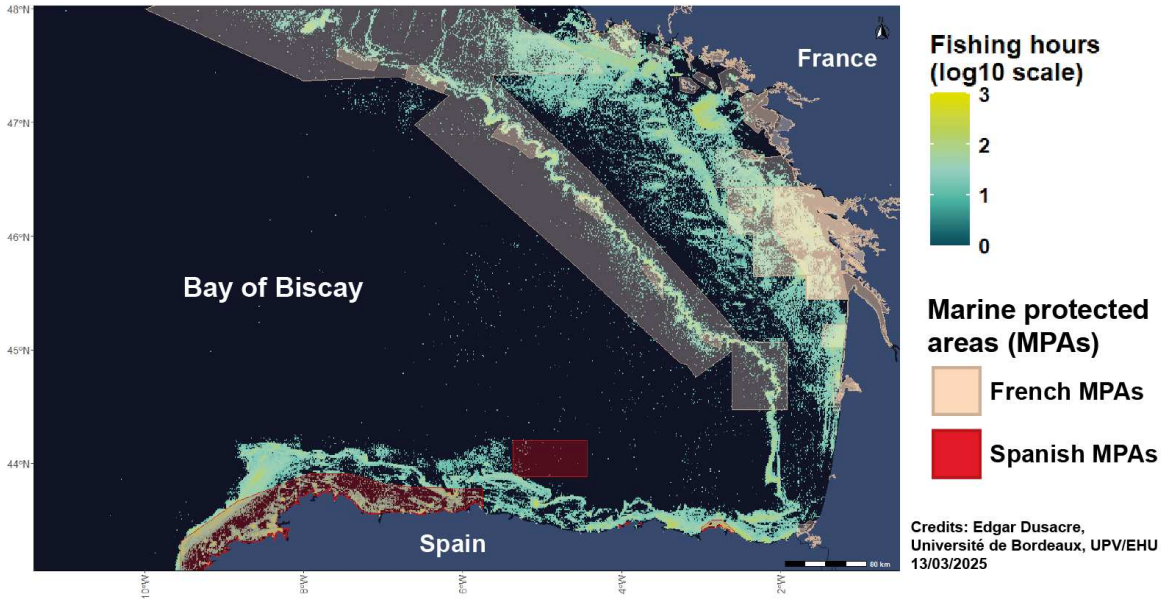

Figure 1.2: Spatial distribution of Marine Protected Areas (MPAs) alongside efficient fishing activity in the Bay of Biscay (log10 scale of fishing hours) in 2022 and 2023.
French MPAs are indicated in blue, while Spanish MPAs are represented in red. The efficient fishing activity was taken from GFW (2024) and the MPAs from the French Office for the Biodiversity (2024) and the Ministerio español para la Transición Ecológica y el Reto Demográfico (2025).

From a fishing perspective, the Bay of Biscay is in the FAO's major fishing area n°27 "North East Atlantic" which is divided in 14 subareas. The Bay of Biscay is the "27.8" subarea and is composed of 5 divisions (Figure 1.3) (FAO 2025). The fishing areas were established by the FAO in 1990 for statistical purposes, specifically to facilitate the registration of fishing fleets, quotas, catches, and landings. The coastal waters of the Bay of Biscay, rich in halieutic resources, are included in zones 27.8.a, 27.8.b, 27.8.c, while the abyssal basin is in the 27.8.d and 27.8.e sub-divisions (Figure 1.3). The area encompasses approximately 72 fishing ports, from which 22 are shown on the Figure 1.3. Most of the fishing effort in 2022 and 2023 was accumulated on the continental shelf and on the slope (Figure 1.2). It appears that the main areas of fishing activity overlap with numerous marine protected areas, both in France and in Spain (Figure 1.2). This observation raises questions about the impact of fishing on the Bay of

Biscay, whether in terms of fishery resources and biodiversity or in terms of its contribution to ocean plastic pollution. It is necessary to go back in time to understand the evolution of the fishing industry as well as the use of plastic for fishing gear in the Bay of Biscay over the last 2 centuries.

1.1.2 Historical context of the fishing sector in the Bay of Biscay

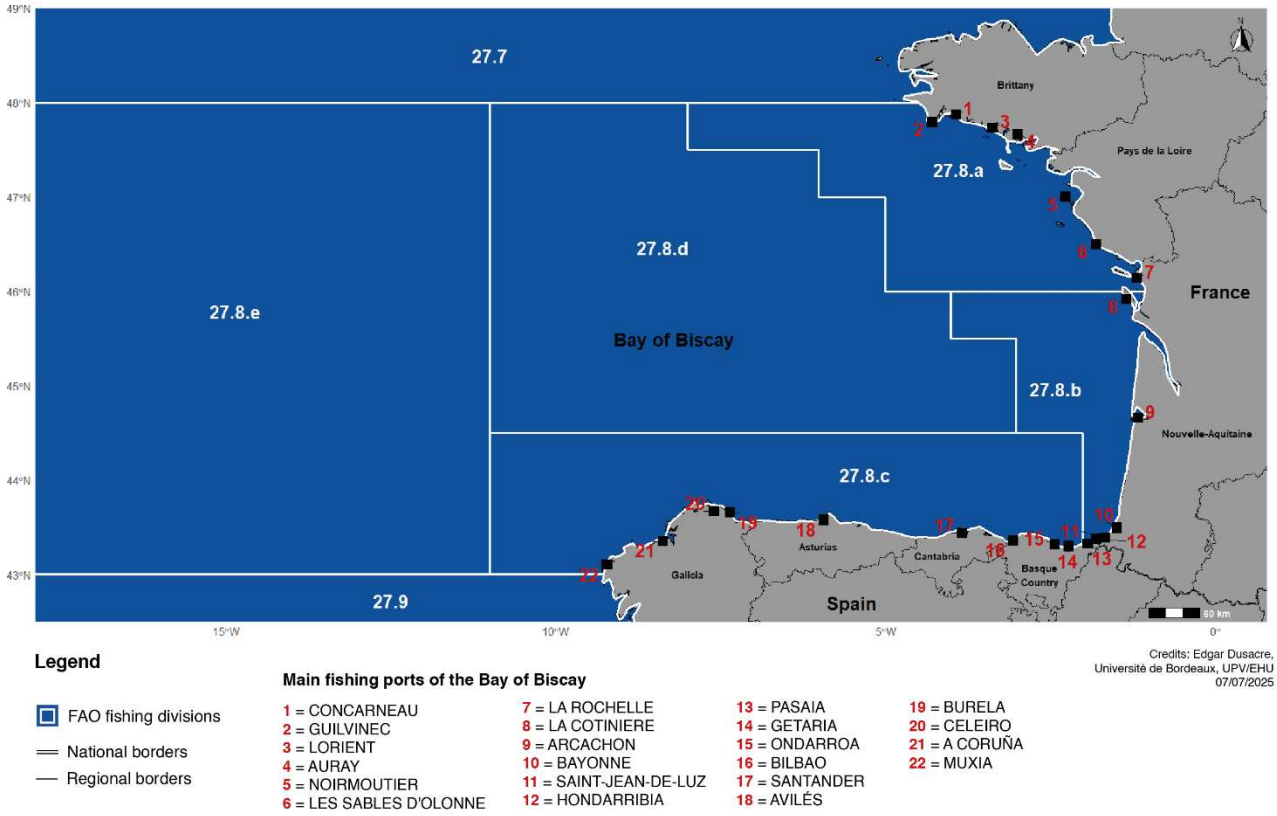

Figure 1.3: The Bay of Biscay and its FAO subareas (27.8) and divisions (27.8.a, 27.8.b, 27.8.c, 27.8.d, 27.8.e). Shapefile of the ports is taken from the European Environment Agency (2025).

1.1.2.1.1 From 1850 to the end of the second world war (WWII)

The transition from sailing to steam power between 1850 and 1910, as well as the creation of the fish cannery sector marked a significant turning point for the fishing fleet in the Bay of Biscay between 1850 and the early 1900s (Moulinier 2015). In the mid 19th century, the predominant vessels were wooden sailing boats, traditional “traineras” and “lanchas boniteras” or “sardinera”, which were used for coastal fishing of fish species like sardine and tuna (Zallo & Antonio 2003; López-Losa 2008; Ondicola n.d.). The first steam trawlers of the world appeared in the port of Arcachon in 1836, followed by the Cantabrian ports around 1880 and La Rochelle port (France) in 1904 (Figure 1.4). In 1908, French Basque ports (Capbreton, Saint-Jean-de-Luz and Hendaye), had tens of steam vessels. The new powered vessels enabled longer journeys, larger catching quantities and high seas and deepwater fishing (Zallo & Antonio 2003; Baulaz & Morin-Repinçay 2015; Ondicola n.d.). At this period,

in the Basque Country and in Cantabria the “sarda” purse seine became the dominant gear for sardine and anchovy fishing, replacing the labor-intensive rowing with mechanized net hauling, which also facilitated the rise of tuna longlining. The species fished during this period of high fish abundance were predominantly sardine, anchovy, sea bass, tuna, sea bream, mackerel, turbot, sole, and hake. Regarding the materials used for the manufacture of fishing gear during this period extending until the 1950s, they were primarily comprised of natural fibers such as horsehair, hemp, pita, or cotton (Zallo & Antonio 2003; Deschamps 2009).

The inter-war period then brought new changes, with the introduction of the fuel boats, the wireless telegraphy for deep-sea vessels, immersion thermometers and salinometers, and the development of larger specialized fishing companies (Moulinier 2015). These companies possessed their own shipyards, fishing boats, fish processing plants and distribution channels.



Figure 1.4: A landing dock for steam trawlers in France.
Taken from Baulaz and Morin-Repinçay (2015)

In 1922, the purse seine named “bolinche” was authorized and boosted the sardine catches from hundreds of tons to thousand of tons in the Basque Country ports. By the same time, the number of trawlers increased, especially in the French ports, and reached several hundreds in the 1930s. This was particularly true for the tuna boats in the port of La Rochelle (Moulinier 2015). Although fish storage, facilitated by the use of ice loaded on board, had been established since the late 19th century, significant storage improvments were introduced onboard and in ports during the 1930s (Moulinier 2015; Lohier 2019). These innovations, coupled with the expansion of the deep-sea fishing fleet, facilitated the development of industrial fishing based on trawling (Moulinier 2015). However, combined with coastal small-scale fishing and the purse seine development, the fishing activity started to create pressures on fish stocks. Consequently, it led to crises between regional fisheries, such as the conflict between Basque and Brittany fishermen in 1926, and foreshadowed the overfishing issues that would emerge in later decades (Zallo & Antonio 2003; Baulaz & Morin-Repinçay 2015; Ondicola n.d.). Nevertheless, the WWII slowed down the development of the fishing industry and its activities, leading to a strong growth of all fish stocks in the Bay of Biscay by 1945 (Moulinier 2015).

1.1.2.2 Post-WWII boom and fishing sector technological revolution

The post-WWII era underwent significant technological advancements in fishing vessel construction and propulsion in the Bay of Biscay. By the 1950s, wooden hulls were increasingly replaced by steel and fiberglass materials that offered greater durability and reduced maintenance costs (Ondicola n.d.). Diesel engines ranging from a few to thousands horsepower began to substitute steam power (Zallo & Antonio 2003), offering improved power efficiency and reliability (Moulinier 2015). In addition to more efficient engines requiring less manpower to operate, boat equipment had improved. In particular, between 1945 and 1970 boats were equipped with onboard renewed water tanks for tuna boats, stern fishing capacities (Figure 1.5), auxiliary engines, radiophony, direction-finding, and depth-finders (Baulaz & Morin-Repinçay 2015; Moulinier 2015; Ondicola n.d.).

In the Bay of Biscay ports, from the early 50s, plastic polymers, and especially the braided polyamide (PA) (nylon), appeared and started to replace natural fibers, thus revolutionizing trawl and purse seine fishing (Deschamps 2004, 2009; Martinussen 2006). The preference for this material was attributed to its enhanced strength, reduced weight, and resistance to



Figure 1.5: Trawl fishing vessel equipped with stern facilities. La Rochelle 1965.
Taken from Baulaz and Morin-Repinçay (2015).

biofouling. These qualities facilitated the usage of larger nets and improved the rate of fish capture (Martinussen 2006; Matsushita et al. 2008). The introduction of PA monofilament nets in 1960 marked the development of modern gillnets (Deschamps 2009). By the year 1960, in Norway, PA constituted 95% of the fishing nets used for cod capture (Martinussen 2006). In the same period the polypropylene (PP) thread was invented and started to be used for buoys and nets. In the Basque Country (Spain), this period was called the “golden age of the sector” with a strong industrial fleet development for deep-sea vessels, promoted by economic support from the state and laws for the renewal and protection of the fishing fleet (Ley 147/1961) (López-Losa 2008). This combination of multiple innovations contributed to the rapid growth of catches in the 1960s and early 1970s. In those post-war years, boats from the ports of the southeastern the Bay of Bay had begun to fish further and further away, reaching the Irish coast, in the North Sea, or off the Senegal coast (Baulaz & Morin-Repinçay 2015; Ondicola n.d.). In general, the fishing effort was greatly intensified, rising from over 520,000 tonnes of fish landed in 1950 to 990,000 tonnes in 1964 (Figure 1.6). This unregulated

intensification of fishing activity had an impact on fish stocks as early as the 50s, with the depletion of the sardines in the Bay of Biscay, and became widespread from the 70s to the 90s (Guénette & Gascuel 2012; ICES 2022).

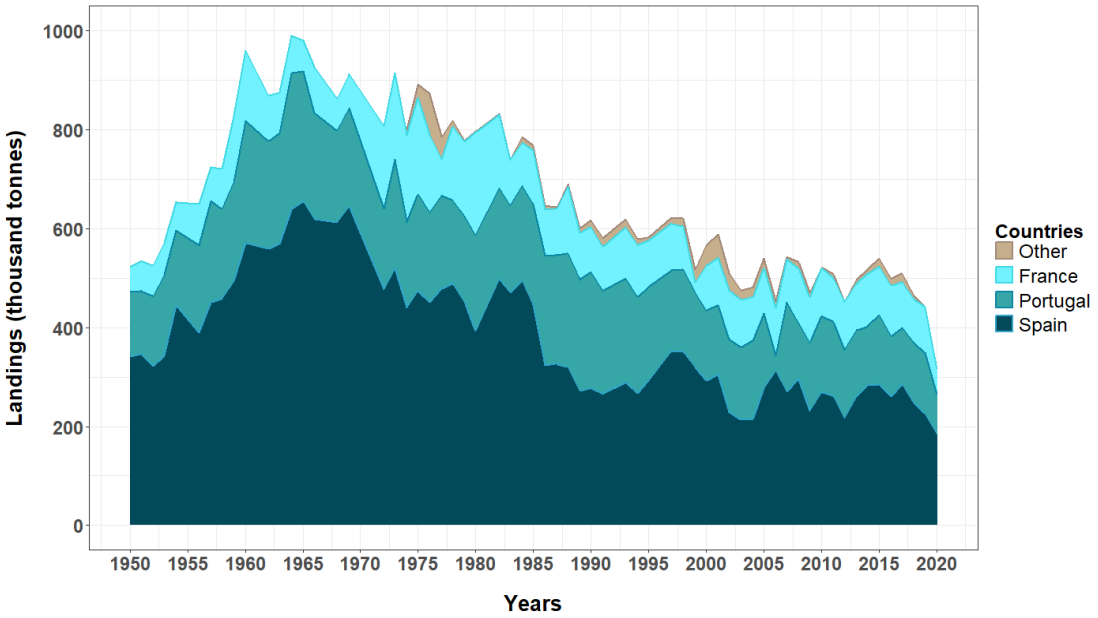

Figure 1.6:The quantity of fish landed in the ports within the fishing region of the Bay of Biscay.
FAO subareas: 27.8.a, 27.8.b, 27.8.c, 27.8.d, 27.8e, 27.9.a, 27.9.b. France, Portugal and Spain were represented individually as they accounted for the largest quantities of fish landings from 1950 to 1990. The "Other" category aggregated all other countries that landed fish in the region. This figure is based on the data of ICES (2022).

1.1.2.3 From the 1970s onwards, a period of crisis in fish stocks and fleet restructuring

During the 1970s, a pivotal transition of the fishing sector of the Bay of Biscay occurred. In the early 70s numerous technological advances had further improved fishing efficiency and yields (Lively & Good 2019). Firstly, two fishing métier were developed, the pelagic trawling and the large-scale gillnetting, thanks to the nets made of PA monofilament and multifilament (Figure 1.7) (Deschamps 2009). In fact, the latters were larger, more resistant and provided higher fishing yields. In addition to PA, high-density polyethylene (HDPE), and PP became predominant materials for trawls, and various gear components, offering superior durability and operational efficiency (Punzón et al. 1999; Baulaz & Morin-Repinçay 2015). In the following years, a mix of these two polymers called polysteel was developed to make more resistant and lighter trawls, bottom seines and ropes. In this period, both sonar and Global Navigation Satellite Systems (GNSS) were introduced. The fishermen also used an increased number of electrical and mechanical gear haulers, facilitated by the installation of auxiliary engines. Moreover, the introduction of the hydraulic net-handling systems enabled the trawlers to switch between bottom and pelagic trawling (Figure 1.7). In this period, the fishery became more specialized in trawling, replacing traditional methods such as the "bolinche" (purse seine net) (Zallo & Antonio 2003; López-Losa 2008; Baulaz & Morin-Repinçay 2015; Ondicola n.d.). It was also in this period that the ports of Nouvelle-Aquitaine (Southwest France) (Figure 1.3) declined in terms of catches and number of boats in favor of the ports of Brittany (Moulinier 2015). However, overall, fishing effort continued to rise in the area,

increasing the pressure on fish stocks (Guénette & Gascuel 2012). This continuous increase caused the beginning of "The Great Fishing Crisis" by the end of the 70s (López-Losa 2008; Baulaz & Morin-Repinçay 2015). This multifactorial crisis was mainly due to fish resource depletion and to poor regulation of the fishing activity. However, the crisis was also influenced by: (i) The oil crisis of 1970. (ii) The extension of the marine territorial waters of Spain that prevented French vessels from accessing traditionally productive Spanish continental shelves. (iii) the decrease of the fish quality (species richness, smaller sizes, lighter weight). (iv) The fall in in prices for seafood products.

Therefore, the 1980s were characterized by fleet reconfiguration and implementation of initial regulatory frameworks. Particularly, the creation of the "Blue Europe" program by the European Union introduced Total Allowable Catches (TACs) and fishing quotas as systematic approaches for fish stock management. Therefore, in the Basque country (Spain), following the country's entry into the European Union and numerous changes in fishing regulation, the number of deep-sea vessels and especially trawlers registered in Basque ports fell from 306 in 1975 to 121 in 1985 (López-Losa 2008). Specialized fleet changes occurred in ports like Pasaia,

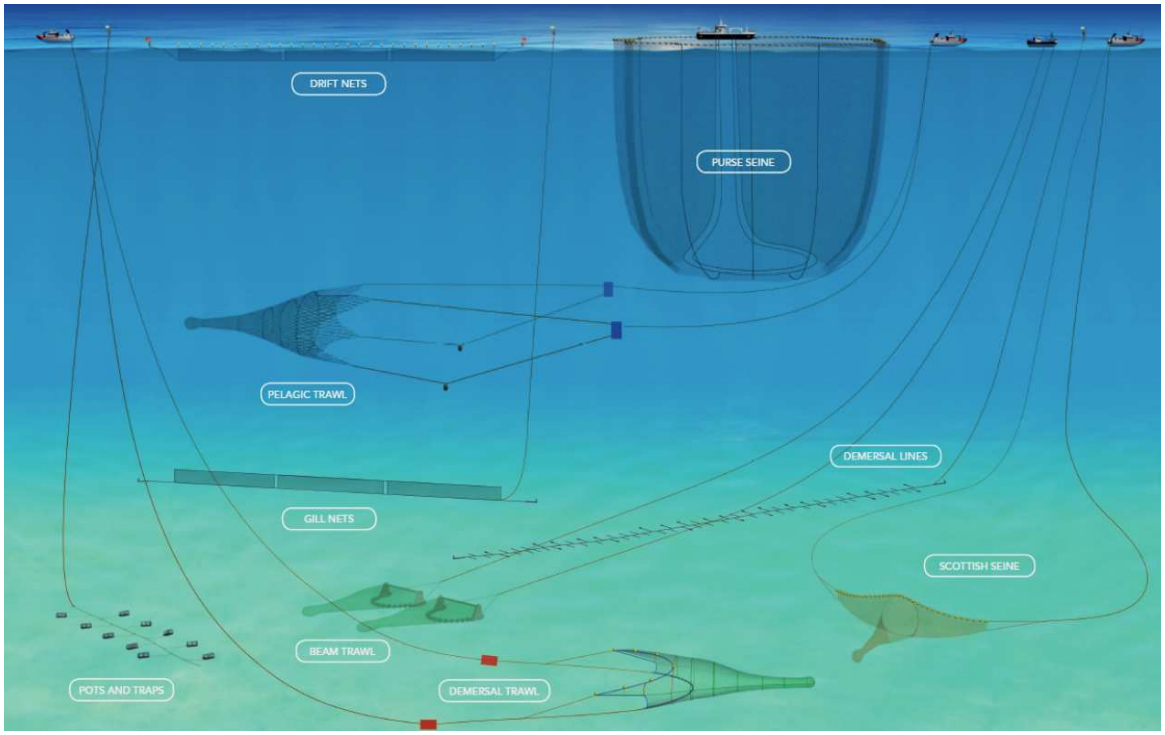

Figure 1.7: Illustration of some fishing gear used in the Bay of Biscay.
It should be noted that drift nets were banned in the European Union waters since 2002. Demersal lines are also called set longlines. The figure does not show some fishing gear as drifting longlines, trolling lines, dredges and some gear used by the poles and lines fishing métiers. The image is taken from Weissenberg (2015).

Ondarroa, Bermeo, Arcachon, Saint-Jean-de-Luz, and Lorient becoming predominantly dominated by small-scale fisheries. Consequently, the design of gillnetters improved, making it possible to carry more nets on board (several tens of kilometers of length), while using less manpower (Baulaz & Morin-Repinçay 2015).

The year 1990 represented a critical inflection point characterized by resource depletion (Figure 1.6) and subsequent fleet reductions. Quantitative data illustrates this decline: the

global fish landing for France, Portugal and Spain went from approximatly 900,000 tons in 1970 to 620,000 tons in 1990 (Figure 1.6) (ICES 2022). Ports of Saint-Jean-de-Luz and Hendaye underwent 60% reduction in pelagic trawling capacity within six years (600 fishermen and 100 vessels in 1992 versus 320 fishermen and 62 vessels by 1998 (Ondicola n.d.). Hake declined to 11.1% of landings by 1992 compared to 1970 and jack mackerel/horse mackerel landing went from 160,000 tons in the end of the 70s to 50,000 tons in 1990. The sardine stock, which had improved up to the mid-1980s after falling in the 1950s, was falling again (ICES 2022).

In reaction, the European Union promoted regulations targeting pelagic trawling practices identified as causing significant stock depletion. The European Union also reduced the use of drifting gillnets by prohibiting to keep on board or to use driftnets whose individual or total size exceeded 2.5 km (Sala 2016). Moreover, the implementation of the “Mellick Plan” resulted in the removal of approximately 250 vessels between 1991 and 1995 in France (Léauté 2000). In 2002, the use of all drifting gillnets was permanently prohibited for the capture of highly migratory species, such as tuna and swordfish, within European Union waters (Sala 2016). Therefore, the fleet fishing in the Bay of Biscay moved towards a coastal fishery with less fish landings, but without a drastic drop in boat size and engine power (Baulaz & Morin-Repinçay 2015). There was also a resurgence of fishing *métier* that were neglected during the 1960s and 1970s, such as the utilization of the "bolinche" purse seine made of PA. Moreover, new technological innovations appeared with the implementation of square mesh, escape windows, and ground gear modification designed to reduce bycatch and sea floor impacts. In 1999, the invention of the ultra-high molecular weight polyethylene (UHMWPE) (sold under the brand name “Dyneema”) improved net drag (increasing their resistance in water), and gear durability and reduced energy consumption, especially for trawlers and bottom seiners (Ramos 1999; Nyatchouba Nsangue et al. 2020).

From 2010, small-scale and artisanal fishing became predominant in the Bay of Biscay, with industrial fishing significantly diminished across the area. Regulatory restrictions expanded to include gillnet size limitations and specialized fishing prohibitions (Sala 2016). Diversification strategies emerged, including targeting other species, "pescatourism", “algae fishing” and other activities as supplementary financial incomes (Baulaz & Morin-Repinçay 2015; CIDPMEM 64-40 2021). Franco-Spanish collaboration was developed through French vessels with French crews selling catches in Spain. The reformed Common Fisheries Policy implemented in 2013 has further regulated fishing by introducing multi-annual plannification, the obligation to land species even if quotas are exceeded, and a management approach based on the precautionary principle (European Parliament 2013). The implementation of these regulations has resulted in a moderate recovery of fish stock status in the Bay of Biscay. In 2021, it was reported that approximately 85% of studied stocks were fished at or below fishing maximum sustainable yield (MSY). However, these fish stocks fished at or below the MSY represented only 45% of the total landings. Moreover, only 30% of the stocks have full analytical assessment (ICES, 2021). 40% of the fish stocks studied were still below the threshold in which fishing mortality must be reduced to restore the stock. Overall, fish landings stayed between 330,000 and 413,000 tons from 2010 to 2018. From 2019 onwards, they declined because they were clearly impacted by the COVID-19 pandemic (Figure 1.6). Even if overfishing has diminished in the Bay of Biscay since the 2000s and stocks are restoring,

fish are smaller than in the past, and certain practices such as bottom trawling on vulnerable sea floors, as well as catch discards, by-catches and abandoned, lost or otherwise discarded fishing gear (ALDFG) remain as important issues to be addressed (Guénette & Gascuel 2012; Ravard et al. 2014; Lively & Good 2019; Véron et al. 2020).

1.1.3 Bay of Biscay's fishing activity since 2022

1.1.3.1 Fishing situation pertaining to the global Bay of Biscay

Although 2022 had suggested a return to pre-pandemic catch levels (250,000 tons), 2023 was a black year, with the lowest catch since 1990 (210,000 tons) (European Commission & Eurostat 2013, 2015, 2024). Fishing vessels from the ports of North of Spain (Basque Country, Cantabria, Asturias, Galicia) (Figure 1.3) caught 65% of the fish fished in the Biscay Bay in 2023, followed by France with 34%. Other countries (Belgium, Denmark, Ireland, Netherlands, Poland, Portugal, United Kindgom) catched 1.4%.

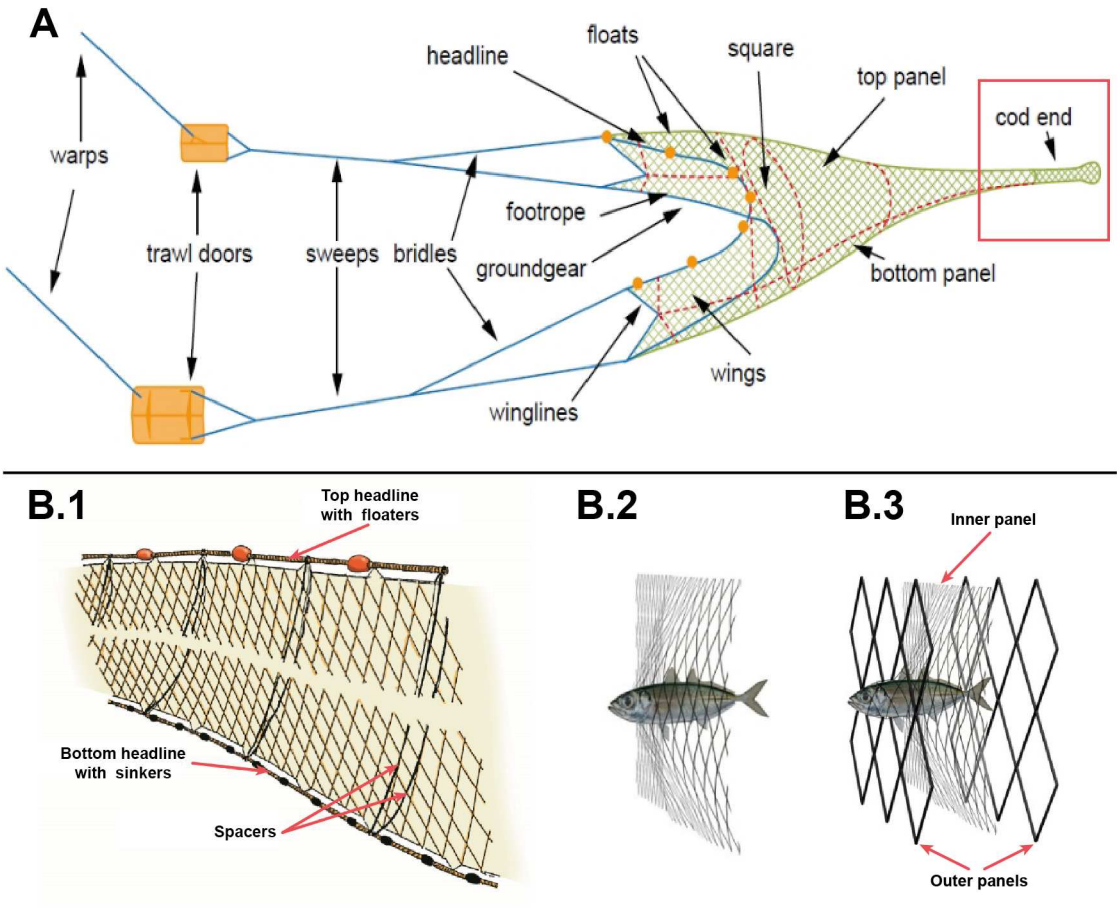

Figure 1.8: Structure of trawls (A) and gillnets (B.1) and catching principle for a straight gillnet (B.2) and a trammel net (B.3). The Red square at the right of the image (A) indicates the cod end of the trawl. Straight gillnets have only one panel with a unique mesh size while the trammel nets have 3 panels. The outer one with a larger mesh and the inner panel with smaller mesh. Modified from Weissenberg (2015).

Since the early 2000s, the polymers used in the manufacture of FNs have undergone minimal changes (they are mainly made of PA, polyethylene (PE), PP, polysteel and UHMWP). Most of these primary polymers, monofilament threads or manufactured FNs, are coming from Asia. In Europe, and more specifically in Spain and France, the companies that manufacture and sell the fishing gear are divided into two types. The companies that extrude the polymeric fibres (e.g., Redesmar, Momoï, Sicor Fishing and Marine, Garware technical fiber) and the fishing gear designer, manufacturer and seller companies (e.g., Eurored, Redesmar, Momoï, Sicor Fishing and Marine, Garware technical fiber, Naberan, Sumipesca, Tecnored, Le Drezen, ETS A. Mondiet, Alprech Filets). Concerning the three most used fishing gear in the Bay of Biscay, i.e., bottom trawls, straight gillnets and trammel nets, the first is mostly made of PE, PP and polysteel and the others of PA. The bottom trawl is a three dimensional funnel-shaped net opened front, and towed by a boat (or two boats), allowing active fishing. It is made of various panels with different meshes and colors (Figure 1.8) (Weissenberger 2015). The part of the gear studied in this PhD work was the cod end (Figure 1.8). On the contrary, gillnets are fixed gear that result in a passive fishing. There are gillnets of various structures, but the Bay of Biscay the straight gillnets and trammel nets are mostly used (Figure 1.8). The latter have three net panels of different mesh. The panels themselves are made of PA, while the ropes and spacers are usually made of PE or PP. Although in recent years there has not been much change in the polymers, efforts have been undertaken to modify FNs and especially trawls, to reduce fuel consumption, their impact on the sea floor and to enhance their selectivity, particularly through the integration of fish escape mechanisms or changes in the mesh size or shape (Sala et al. 2023; Bates 2025; Krag et al. 2025; Petetta et al. 2025).

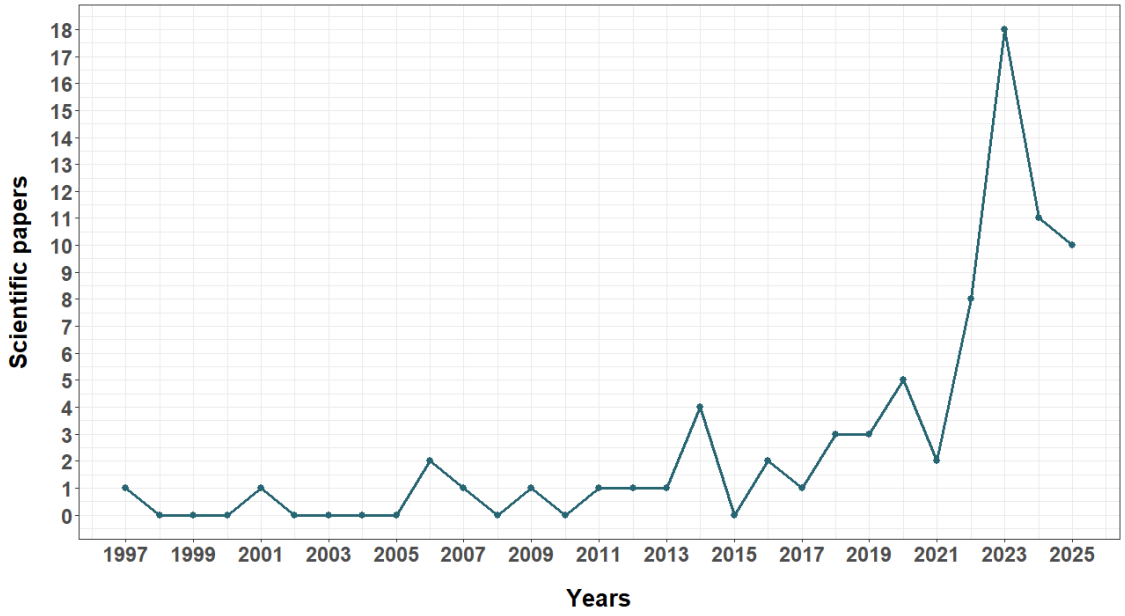

Figure 1.9: Number of scientific papers on the topic of biobased and biodegradable fishing gear and fishing nets between 1997 and 2025.
Results were extracted from the Scopus browser (07/07/2025). The following keywords were used (TITLE-ABS-KEY(biodegradable AND "fishing net") OR TITLE-ABS-KEY(biobased AND "fishing net") OR TITLE-ABS-KEY(biodegradable AND "fishing gear") OR TITLE-ABS-KEY(biobased AND "fishing gear")).

However, with increasing environmental concerns since the 2010s, and in particular the recognition of the global threats of plastic pollution (Kedzierski 2017), fishing gear polymers and additive compositions are expected to experience a new transformation over the next few years to reduce their environmental impacts. Therefore, research teams and private companies have set up efforts to develop biobased and biodegradable fishing gear. Research on Scopus revealed that five times more papers were published on this topic these last 10 years (63 scientific papers between 2015-2025) than during the 17 previous years (13 scientific papers between 1974-2014) (Figure 1.9). A large variety of biobased and biodegradable polymers were developed and tested in recent years. Those most widely used in the fishing industry were polybutylene succinate (PBS) (Deroiné et al. 2019), polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT) (Kim et al. 2016; Le Gué et al. 2023, 2025), polybutylene succinate co-adipate-co-terephthalate (PBSAT) (Grimaldo et al. 2018; Cerbule et al. 2022; Le Gué et al. 2024), polylactic acid (PLA) (Yu et al. 2023) and adipate-co-butylene succinate-co-ethylene adipate-co-ethylene succinate (PBEAS) (Park et al. 2023). In the European Union, manufacturers developing or marketing FNs as "biobased" must ensure the material is “derived from biomass”, although no minimum biobased content thresholds are required by EU law. While third-party verification (e.g., via EN 16640/EN 16785) is voluntary, certifications like “OK Biobased” (requiring ≥50% biobased carbon) are often pursued for market credibility (CEN-CENELEC 2016, 2017, 2018). The proposed “EU Green Claims Directive” (not yet ratified) may introduce stricter requirements for substantiating claims in the future. For “industrial compostable” FNs, manufacturers must comply with the EN 14995 standards (non-packaging plastics) which require ≥90% biodegradation at 58±2°C within 6 months, disintegration into particles <2 mm after 12 weeks and low toxicity on germination and growth of mono and dicotyledons (CEN-CENELEC 2006). Claims of “biodegradability in marine environments” refer to ISO 22403 or NF EN ISO 19679 guidelines and imply ≥90% conversion to CO_2/biomass at 20–30°C over 24 months. Nevertheless, these standards do not require ecotoxicity testing, do not regulate produced particle size or chemical leaching, are voluntary and lack EU legal recognition (International Organization for Standardization 2020; International Organization for Standardization et al. 2020). While French and Spanish institutions recommend these guidelines for research and development, they are not mandatory for market claims.

Benefits of the implementation of biobased and biodegradable polymers in FNs manufacture would be multiple. First, it would enable a more effective management of end-of-life FNs through recycling and industrial composting (Basurko et al. 2023; Kim et al. 2023a). Second, they could potentially mitigate the risk of ghost fishing of ALDFG into the oceans, via faster degradation. This could be also a cost-saving solution for fishermen considering the substantial economic cost of lost fishing gear and their negative environmental impacts (Drakeford et al. 2023b). Nevertheless, to date, the scientific community has not reached a consensus regarding whether biodegradable fishing gear can completely degrade without generating microplastics, nanoplastics, or additives harmful to aquatic environments. This applies to both industrial composting and natural environmental biodegradation (Agostinho Beatriz et al. 2022; Le Gué 2024). Moreover, from 2022 to 2025, there was no documented evidence indicating the use of biobased and/or biodegradable FNs by fishermen in the

Southeastern Bay of Biscay. It appeared that the main constraint on their adoption extend beyond economic factors, being primarily attributed to the lack of commercial offer and to concerns regarding fishing efficiency (Drakeford et al. 2023b, 2023a; Savina et al. 2024). Therefore, albeit these new materials are promising, there are still challenges to be adressed, particularly their fishing efficiency and their toxicity during degradation.

1.1.3.2 Fishing situation pertaining to the Southeastern Bay of Biscay

Concerning the fishing fleet of the study area of the Southeastern Bay of Biscay in 2023 (FAO subareas 27.8.b, east of the 27.8.c), 1041 vessels fished actively, from which 529 were French and 487 Spanish. The other were from Belgium, Ireland, Portugal and United Kingdom (MTE France 2019; CIDPMEM 64-40 2021; European Commission 2024; MAPA Spain 2024; DAFM Ireland 2025). 56% of these vessels had a Length Overall (LOA) below 12 m. This size threshold is important to consider since the fishing boats of 12 m or more must be equipped with a device enabling competent authorities to automatically locate and identify them. However, some vessels might be exempted from this requirement if they: (i) are between 12 m and 15 m LOA and operate exclusively within the territorial seas of the flag Member State; or (ii) never spend more than 24 hours at sea from the time of departure to the time of return to port (Council of the European Union 2009; European Commission 2021). The “devices enabling to automatically locate and identify fishing vessels” are the automatic identification system (AIS) and the Vessel Monitoring Systems (VMS). The data obtained from these systems allow to describe the fishing fleet in terms of “efficient fishing activity”, which is the time in hours spent by boats in fishing. Based on data extracted from the Global Fishing Watch platform (2024), when compared to 25 other fishing areas of similar dimensions (70,000 km²) in 2023, the SE BoB fishery was ranked 7th for efficient fishing activity and 10th for the number of boats. Concerning the boats equipped with AIS in the SE BoB, gillnetters represented 30% of the efficient fishing activity. The second fishing gear most used was the bottom trawls (28%), followed by the set longlines (16.4%) and the purse seines (14.2%). This latter gear was used to catch most of the fish (44%, 26,000 tons) in the area. This gear was used mainly to fish European anchovy, Atlantic Mackerel and Sardines (Figure 1.10). The bottom trawls were used to catch 26% of the fish, including a wide diversity of species. Gillnets were used to catch 18% of the captures; mainly European hake, Common sole and Meagre (Figure 1.10). In 2023, European Anchovy was the most fished species (9,400 tons), followed by the Atlantic Mackerel (9,800 tons) and the European Hake (7,400 tons) (Figure 1.10). Based on the 2024 report on the status of fishery resources landed by French hexagonal fishing fleet in 2023 (Vermard & Ulrich 2025), from the 14 most caught species, four were in “good state” (European Anchovy, European Hake, BlackBellied Angler, Megrims spp.), and five were “restorable” (Albacore, Sardine, Common Sole, Wedge Sole, Seabass) (Figure 1.11). However, for the sardine in the subarea 27.8.c the stock was overfished. The Atlantic Mackerel and the Blue Whiting were also overfished in 2023. The Common Cuttlefish and the Meagre were not evaluated (Figure 1.11) (Vermard & Ulrich 2025).

Table 1.1: Efficient fishing activity (h) and number of boats in 25 regions of the world in 2023, plus the Southeastern Bay of Biscay. Marine areas were ordered by effective fishing activity (h). Data obtained in the plastfrom GFW (2024).Bold row indicates the Southeastern Bay of Biscay. "Lat." and "Long." Mean latitudes and longitudes

Marine Area	Region	Lat.; Long.	Number of boats	Effective fishing activity (h)
Tasman Sea	Australia	-34.2; 152.4	31	14169
Gulf of Alaska	USA	57.7; 152.5	243	20724
W Indian Ocean	Madagascar	-13.3; 48.3	88	20856
E Indian Ocean	Indonesia	-7.0; 106.5	61	32311
Benguela Current	Namibia	-22.9; 14.6	91	48366
Sea of Okhotsk	Russia	59.6; 151.0	233	72355
East Pacific	Canada	48.5; -125.6	190	74535
Bering Sea	USA	53.9; -166.6	250	76624
Baffin sea	Greenland	48.9; -64.4	95.2	97517
Baltic Sea	Poland	54.4; 18.8	386.1	105564
Humbolt Current	Perou	-12.4; -78.4	903	132038
Norwegian Sea	Norway	62.5; 6.3	1025	133599
Gulf of Maine	USA	43.7; -70	557	150009
Gulf of Guinea	Guinea	9.5; -14.0	102	154139
Gulf of Mexico	USA	29.1; -95.0	351	162110
Celtic Sea	Ireland	51.8; -9.0	421	223967
Arabian Sea	India	19.0; 73.0	648	246191
Gulf of Thailand	Thailand	12.9; 101.0	190	247740
Barents Sea	Russia	69.0; 33.0	1763	275070
SE BoB	**France/Spain**	**44.4; -2**	**670**	**401839**
North Sea	Denmark	57.8; -10.8	1022	478628
Argentinian sea	Argentina	-38.1; -57.6	762	530647
Adriatic Sea	Croatia	43.5; 16.6	964	697613
Persian Golf	Kuwait	27.5; 50.7	1494	990916
South China Sea	China	20.0; 110.3	6539	2038170
Yellow Sea	South Korea	34.8; 125.8	10073	2995167

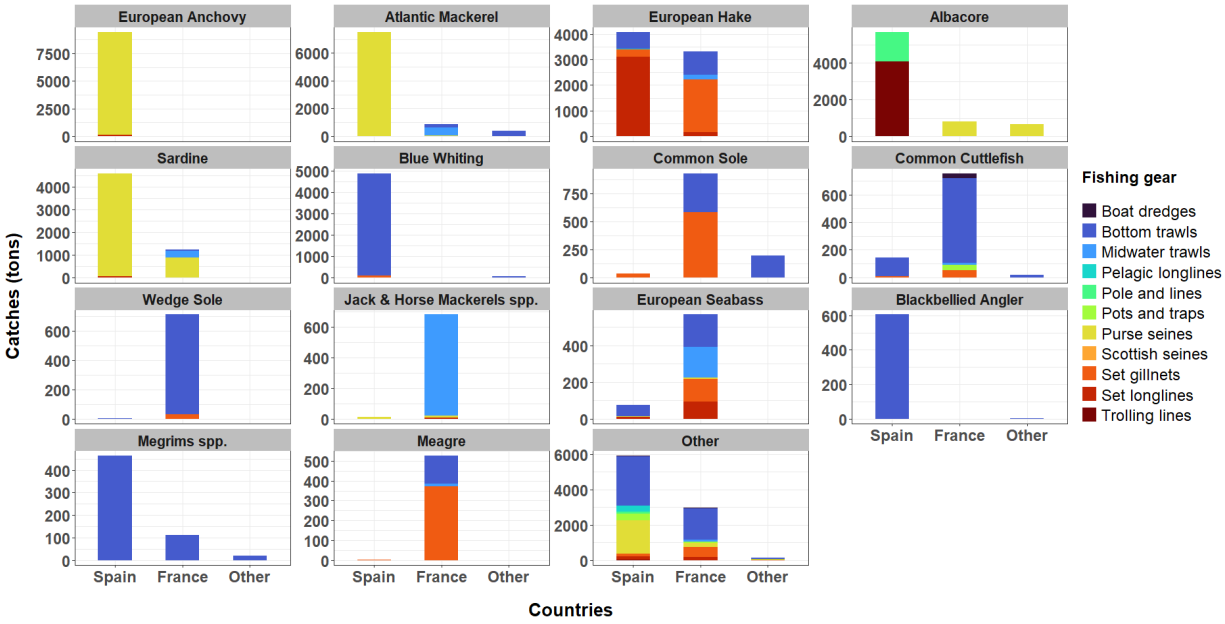

Figure 1.10: Catches (tons) of the 14 species caught in the Southeastern Bay of Biscay in 2023 (FAO subareas 27.8.b, east of the 27.8.c) by country (Spain, France, and others) and fishing gear.
The species at top left was the most caught in 2023. The rest of the plots can be read from left to right and from bottom to top. Data were extracted from the Eurostat database (European Commission and Eurostat 2013, 2015, 2024). The country category "Other" includes Belgium, Ireland, Portugal and United Kingdom. The Fish species category "Other" includes catches of 210 other fish species.

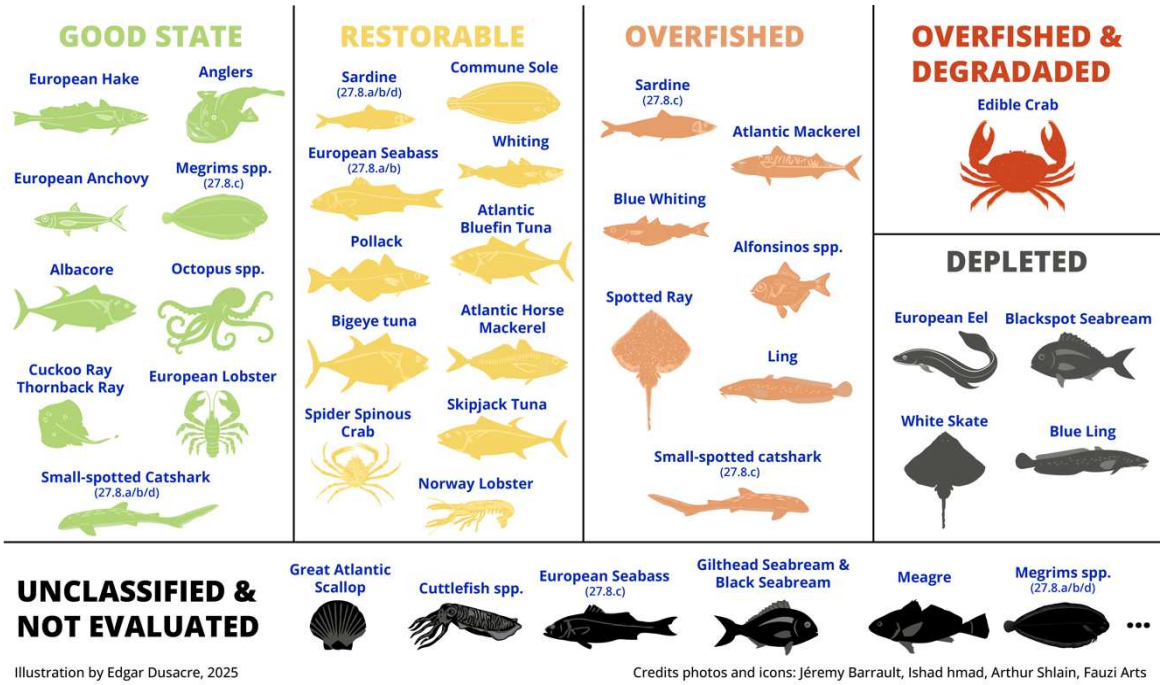

Figure 1.11: : Stock status of key commercial marine species in the Bay of Biscay (sub-divisions 27.8.a, 27.8.b, 27.8.c, 27.8.d).
Good state (green), Restorable (yellow), Overfished (orange), Overfished & degraded (red), Depleted (gray), and Unclassified/Not evaluated (black). Illustration based on the data published by Vermard and Ulrich (2025).

1.2 Fishing gear: a contributor to the global marine plastic pollution

1.2.1 From macroplastics to nanoplastics: a ubiquitous pollution

1.2.1.1 What is called plastic?

Plastic revolutionized all human activities after the WWII. This was mainly due to its great diversity, lightness, plasticity, resistance and advantageous production and sales costs (Kedzierski 2017; Chalmin 2019). Plastics are mostly derived from oil-based resources and consist of a polymeric macromolecular structure characterized by "a large number of repetitions of carbon groups, linked by strong covalent chemical bonds in sufficient quantity to result in a set of properties that do not vary substantially with the addition or removal of a single or small number of constitutional units" (International Organization for Standardization 2013). From a chemical perspective, these molecular chains often have hydrophobic hydrocarbon backbones, providing a barrier against water penetration, and thus reducing the likelihood of hydrolytic degradation. Additionally, polymers can vary in polarity depending on the specific monomers used, which affacets their interactions with the environment (Baur et al. 2019). For instance, some plastics like PE and PP are highly hydrophobic due to their non-polar aliphatic chains, while others like PA exhibit higher polarity due to the presence of amide linkages (-CONH-). This property makes PA more susceptible to water and hydrophilic compounds absorption compared to PE and PP. This polarity also contributes to its high tensile strength and resistance to abrasion (Baur et al. 2019).

In addition to their polymeric matrix, plastics are composed of additives because plastic items are hardly usable without additives (Pritchard 1998). Therefore, they are often complemented with functional additives, colorants, fillers and reinforcement fibres, among others (Hahladakis et al. 2018; Wagner et al. 2024). These additives facilitate the manufacturing process and confer additional properties to plastics, including increased rigidity, superior mechanical strength, improved aesthetic qualities such as color and sheen, greater resistance to heat, enhanced protection against photooxidation and hydrooxidation, reduced sensitivity to moisture (Hahladakis et al. 2018). The quantity of additives can vary widely between plastic items, ranging from 0.001% to 60% (van Oers et al. 2012; Maddela et al. 2023). The fillers (additives designed to change polymer physical properties or to lower costs than the base polymer resins) are 50% of the market. The plasticizers (additives able to separate polymer molecules to increase flexibility and ductility of the plastic) are second with 22% of the market (18% phthalates), followed by performance additives (12%) (van Oers et al. 2012). Recently, it has been estimated that more than 16,000 chemicals are related to the manufacture, use and degradation of plastics (Wagner et al. 2024). These compounds include the additives (intentionally added substances), the non-intentionally added substances (NIAS) which are coming from the impurities in the manufacturing process and the by-products formed during uncontrolled chemical reactions (Wagner et al. 2024). Most of the additives, added during the injection, extrusion, moulding or shaping of the polymers, are mainly physically entrapped within the polymer matrix without being covalently bound to it (Gewert et al. 2015; Cheng et al. 2020; Dimassi et al. 2022). However, certain additives are bound through hydrogen bonds,

electrostatic interactions, cation ligand interactions, π-π interactions, hydrophobic interactions or van der Waals forces (Carrega & Verney 2017; Atugoda et al. 2021). All these properties make plastics highly durable over their entire life cycle. This durability results in the accumulation of plastics at their end-of-life or when they are discarded into the environment. Therefore, because the plastic industry was not sufficiently developed to manage this amount of plastic waste efficiently and properly, plastic has become a major source of pollution in all environmental compartments (Geyer 2020; Kedzierski et al. 2020). In fact, some scientists were pushing for the plastic pollution to be considered the "Global Boundary Stratotype Section and Point" (also called "golden nail") of the geological epoch that would be named "Anthropocene" (Taffel 2016; Praet 2023).

1.2.1.2 Definition of macroplastics, mesoplastics, microplastics and nanoplastics

Macroplastics, mesoplastics, microplastics and nanoplastics refer to different sizes of plastic debris that make plastic pollution on earth. Plastic debris include all particles that respect three criteria (Figure 1.12). Then, the main difference between the four types of plastic debris is their size. Since 2019, most of the scientific community agreed around the size ranges given by Hartmann et al. (2019). Therefore, macroplastics represent the debris of plastics from which the largest dimension is > 1 cm, the mesoplastics 1 to <10 mm, the microplastics 1 to < 1,000 µm and the nanoplastics 1 to < 1,000 nm (Figure 1.12). Then, plastic debris can be classified according to their shape and structure, their color and finally their origin. The term "origin", particularly applied to microplastics, denotes "primary" or "secondary" plastic debris. Primary ones refer to "microplastics intentionally produced in that size range", while secondary are "formed in the environment through fragmentation or through wear and tear of plastic-containing items" (Hartmann et al. 2019). The difference between primary and secondary microplastics will be further developed in the section 1.2.1.4.

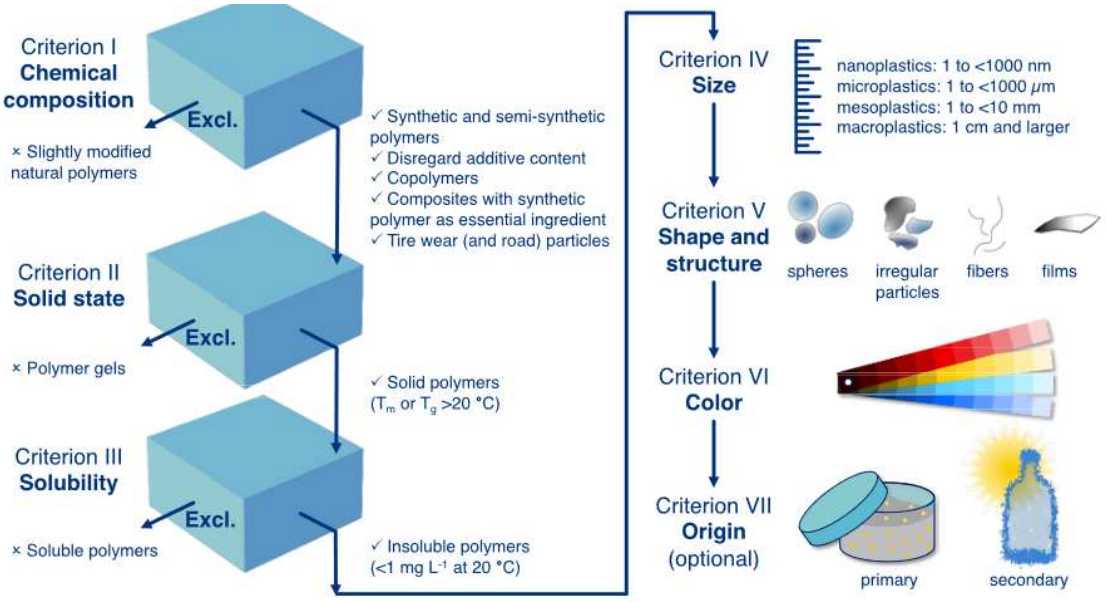

Figure 1.12: Proposed and frequently cited definition and categorization framework of plastic debris made by Hartmann et al. (2019). Excl. = excluded.

1.2.1.3 Macroplastic pollution

From a quantitative perspective, the accumulated world plastic production was estimated to be 9,900 million tonnes of plastic between 1950 and 2017 (Geyer 2020). Of those, 2,900 million tons were considered to be still in use. 7,000 million tons of waste were generated from which 5,300 were discarded in landfills or in the open environment, 1,000 million tons were incinerated and 700 million were recycled (Geyer 2020). If the actual trends follow the projections for 2050 estimate, a global production of 33,000 million tons and generation of 26,000 million tons of waste will be reach. Discharges in landfills and open environments are estimated to continue to grow but at lower rate, while incineration and recycling are predicted to increase (Geyer 2020). From the wastes discarded in landfills, it is difficult to estimate the exact quantity of plastics that spilled out of them, but the long-term impact of landfill sites in the plastic pollution has been highlighted multiple times in the scientific literature (Brand et al. 2018; Siddiqua et al. 2022; Wojnowska-Baryła et al. 2022; Nguyen et al. 2025). It was estimated that the mismanagement of plastic waste resulted in the release of 8.7 million tons of macroplastics into the environment in 2017, representing 2% of the total production (Schwarz et al. 2023). From this quantity, most was estimated to be in residential soils (soils around cities and populated areas) (3.6 Mt), in surface waters (rivers, lakes, canals, streams) (3.2 Mt) and on the side of the roads (1.1 Mt) (Figure 1.13). Oceans and agricultural soils received a similar quantity of 630,000 tons of macroplastics each, whereas natural soils (rural and non-populated areas) received 170,000 tons (Schwarz et al. 2023) (Figure 1.13).

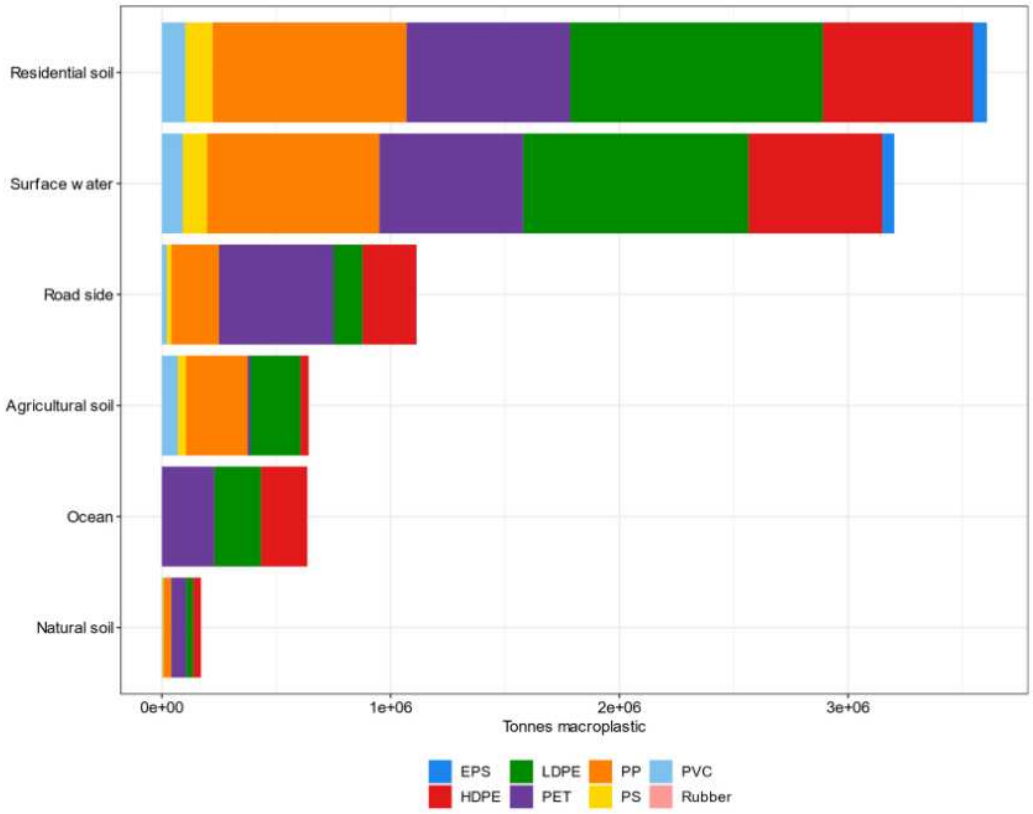

Figure 1.13: Macroplastic emissions per environmental compartment and per polymer between 1950 and 2017. Figure taken from Schwarz et al. (2023).
EPS = expanded polystyrene, LDPE = low density polyethylene, PVC = polychlorure vynile, PET = plyethylene terephtalate, PS = polystyrene.

The origins of these plastics were diverse with about 90% coming from packaging. The rest (10%) was mainly macroplastics coming from the fishing, agriculture and textiles sectors (Schwarz et al. 2023). Focusing on the ocean, it is currently estimated that between 0.8 and 4 Mt enter the ocean via rivers annually (Lebreton et al. 2017; Schmidt et al. 2017; Schwarz et al. 2023) and that currently between 1.8 and 12.7 Mt enter the ocean from land yearly (Jambeck et al. 2015; Schwarz et al. 2023). Based on all these data, Schwarz et al. (2023) constructed a model and estimated that, excluding landfill sites, 24.2 Mt of macroplastics accumulated in the ocean between 1950 and 2025 (21.6 Mt in the sediments and 2.6 Mt in surface and water column). Considering the oceanic shores, an additional 15.5 Mt would have accumulated since 1950. In terms of accumulation zones, the oceanic gyres are known to accumulate macroplastics in the surface and sub-surface. The North Pacific Gyre was estimated to be the one with the highest concentration (Figure 1.14) (Barboza et al. 2019; Isobe et al. 2019; Kaandorp et al. 2023). The South Atlantic Gyre, the Gulf of Guinea, the Bay of Bengal, the Meridional China Sea, the Mediterranean sea and the Caraibean sea were also considered as hotspots of macroplastic accumulation (Figure 1.14) (Isobe & Iwasaki 2022). Macroplastics were also found in deep-sea environments in concentrations ranging from few or no plastics per square kilometer (Antarctica) to 30,000–120,000 items/km^2 (Tyrrhenian Sea) (Ioakeimidis et al. 2019). All these data confirm that macroplastics are ubiquitous in the environment, especially in the marine environment. In addition to be present in most abiotic compartments on earth, macro and mesoplastics are ingested by most macroorganisms (Ryan 2019; Jâms et al. 2020; López-Martínez et al. 2021; Galgani et al. 2022; Kühn et al. 2022). The size of the plastic ingested depends on the size of the organisms with larger animals ingesting larger plastics with a ratio “body size:plastic size” of approximately 20:1 (Jâms et al. 2020).

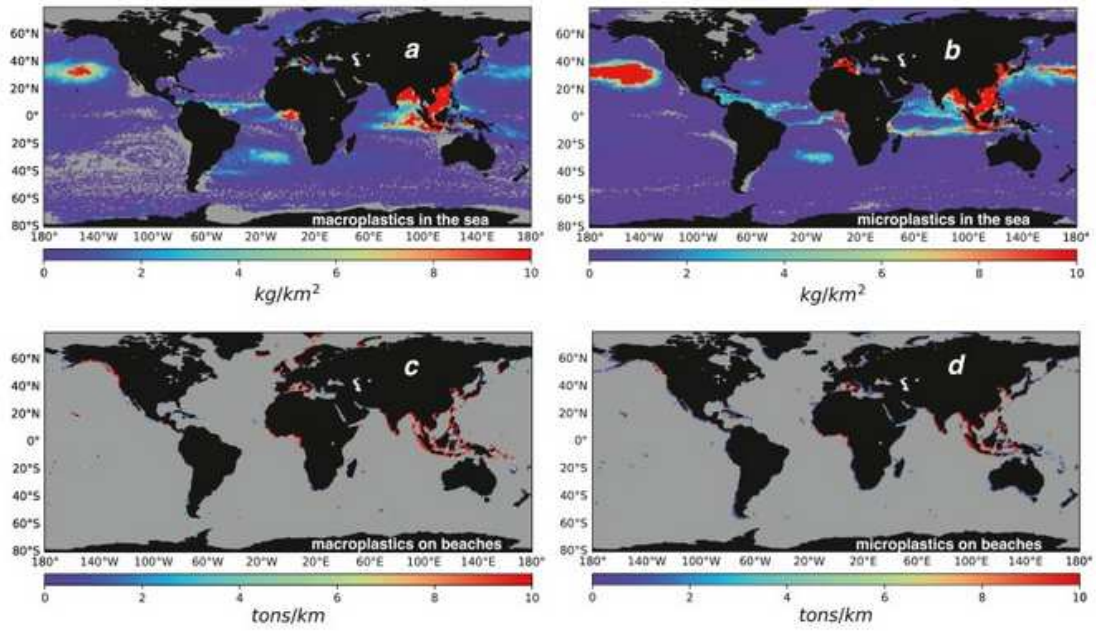

Figure 1.14: Abundance of ocean plastics in 2017 estimated using a particle tracking model with fragmentation timescale of three years.
The mass per unit area is represented by the color sclae within each one-degree latitude and longitude box. The map (a) and (b) represents the average quantities (kg/km^2) of macroplastics and microplastics in oceans, respectively. (c) and (d) show the average quantities (tons/km) of macroplastics and microplastic on beaches, respectively. Maps made by Isobe et al. (2019).

Focusing on the Bay of Biscay, a sampling in the NW Cantabrian Sea between 2013 and 2014 estimated the surface macro and mesoplastic concentration to be between 34,000±32,000 items/km^2 and 176,000±278,000 items/km^2 (Gago et al. 2015). In 2017 and 2020, other samplings calculated similar average macro and mesoplastics abundance at the surface of the southeastern Bay of Biscay (66,895±182,251 items/km^2) (Basurko et al. 2022). The high standard deviations of macroplastic concentrations were mainly explained by seasonal variations of plastic accumulation in the Bay of Biscay (Mendoza et al. 2020; Ruiz et al. 2020; Basurko et al. 2022; Alfaro-Ortega et al. 2025). Weaker currents during spring and summer tend to retain the litter in the Cantabrian Sea and the southeastern Bay of Biscay. On the contrary, during the autumn and winter seasons, the predominant northward current in the Bay of Biscay facilitates the transportation of marine waste out of the southeastern region. Subsequently, plastic debris is conveyed into the deep seas through submarine canyons, particularly the Capbreton canyon, or via the Aquitaine shelf, eventually reaching the northern coast of France (Pereiro et al. 2019; Mendoza et al. 2020). Concerning the sea floor of the Bay of Biscay, Van den Beld (2017), found plastic litter in the 15 canyons they studied with highest concentrations in the Arcachon Canyon. Comparing the “Greater North Sea”, “Celtic Seas”, and the “Bay of Biscay and Iberian Coast”, the latter exhibits the greatest likelihood for the presence of marine litter on the sea floor (Barry et al. 2022). Concerning the marine litter on beaches, the OSPAR monitoring on European Atlantic beaches revealed that 360 items/100 m were washed up between 2018 and 2020. This value is 78% higher than the EU median total count threshold (Lacroix et al. 2022; OSPAR Commission 2023). Albeit macroplastics are ubiquitous in the oceans, including the Bay of Biscay, they represent only the tip of the iceberg. Indeed, once in the natural environment, plastics can degrade and produce microplastics (MPs) and nanoplastics (NPs) and can also release their plastic chemicals and sorbed pollutants.

1.2.1.4 Microplastic pollution

The ubiquitous presence of microplastics (MPs) in all environmental compartments is now a recognized scientific fact (Kedzierski 2017; Borrelle et al. 2020). The current challenges are twofold. First, to figure out where they really come from and how many of them there are in the various compartments. And second, how they impact the environment and human health. About their origins, they are classified into two distinct categories, each with unique formation pathways and ecological implications. On one hand, the primary MPs (defined in section 1.2.1.2), that include microbeads incorporated into personal care products (Fendall & Sewell 2009), pre-production plastic pellets or "nurdles" (Cole et al. 2011), microfibers in synthetic textiles (Browne et al. 2011), and plastic blasting media (2.40-0.12 mm) (Andrady 2011; Raptor Blaster 2019; Airblast Eurospray 2020). Quantitative assessments by Boucher and Friot (2017) estimated global primary MPs emissions at 1.5 million tonnes annually, with 98,000 tonnes directly entering aquatic ecosystems every year. These primary MPs predominantly enter aquatic systems through wastewater treatment facilities. Murphy et al. (2016) determined that even advanced treatment processes remove only 75 to 90% of MPs, allowing significant quantities to bypass filtration. On the other hand, MPs, conversely, represent a substantially greater environmental concern due to their formation mainly in the environment through the fragmentation of larger plastic. Researches indicated that secondary MPs could constitute approximately 69 to 92% of marine microplastic pollution (Eunomia 2016; Boucher & Friot 2017).

Plastic degradation in aquatic environments encompasses complex interconnected physico-chemical and biological processes that transform macroplastics into progressively smaller particles, the MPs and NPs. The degradation process mainly begins with photooxidation, a primary mechanism where ultraviolet radiations initiate free-radical reactions through

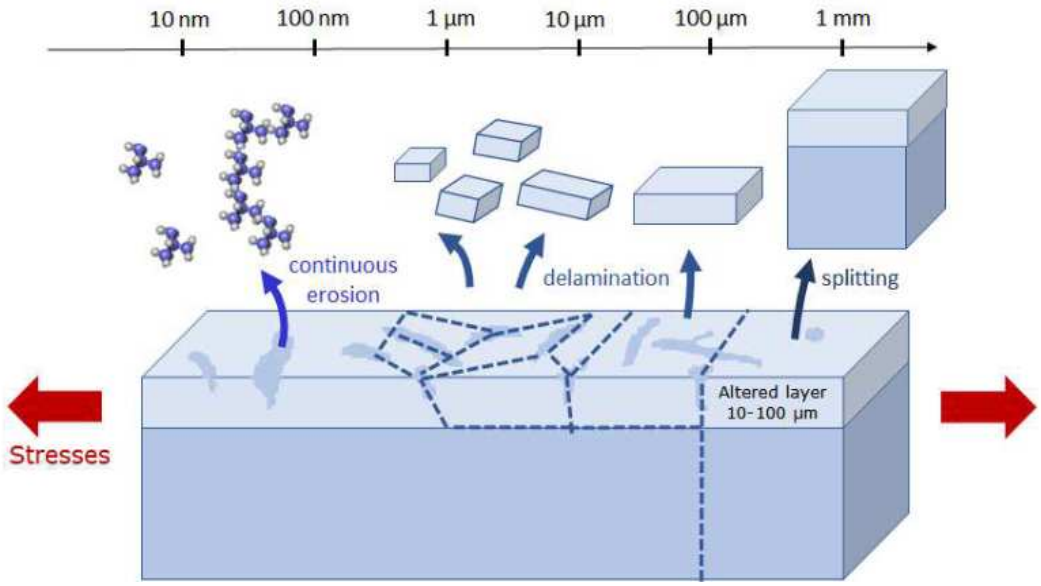

Figure 1.15: Schematic representation of the three degradation mechanisms, splitting or bulk fragmentation, delamination and erosion, and their resultant by-products.
Sizes (scale at the top of the illustration) of the produced products depend on the degradation modes. Fractures are expected to manifest within the plastic item from the zones in light blue and dotted lines. Illustration taken from George et Fabre (2021).

hydrogen removal from the polymer backbone (Gewert et al. 2015). For the main thermopolymers (PE, PP, PA), this process follows a cyclic autocatalytic sequence (initiation, propagation, termination) creating peroxy radicals that extract hydrogen from polymer chains, generating hydroperoxides and causing chain scission (François-Heude et al. 2015; Masry et al. 2021; Andrady 2022). This photodegradation is diffusion-limited, creating a heterogeneous oxidized surface layer of approximately 10 to 100 µm thickness while the underlying material remains relatively unaffected (Figure 1.15) (Shyichuk et al. 2004; Nagai et al. 2005; Pliquet et al. 2021; Andrady 2022). Environmental variables (e.g., irradiation, temperature, oxygen availability), substantially influence degradation rates, with studies consistently showing faster thermo-oxidative degradation in air than in water due to greater oxygen availability and higher temperature (Pliquet et al. 2021; Andrady 2022). The weakened surface layer develops microcracks, facilitating fragmentation enhanced by mechanical abrasion. Bulk fragmentation occurs where cracks propagate deeper, causing larger pieces to split into fragments typically around 1 mm (Figure 1.15). Delamination, on the other hand, involves thin surface layers disintegration into numerous smaller particles (< 150 µm) (Figure 1.15) (George & Fabre 2021). Recently, George et al. (2024) proposed the "sugar lump model", which postulates a critical size threshold (approximately 1 mm) below which plastic fragmentation becomes less likely to occur. This threshold-based fragmentation model provides a physical explanation for the observed deficit of particles between 1 mm and 150 µm in oceanic samples.

In addition to physico-chemical variables, biotic processes have a role in plastic degradation into smaller particles, especially for biodegradable plastics. Biofilm formation on plastic surfaces can both inhibit photodegradation by absorbing up to 99% of UV radiation (Weinstein et al. 2016) and facilitate biodegradation through microbial enzymatic activities. The biodegradation process involves sequential stages of bioattachment, deterioration, fragmentation, assimilation, and mineralization (Ahmed et al. 2018; Kothawale et al. 2023). Microorganisms use various enzymes including lipases, proteinases, and peroxidases to break down polymers until full mineralization (Zhou et al. 2022; Cai et al. 2023). However, some studies showed that microorganisms activity could lead to an uncomplete degradation of the plastic items, including biodegradable ones, and therefore produce microplastics, nanoplastics and additives (González-Pleiter et al. 2019; Tong et al. 2022).

Concerning the quantities of microplastics in the environments, they are ubiquitous similar to macroplastics, but they occur at higher quantities in terms of number of items (Cole et al. 2011; Eriksen et al. 2014). Ryberg et al. (2019) and Schwarz et al. (2023) estimated that between 0.8 and 5.2 Mt of MPs were released in the environment in 2015 and 2017, respectively (differences are derived from the different methodologies used). According to a recent material flow analysis, tyres were estimated to represente the major source of MPs emission into the environment (Schwarz et al. 2023). Furthermore, these authors estimated that roadsides constitute the area with the highest quantity of microplastics. Second and third MP emission sources were estimated to be packaging and agriculture. In terms of accumulation, Schwarz et al. (2023) estimated that between 1950 and 2025, natural soils around cities and populated areas (430 Mt of MPs) and subsurface soil (325 Mt of MPs) were the largest final sinks of MPs. Considering the marine environment, beaches would have

accumulated 15 Mt of microplastics, Ocean sediments 2 Mt and ocean surface water and water column 220,000 tons. Similarly to macroplastics, MPs mostly accumulated in the North Pacific Ocean Gyre, in the Bengal Bay, Meridional China Sea and the Mediterreane Sea (Figure 1.14).

The ubiquitous presence of MPs, their substantial quantities, their large spectra of sizes and their high diversity of shapes make the MPs bioavailable for most of the aquatic species (Tosetto et al. 2016; Barboza et al. 2019; Botterell et al. 2019; Zitouni et al. 2020; Kim et al. 2024). Ingestion of MPs has been widely reported and roughly all aquatic species studied had at least one MP in their digestive tract or tissues (Setälä et al. 2014; Covernton et al. 2021; Lefebvre et al. 2024). In 2020, Mendoza et al. did a meta-analysis of the MP quantification in the different compartments of the Bay of Biscay. Main results are shown in Figure 1.16. Compared to the worldwide data, in the review of Mendoza et al. (2020), the Bay of Biscay was considered as medium level polluted area in terms of MP concentration, even if comparisons are difficult due to the lack of standardization in quantification methods and units.

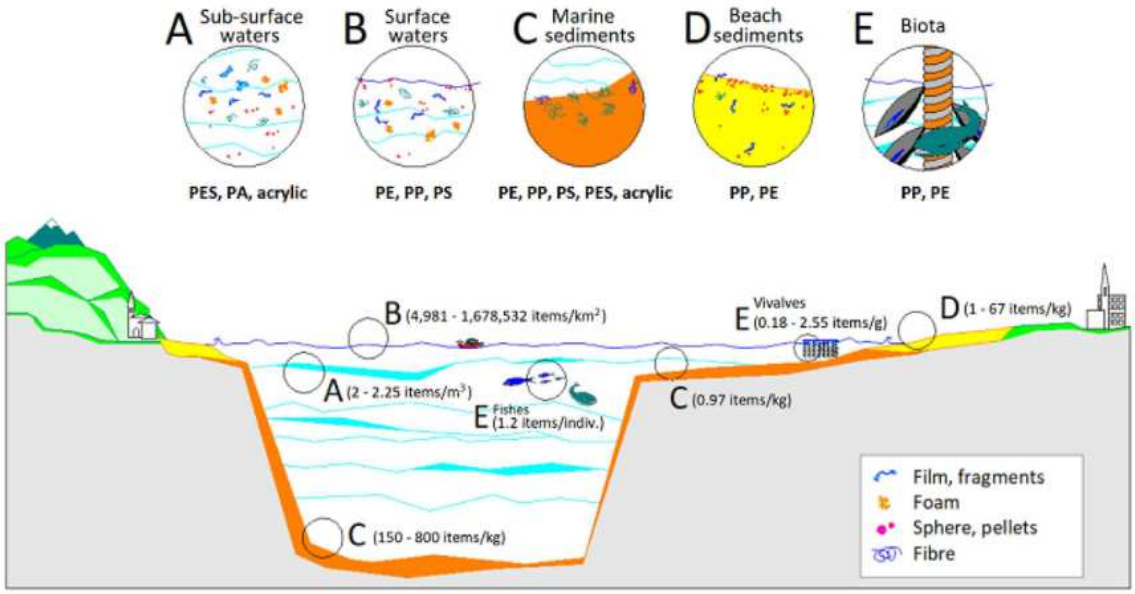

Figure 1.16: Meta-analysis of the microplastic quantifications in the different marine compartments of the Bay of Biscay. Main polymers of the microplastics quantified are also shown. Illustartion taken from Mendoza et al. (2020).

1.2.1.5 Nanoplastic pollution

Nanoplastics are estimated to mainly come from the degradation of larger plastics into nanoplastics (<1 µm), it can occurs through multiple pathways, including progressive fragmentation of plastics (Dawson et al. 2018; Blancho et al. 2021; Monira et al. 2025). NPs can be produced by direct generation of nanoparticles from macroplastics or large microplastics through splitting, surface delamination and continuous erosion processes without passing through the small microplastic stage (Figure 1.15) (Gigault et al. 2016; Song et al. 2020; Monira et al. 2025). Further, complete mineralization to carbon dioxide and dissolved organic carbon has been observed under laboratory conditions, demonstrating that UV degradation can fully oxidize PS, PE, PP into CO_2 and partially convert them into dissolved

organic carbon (Ward et al. 2019; Zhu et al. 2020). This process appeared to be significantly slower in natural marine environments due to lower dissolved oxygen levels and rapid biofouling (Andrady 2022). Furthermore, even if complete mineralization eventually occurs, full degradation of plastics that are less photodegradable such as PE may require decades to centuries to fully degrade and could continously release hazardous organic compounds during their degradation (Zhu et al. 2020). Nanoplastics (NPs) are ubiquitous contaminants across diverse environments (aquatic, soils, atmosphere) (Alimi et al. 2018; Toussaint et al. 2019; Davranche et al. 2020; Patil et al. 2022), even if quantitative assessments remain constrained by significant analytical limitations (Gigault et al. 2016; Ter Halle et al. 2017; Schwaferts et al. 2019). During an expedition in the North Atlantic Subtropical Gyre Ter Halle et al. (2017), successfully identified PE, PS, PVC and PET NPs in surface water samples using Dynamic Light Scattering and Pyrolysis (Pyr), Thermodesorption and Thermochemolysis Coupled to Gas Chromatography-Mass Spectrometry (GC-MS). NPs were also identified in sand of the Guadaloupe island by DLS and Pyr-GC-MS (Davranche et al. 2020). Materić et al. (2020, 2022) successfully quantified NPs in pits of the Alps with average concentration from 4.6±0.9 to 23.6±3.0 µg of PET/L of snow and in seawater samples from the Dutch Wadden sea, they found in average 4.2 µg of PS/L. These authors used the thermal desorption-proton transfer reaction-mass spectrometry method. Finally, a promising recent study quantified NPs in municipal wastewater treatment plants with a consistent methodological approach to prepare the samples and used the Pyr-GC/MS method to quantify the NPs (Okoffo & Thomas 2024). The authors quantified PA 66, PA 6, PE, PP and PET in concentrations from 0.04 µg/L to 7.3 µg/L. In terms of ingestion, it has been proven that diverse aquatic organisms (e.g., *Danio rerio*, *Mytilus galloprovincialis, Paracentrotus lividus, Crassostrea gigas, Amphibalanus amphitrite, Artemia franciscana, Tigriopus japonicus*) can ingest NPs (Ferreira et al. 2019; Katsumiti et al. 2021; González Soto 2022; Pelegrini et al. 2023; Martínez-Álvarez et al. 2024). Moreover, due to their nanometric size, these particles have a higher translocation capacity into tissues compared to microplastics (Paul-Pont et al. 2018; Shen et al. 2019).

Even if these first data show that NPs are a non-negligible part of the plastic pollution, significant analytical challenges remain to obtain accurate estimates of environmental concentrations. Current analytical limitations include insufficient standardization of sample preparation protocols, with few studies showing recovery rates and procedural blanks (Cai et al. 2021). Therefore, developing standardized protocols that combine chemical identification and quantitative assessment for all NP sizes is crucial for evaluating their distribution and ecological impact on marine ecosystems.

1.2.1.6 Plastic chemicals pollution

In addition to macroplastics, mesoplastics, microplastics and nanoplastics, plastic pollution is also a problem of associated chemical compounds. Indeed, as discussed in section 1.2.1.1, plastics contains additives and NIAS that can be released during their degradation (Hahladakis et al. 2018). The release of additives from degrading plastics occurs through several key mechanisms often grouped under the word "migration". For example, the release of flame retardants from microplastics occurs through: internal movement within the plastic matrix, transport across the plastic-medium boundary, and diffusion into the environmental medium (Cheng et al. 2020). Multiple factors including porosity and thickness of the polymer, irradiation, temperature, abrasion and the characteristics of matrices surrounding of the plastic significantly influence this release process (Paluselli et al. 2019; Luo et al. 2020; Sørensen et al. 2021). Notably, it has been shown that phthalates leaching can be increased by a factor of 2 to 5 when plastics are subject to UV exposure (Luo et al. 2022; Viljoen et al. 2023). Defining a general pattern of additives leaching is hard because migration rates are highly dependent on plastic polymers and additive characteristics (Hahladakis et al. 2018; Maddela et al. 2023). However, it seems that leaching is generally driven by concentration gradients (Da Costa et al. 2023). Among the 16,000 chemicals referenced in plastic literature, a proof of release exists for only 1,571 chemical compounds (Figure 1.17).

Plastics in aquatic environments not only release their inherent additives but they also function as vectors for environmental pollutants through adsorption and absorption processes. The sorption capacity of plastics tends to vary according to different factors e.g., weathering, hydrophobicity, crystallinity, polarity, non-specific van-der Waals interactions, π-π interactions, matrix characteristics, plastic color, and compound molecular structure, among other (Figure 1.18) (Atugoda et al. 2021).

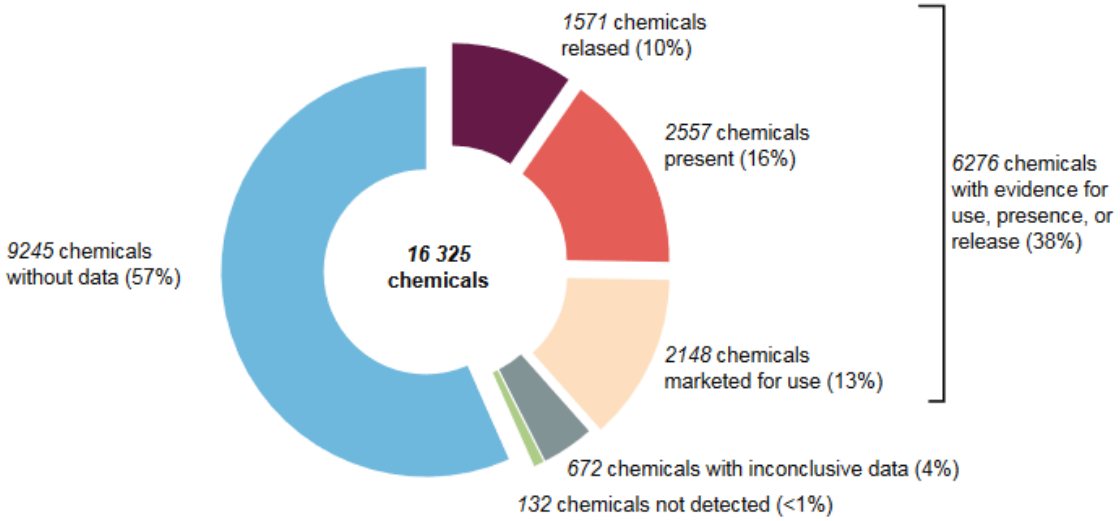

Figure 1.17: Numbers of chemicals with evidence for use, presence, or release from plastics. Chemicals that have been studied but not found or that yielded inconclusive outcomes are also included.
There is significant overlap in use, presence, and release data. Therefore, the strongest evidence for each chemical is highlighted in this graphic. Graphic taken from Wagner et al. (2024).

However, the current understanding is insufficient to explain accurately the different patterns (Hu et al. 2022; Da Costa et al. 2023). Hüffer and Hofmann (2016) showed that PS sorbed more aqueous chemical compounds, followed by PVC, PE and PA. They also highlighted that PE mainly absorbed the compounds in the bulk, while PS, PVC and PA MPs adsorbed them onto their surface. Interestingly, Wang et al. (2018) also emphasized that the sorption capacity of PE fibers for phenanthrene and phenol was 1 to 2 times higher that those of PA fibers. They higlighted that the sorption capacity of PE was higher than that of marine sediments. In this latter study, smaller PE and PA fibers exhibited a higher sorption than larger particles. A recent study compared the sorption capacity of PE, PS and biodegradable polybutylene adipate terephthalate (PBAT) MPs, and showed a higher sorption ratio for PBAT (Zuo et al. 2019). PBAT MPs were the particles that released the highest quantity of phenanthrene. The authors concluded that elevated salinity and lower organic matter fouling of plastics enhanced the sorption of environmental pollutants. One significant concern regarding plastic additives and NIAS, as well as pollutants sorbed onto/into plastic materials, is the potential release of these substances into organisms upon the ingestion of plastic debris (González-Soto et al. 2019; Le Bihanic et al. 2020; Cormier et al. 2021). Bakir et al. (2014) demonstrated that simulated gut conditions, including low pH and high temperature, significantly increase the desorption rates of hydrophobic organic contaminants from MPs compared to seawater conditions. Cormier et al. (2022) reported that sorbed perfluorooctanesulfonic acid spiked on MPs underwent desorption at a rate of 70% to 80% when subjected to simulated digestive intestinal conditions. Recently, a review concluded that additives of plastics were present in every abiotic and biotic environmental matrix including sediments, soils, wastewaters, landfill leachates, freshwaters, marine waters, organisms and humans (Maddela et al. 2023).

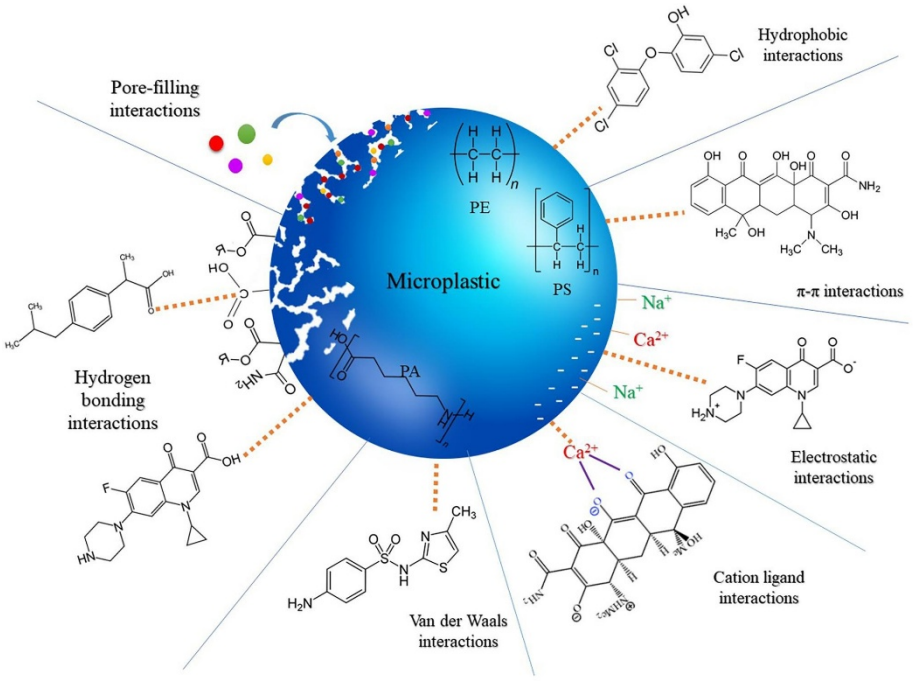

Figure 1.18: Schematic illustration of the main interactions between contaminants and microplastic surfaces.
These mechanisms govern contaminant adsorption onto microplastics in aquatic environments. Taken from Atugoda et al. (2021).

1.2.2 Contribution of fishing gear to plastic pollution

1.2.2.1 Macroplastics perspective

Plastic fishing gear used worldwide was estimated to be approximately 2.1 Mt in 2018 (Kuczenski et al. 2021). In the European Union, FG used was estimated at 33 Kt in 2018, which would represent 2% of the FG used worldwide (Table 1.2).

Table 1.2: Annual quantity of used fishing gear (tons) in the European Union (27 countries – 2020).
Data are based on the sum of produced and imported fishing gear by country minus the exportations. Data includes two product categories from the PRODCOM list. Product 13941233 "Made-up fishing nets from twine, cordage or rope of man-made fibres (excluding fish landing nets)" and product 13941235 "Made-up fishing nets from yarn of man-made fibres (excluding fish landing nets). Data of some EU countries were not available in the European statistics. Data taken from European Commission & Eurostat (2025).

Countries	2018	2019	2020	2021	2022	2023
France	1,868	1,961	1,456	1,729	1,739	1,806
Spain	14,207	13,628	10,856	15,287	11,564	11,970
EU28	33,262	32,175	27,748	30,897	27,020	27,189

FG lifespan is difficult to determine because it is dependent on the fishing métier and region of the world. However, a literature review allowed to estimate that bottom and pelagic trawls are usually renewed every 0.5 to 10 years (Deshpande et al. 2019; DLAL Project 2020; Chambers et al. 2021; Nyatchouba Nsangue et al. 2023). Purse seine lifespan is similar, with a renewal every 3.2 to 10.2 years (Deshpande et al. 2019; Andrés et al. 2021; Basurko et al. 2023). The trawls and purse seines are continuously repared and panels (Figure 1.8) are changed when damaged (Viool et al. 2018; Gilman et al. 2023). Gillnets and longlines have shorter average lifespan of 0.25 to 3.0 years (Kim et al. 2016; Deshpande et al. 2019; Grimaldo et al. 2020; DG MARE 2021; Basurko et al. 2023). When damaged gillnets are often renewed entirely or large panels are changed. Regarding the repair of longlines, few information was available in the scientific literature. Sociological studies involving fishermen would be needed for a more comprehensive understanding. Finally, pots and traps are the gear with the longest lifespan ranging between 2 and 25 years (Breen 1987; Stevens 2014; Deshpande et al. 2019; Chambers et al. 2021; Basurko et al. 2023). At their end-of-life, fishing gear, due to their degradation rate and, or structural complexity are rarely well managed and are mainly stored in sheds, on harbors docks, put in landfills or incinerated (Bertling & Nühlen 2019; DLAL Project 2020; Schwarz et al. 2023). However, in European Union, since the adoption of the directive on the "Strategy for Plastics in a Circular Economy", countries are pushed to set up a strategy to better manage the fishing gear at their end-of-life (European Commission 2018). Most of the efforts are currently directed to collect the end-of-life FG more appropriately and to set up a specialized recycling sector (Bertling & Nühlen 2019; Mondragon et al. 2020; Juan et al. 2021; Kozioł et al. 2022; Nguyen & Brouwer 2022). An alternative solution involves the development of biodegradable FG, which can be converted into compost upon reaching their end-of-life stage, as described in section 1.1.3.1.

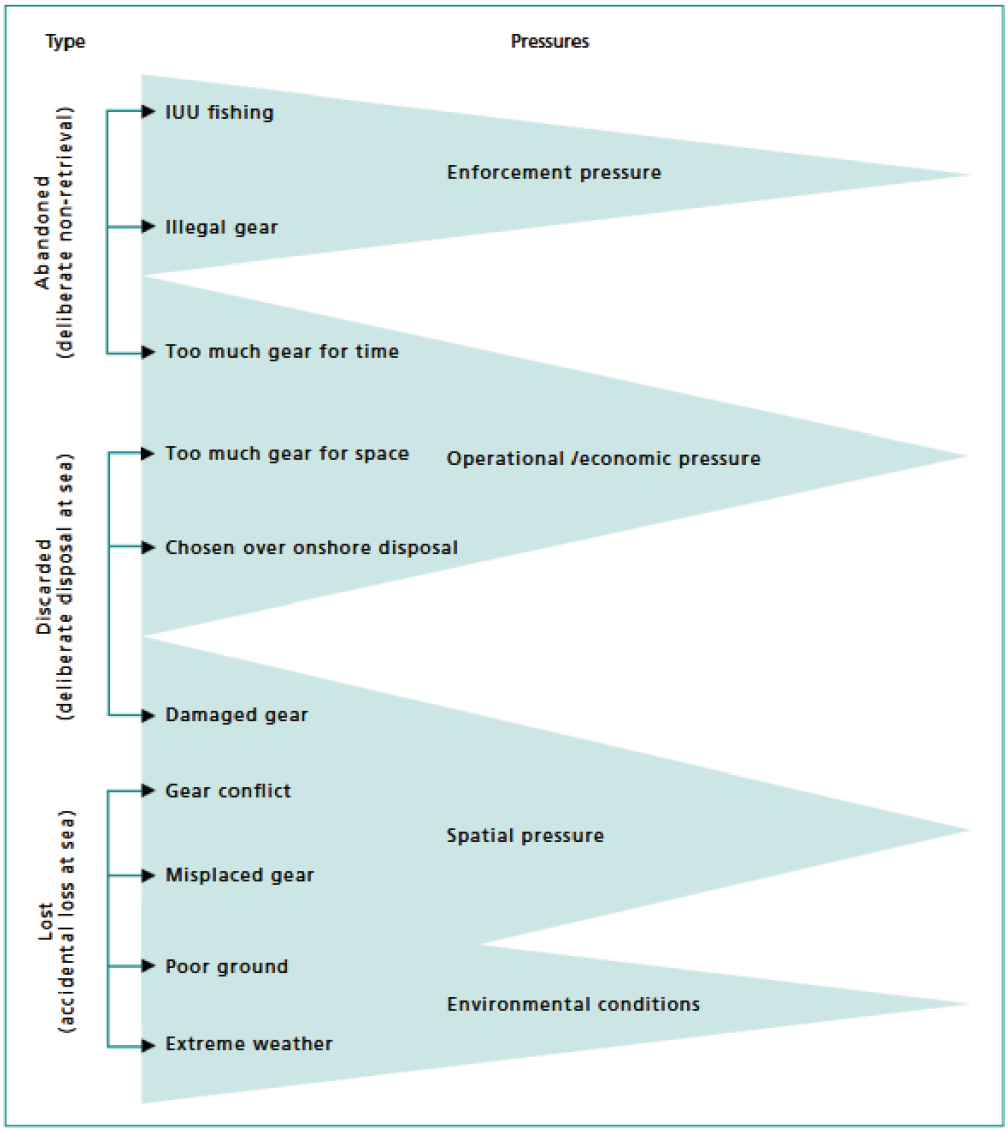

Figure 1.19: Shematic summary of the reasons why fishing gear are abandoned, lost or otherwise discarded. IUU fishing means illegal, unreported and unregulated fishing. Taken from Macfayden et al. (2009).

Fishing gear can be lost by fishermen during their operation. When they are abandoned, lost or intentionally discarded in the environment, they become the known derelict "Abandoned, Lost or Otherwise Discarded Fishing Gear" (ALDFG) (Macfadyen et al. 2009; Richardson et al. 2019). There are three main reasons leading to ALDFG (Figure 1.19). Bad weather is the first reason (Macfadyen et al. 2009; Richardson et al. 2019, 2021). The second reason is the gear conflict between different fishing *métier* (usually mobile *métier* versus fixed *métier*) due to spatial pressures because fishing activity are usually concentrated in the same marine areas. The third main reason is the loss of gear due to sea floor obstacles. Other reasons are discarding by illegal fishermen under enforcement pressure due to police controls and damage or loss due to wildlife entanglement and operator errors (Richardson et al. 2021; Apete et al. 2024).

Annually, taking into account only the lost FG (and not the abandoned and discarded one) the median was estimated to be 48,400 tons (95% confidence interval: 28.4-99.5 kt), which would represent 2.3% of the FG produced worldwide (Kuczenski et al. 2021). However, this

percentage is not the same for all the fishing métier. Indeed, some gear are more likely to be lost than others (Table 1.3). Using an approach based on sociological surveys, Richardson et al. (2022) similarly estimated the loss of gear but in terms of surface of net, length of longlines and count of pots and traps (Table 1.3). They found a similar percentage for trawl net (3.6%) in terms of surface lost, and for mainlines of longlines (3.4%). However, for purse seine the estimated loss rate was half (1.5%). For gillnets, the discrepancy was even more pronounced, with a loss rate 3.4% (0.8% versus 5.1%). Comparing the quantities of lost FG of the two studies, Richardson et al. (2022) found Purse seines > Gillnets > Trawls, while Kuczenski et al. (2021) found Purse seines > Trawls > Gillnets. One advantage of the estimation conducted by Richardson et al. (2022) is the inclusion of data derived from fishermen surveys, allowing for the incorporation of small-scale fisheries (as defined in section 1.1.2.3). On the contrary, Kuczenski et al. (2021) did not account for the latter sector.

Table 1.3: Comparison of global annual gear loss estimates in weight or surface, length and number of items between the studies of Kuczenski et al. (2021) and Richardson et al. (2022).
"FAD" means "fish aggregating device". "CI" means confidence intervals. CI are in the same unit that the weight or size loss. Empty cells mean that the fishing gear was not taken into account in the study.

	Kuczenski et al. (2021)			Richardson et al. (2022)		
Fishing gear	**Percent lost**	**Weight lost**	**95% CI (0.05-0.95)**	**Percent lost**	**Size lost**	**95% CI (0.05-0.95)**
Gillnets	5.1%	3,070 t	893 – 16,400	0.8%	2,963 km^2	1,153 – 4,773
Purse seines	3.1%	56,200 t	30,700 - 128,000	1.5%	75,049 km^2	49,116 – 100,981
Trawls	3.3%	6,970 t	5,280 – 9,880	3.6%	218 km^2	32 - 478
Longlines	4.2%	2,230 t	1,720 – 3,170	3.4%	16,309,856 km	128,549 – 38,793,025
Pots & Traps				0.7%	25,382,742 items	16,198,663 – 34,566,822
Drifting FADs	51.3%	2,020 t	1,490 – 2,620			

Macro ALDFG would contribute about 6% to the annual new emissions of macroplastic in the oceans according to Schwarz et al. (2023). To the best of our knowledge there were no published estimations of the quantity of fishing gear used and lost in the BoB. Consequently, further research is required to improve the management of this type of pollution and to contribute to the sustainability of the fishing sector. Concerning field studies and quantification of ALDFG in beaches, ocean surface and sea floor, multiple works have been published. Table 1.4 presents a non-exhaustive list of quantification of ALDFG in the marine environment. These studies were carried out with different methods of sampling and used different units to share the results. In Europe methods and units are standardized following guidelines from the OSPAR Commission and the Marine Strategy Framework Directive (MSFD) (OSPAR Commission 2010; MSFD Technical Subgroup on Marine Litter et al. 2013). It was estimated that the mean quantity of fishing gear accumulated in the European marine environment (encompassing beaches, sea surface and sea floor) was 34±23% (n=31) of the marine litter (Table 1.4). For the Bay of Biscay, the percentage was slightly higher accounting for 37±20% (n=12).

Table 1.4: Abundance of ALDFG across various marine compartments (beaches, sea surface and sea floor) around the world between 1994 and 2022.
Bold text highlights data from the Bay of Biscay region. (/) indicates data that was not reported in the original study. "MPAs" means Marine protected Areas and "SE" means standard error.

Compartment	Year	Site	All Fishing gear (%)	All Fishing gear	Unit	Reference
Beaches	2022	Bengladesh - North	38.08	198	items/m²	(Chowdhury et al. 2024)
		Bengladesh - South	28.79	369		
	2021-2022	Southwestern Madagascar	23.73	729	items	(Raharinaivo et al. 2025)
			/	2	item/m²	
	2022	Galápagos	/	0.09 (SEM = 0.02)	items/m² (mean)	(Muñoz-Pérez et al. 2023)
	2016	European beaches	15	60381	items	(MSFD Technical Group on Marine Litter et al. 2017)
	1994-2018	England MPAs	12.6	/	/	(Nelms et al. 2020)
	2015	Cornwall - North (Constantine Bay)	/	53	items	(Turner 2017)
			/	41	g	
		Cornwall - South (Whitsand Bay)	/	100	items	
			/	126	g	
	2018-2020	North East Atlantic		36	items/100 m (median)	(Lacroix et al. 2022)
		North Sea	25	/		
		Bay of Biscay		**51**		
	2018-2020	English Channel	13.9	74	item/100m (median)	(Gérigny et al. 2022)
		Celtic sea	30	39		
		Bay of Biscay	**29.3**	**130**		
		Bay of Biscay - North	**48.4**	**210**		
		Bay of Biscay - South	**20.4**	**82**		
		Western Mediterranean	1.5	12		
	2008-2017	**Bay of Biscay - Basque country**	**16.8**	**/**	/	(Granado et al. 2019)
	2016	**Bay of Biscay -Asturia**	**12**	**0.35**	item/m² (mean)	(Rayon-Viña et al. 2018)
Sea surface	2019	North Pacific subtropical gyre	12.9	781.0	items	(Lebreton et al. 2022)
			7.4	37.9	kg	
	2015-2016	North Pacific subtropical gyre	46	36340	tons	(Lebreton et al. 2018)

Continuation Table 1.4

	2018	**Bay of Biscay**	**48.6**	**100**	kg	(Ruiz et al. 2020)
			29.3	**163**	items	
	2015-2020	English Channel	/	0.02±0.24	item/km² (mean)	(Gérigny et al. 2022)
	2015-2020	Celtic sea	/	0.01±0.06		
	2015-2020	**Bay of Biscay**	/	**0.02±0.12**		
	2015-2020	Western Mediterranean	/	0.01±0.06		
Seafloor	2019-2021	Laccadive Sea	/	53	items	(Viswambharan et al. 2025)
	2020	Southwest Nova Scotia	98	6923	kg	(Goodman et al. 2021)
	2023	Barent sea	/	118	items	(Vodopia et al. 2024)
	2014	Sicily	77.9	6	items/m²	(Consoli et al. 2019)
	2009-2010	**Bay of Biscay**	**16.2**	**/**	**/**	(Van den Beld et al. 2017)
		Bay of Biscay - Ars Canyon	**66.7**	**/**	**/**	
	2018-2020	Greater North Sea	50	4995	items	(Barry et al. 2022)
		Celtic Sea	52	1482		
		Bay of Biscay	56	**1520**		
	2021	**Bay of Biscay - North**	**53**	**46**	items	(Gerigny et al. 2023)
	2022-2023	North Sea	12	/	items	
	2015-2020	English Channel	/	44±277	item/km² (mean)	(Gérigny et al. 2022)
	2015-2020	Celtic sea	/	15±91		
	2015-2020	**Bay of Biscay**	/	**69±240**		
	2015-2020	Western Mediterranean	/	25±94		
	2011-2013	**Bay of Bisacy - Basque Country**	**20**	**60**	kg	(Garmendia & Quincoces 2019)
			25.6	**251**	items	
	2006-2010	**Bay of Biscay - Cantabrian Sea**	**74**	**7**	item/km²	(Lopez-Lopez et al. 2017)
Beaches & Sea surface	2019-2021	Hawaiian Islands	/	136	items	(Royer et al. 2023)
	2019-2022	Palmyra Atoll	/	31	items	
	2019-2023	Central North Pacific Ocean	/	14703	kg	

1.2.2.2 Microplastics, nanoplastics and associated chemicals perspective

In contrast to the well-documented contribution of macro ALDFG to plastic pollution, the literature on the contribution of degradation products of FG including MPs, NPs, and additives is considerably more limited. To the best of our knowledge, no studies have been published focusing on NPs and fishing gear. Furthermore, we are aware of only two studies that quantified MPs originating from FG. The first study, published in 2018, demonstrated that following a Linitest© washing procedure, several hundred microfibers were generated per gram of nets composed of PA, HDPE, and PP (Montarsolo et al. 2018). On the contrary, focusing on ropes, Napper et al. (2022) highlighted that conventional used ropes produced microfragments of plastics and not fibers. The authors estimated that during hauling, new fishing ropes (PP) can release 14±3 microfragments per meter of rope. The emission was 51 times higher (720±51 microfragments per meter of rope) when hauling with a two-year-old rope. They also found that polysteel ropes emitted 2.8 more MPs than the PP ropes. Le Gué et al. (2024) studied the degradation of FNs but did not quantify the MPs and NPs released. They reported in their publication that the strength of biodegradable PBSAT gillnets were half lower that of PA ones. The authors also highlighted that the highest lost of mechanical properties was at the nodes, for both PA and PBSAT gillnets. Subsequently, the same research group investigated the degradation behavior of biodegradable PBS-PBAT and HDPE ropes in marine environments (Le Gué et al. 2025). Their findings indicated that at temperatures of 4°C and 15°C, the tensile strength of the PBS-PBAT net remained stable even after 18 months submerged in seawater. However, a noticeable reduction in tensile strength was observed at a temperature of 25°C, starting after 5 months in seawater. Moreover, at elevated temperatures of 40°C and 60°C, pronounced degradation and a significant loss of mechanical strength were evident, occurring after 12 months and 4 months in seawater, respectively. In contrast, HDPE filaments exhibited no significant changes under the same conditions (Le Gué et al. 2023). Brakstad et al. (2022) conducted laboratory seawater-sediment weathering of biodegradable PBSAT and conventional PA monofilaments. Their findings corroborated previous research by demonstrating the progressive weakening and degradation of PBSAT filaments over time, while the PA filaments remained unaltered. Interestingly, they showed that the developed microbial community differed significantly between the two filaments. Finally, utilizing pyrolysis-gas chromatography/mass spectrometry, they identified compounds leached during the degradation process.

The study of Vilke et al. (2024) identified 6 trace metals (Mn, Fe, Ni, Cu, Zn, Pb) in leachates of beached ALDFG. Fe (546 µg/L), Zn (123 µg/L) and Mn (30 µg/L) were the three most abundant trace metals. In the study of Turner et al. (2017) trace metals were identified in leachates of beached ALDFG. Nevertheless, Br at a concentration of 2,420 µg/L, Cd at 1,460 µg/L, and Cr at 909 µg/L were the most prominently abundant metals (Turner 2017). These metals were also shown to be able to leach from fishing gear when incubated 7 days in a medium that mimicked the digestive track of a seabird (Turner 2017). Vilke et al. (2024) also quantified 20 organic compounds in leachates of beached ALDFG, including intermediates of plastic manufacture, plasticizers, UV stabilizers, flame retardants, antioxidants, flavouring agents, dyes and preservatives. In another study, 26 additives were found after artificial aging of FNs, especially placticizers and antioxidants (Chen et al. 2023). However, compared to bags and foam, nets

appeared to leach fewer organic compounds. Particularly, it was shown that the conditions resulting in the greatest leaching of additives involved the combination of UV irradiation, wave simulation, and shoal conditions. In another study, researchers identified five to seven organic compounds within three different PVC fishing lures (Beggel et al. 2025). They encompassed three phthalates, one phosphorylated flame retardant, and one sodium sulfate salt as the primary filler. Nevertheless, following a 96-hour period of agitation in freshwater, no organic compounds were identified in the leachates (Beggel et al. 2025). Finally, very recently a team studied the Cu, Pb and polycyclic aromatic hydrocarbons (PAHs) adsorption capacities of FNs (Almeida et al. 2024). Interestingly, distinct adsorption patterns depending on the type of pollutants, polymer composition, diameter, and structural form were observed. The braided and thicker filaments demonstrated a higher adsorption of Cu and Pb. With respect to PAHs, the PA nets exhibited a greater adsorption capacity, and this adsorption was positively correlated with the molecular weight of the nets. In general, thinner nets adsorbed less pollutants. However, it has to be mentioned that the study was based on similar size of FNs and not on equivalent weight. This methodological approach raises questions regarding what the adsorption levels might have been if the adsorption experiment was conducted using equivalent weights for each type of FN, considering that thinner nets would theoretically present a larger exchange surface area. In environmental settings, all FNs demonstrated an increasing trend in the adsorption of Cu, Pb, and PAHs over time. Almeida et al. (2024) hypothesized that this progressive increase might be attributed to the development of biofilm.

The existing body of knowledge presented above and gaps detected highlight the necessity for a more precise estimation of the contribution of fishing gear to MP, NP and additive pollution during their operational use, subsequent loss. Consequently, it is imperative to undertake further research to comprehensively understand the degradation kinetics of fishing gear, particularly in regions subject to intense fishing activities, such as the SE BoB. Moreover, the widespread presence of plastic ALDFGs in the environment, coupled with initial findings indicating the potential release of MPs and additives, raise significant concerns regarding the impact on the aquatic ecosystems.

1.2.3 The known toxic effects of plastic and fishing gear pollution

1.2.3.1 Macroplastics

Over the past 30 years of investigation on macroplastic pollution, the scientific community agreed that macroplastics cause significant harm to organisms through multiple pathways. Habitat degradation through physical alterations constitutes a substantial impact of macroplastics. Notably, they can smother benthic species, by reducing luminosity and creating anoxic conditions through inhibition of gas exchange at the sediment-water interface (Green et al. 2015). This can reduce primary productivity and alter infaunal communities (Kühn et al. 2015). Indeed, Green et al. (2015) highlighted that conventional and biodegradable plastic bags sea floor coverage caused a decrease of 13% of the organic matter in the superficial sediment (4 cm). Moreover, they showed that both types of bags blocked around 50% of the photosynthetically active radiations that reach the sediment surface.

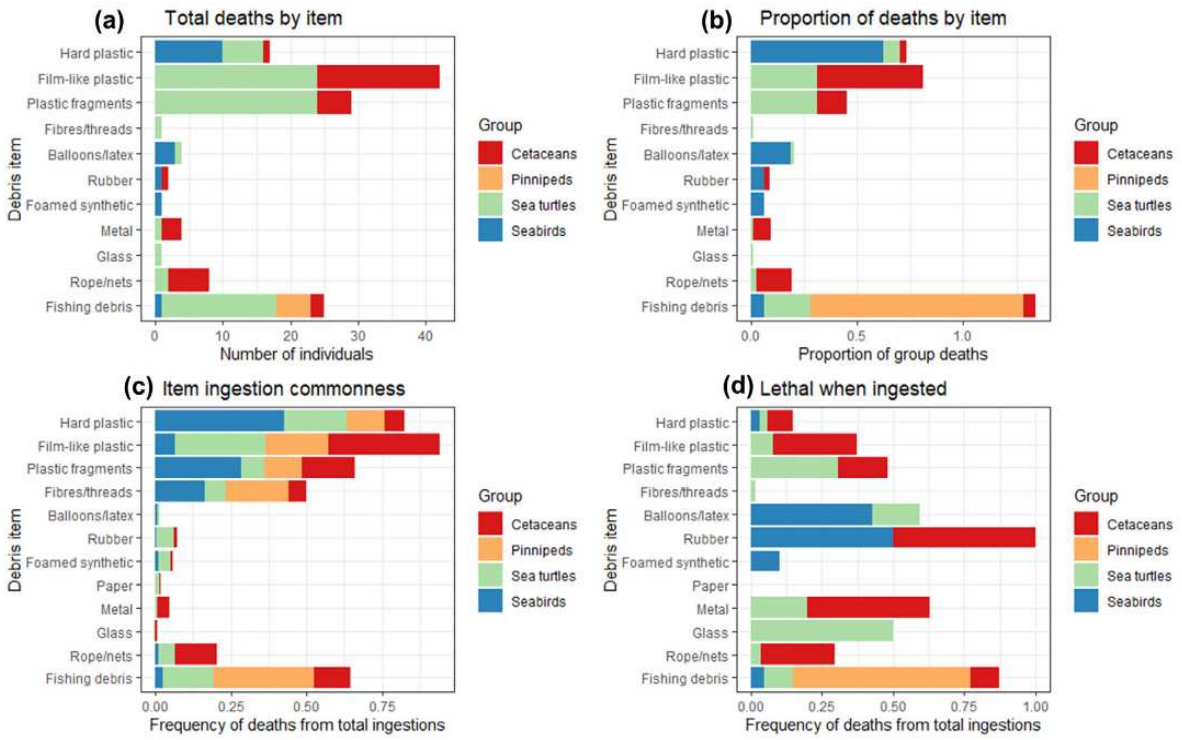

Figure 1.20: Ingestion of marine debris items and mortality of marine megafauna.
The number of individuals (a) and proportion of total individuals (b) within each megafauna group that died following the ingestion or contact with each debris item type. Film-like plastic and fishing debris are responsible for the highest proportion of death when there is a contact or ingestion. Hard plastic, film-like plastic, plastic fragments and fishing debris are the items most commonly ingested by megafauna (c). Rubber items and fishing debris are most likely to be lethal when ingested (d). Taken from Roman et al. (2021).

Concerning direct impacts, ingestion of macroplastics was reported to represent one of the most significant threat to marine organisms, with Kühn & van Franeker (2020) documenting cases of plastic ingestion in 701 marine species globally. Ingestion can cause intestinal blockage, tissue lesions, reduced feeding capacity and altered metabolic processes. Obstruction of the digestive tract was shown to be the primary cause of death from plastic ingestion (Roman et al. 2021). Gastric blockages can result in starvation, while perforations of the gastrointestinal tract can lead to peritonitis and septicemia (Orós et al. 2005; Barreiros & Raykov 2014). The frequency of occurrence of plastic ingestion varies substantially across taxonomic groups. 44% of seabirds (number of species in taxon (n_{sp}) = 409), 52% of marine mammals (n_{sp} =123), 100% of turtles (n_{sp} = 7) and 0% of sea snakes (n_{sp} = 62) were found with ingested plastics (Kühn & van Franeker 2020). In addition to ingestion, the entanglement of organisms with plastics, and in particular fishing gear, was highlighted to be also a major cause of behavior disruption and mortality. Most of the time, entanglement leads to drowning, suffocation, strangulation and starvation of organisms (Kühn et al. 2015; Wilcox et al. 2016). Fishing-related gear (nets, lines, lures, ropes) was reported to be the most common source of entanglement (Kühn et al. 2015; Wilcox et al. 2016; Lively & Good 2019). The entanglement of marine organisms within fishing related items is called ghost fishing and is defined as the phenomenon wherein derelict ALDFG continue to capture both target and non-target species, despite no longer being actively managed by fishermen (Breen 1987). For nets, ghost fishing was estimated to range between 67 to 100 kg of fish/day/net (1.04 to 2.2 fish/100 m^2/day) and 0.04 to 0.5 animals/trap/day. These captures were found to be lethal in 73 to 100% of cases for nets and in 2 to >90% of cases for traps (Lively & Good 2019). Nets were estimated

to continue to fish between less than 60 days to more than 20 years after being lost, while pots would continue to fish between less than 30 days until more than 7 years (Wilcox et al. 2016; Lively & Good 2019). Roman et al. (2021) analyzed 1,328 mortalities across 80 marine species, identifying 11 different classes of debris responsible of deaths (Figure 1.20). From these 11 categories, certain types of macroplastics were disproportionately lethal. Film-like plastics (including plastic bags, sheets, and film packaging), plastic fragments, and fishing debris were responsible for the highest number of deaths (Figure 1.20). Taking into account both entanglement and ingestion, the ALDFG caused the highest proportion of mortality. When considering ingestion, the ALDFG was the second most lethal type of litter, especially for the Pinnipeds (Figure 1.20). ALDFG was also identified as a marine habitat threat. Indeed, they damaged sensitive benthic habitats such as coral reefs or deep-sea corals and seagrass meadows (Kühn et al. 2015) through scouring, breaking, and smothering, as they are often entirely or partially derelict (Macfadyen et al. 2009; Lively & Good 2019).

In the Bay of Biscay, García-Barón et al. (2019) found that fishing gear was the third marine threats (5.3%) behind the exposure to crude oil (10%) and cachexia (52.3%). Looking at the Cetaceans, they showed that they were mainly threathened by fishing bycatch, vessel collisions, and pollution-related threats. On the other hand, seabirds were mostly vulnerable to oil spills, fishing bycatch and marine litter. Finally, in the sea floor of the Bay of Biscay's canyons, it has been suggested that fishing gear caused a substantial pressure on the deep-sea corals (Van den Beld et al. 2017). The plastic wastes identified in these canyons were colonized in 13.6% of the cases. ALDFG was particularly colonized by actinians. This colonization tended to change the naturally present biodiversity and may caused ecological deregulation (Mordecai et al. 2011). A less expected observation is that sedimented ALDFG has been identified as potential refuge areas for certain species, notably fish and crabs. However, this could also have deleterious effects on predators and scavengers which could be trapped while hunting and feeding (Macfadyen et al. 2009).

Macroplastics and especially macro fishing gear were identified to impact mainly marine organisms of large sizes and to cause lesser impacts on small aquatic species such as bacteria, phytoplankton, zooplankton or small fishes (Roman et al. 2021). However, the potential degradation of macroplastics into MPs, NPs and addtives as discussed in sections 1.2.1 and 1.2.2 can adversely impact these organisms.

1.2.3.2 Microplastics, nanoplastics and plastic chemicals

Toxicity of MPs and NPs have been extensively studied these last years and many negative effects on various species were shown. However, mainly primary spherical MPs and NPs have been studied which is not very representative of environmental reality (Paul-Pont et al. 2018; Barrick et al. 2021; Parsai et al. 2022). Indeed, as described in sections 1.2.1.1, 1.2.1.4, 1.2.1.5 and 1.2.1.6, most MPs and NPs present in the environment are coming from degradation of larger plastic particles and they can show different characteristics (e.g., shape, size, aggregation, aging state, etc.), additive composition and concentrations. Therefore, this section will focus on current knowledge about the toxicity of MPs, NPs, and additives/NIAS derived from the degradation of larger plastics, as well as that of their leachates, or extracts.

This approach aims represent more closely environmental conditions of plastic pollution. Indeed, although it is valuable to use a reductionist approach to thoroughly understand the mechanisms underlying MPs, NPs, additives and NIAS toxicity independently, an increasing body of knowledge indicates that mixtures of MPs, NPs and plastic chemicals often exhibit greater toxicity than individual component separately (Zimmermann et al. 2020b; Barrick et al. 2021; Beiras et al. 2021; Na et al. 2021).

1.2.3.2.1 Effects of MPs, NPs and plastic chemicals on bacteria

Bacteria are a useful model organism to quickly screen the toxicity of hazardous materials. Indeed, bacteria possess rapid reproduction rates, allowing for testing across multiple generations within short timeframes. Furthermore, their simple cellular structures facilitate the interpretation of toxicological mechanisms (Dunn 2012; Klein et al. 2021). Additionally, many bacterial species are environmentally relevant, playing crucial roles in ecosystem functioning through biogeochemical cycling and serving as the foundation of aquatic food webs (Dunn 2012; Pencik et al. 2023). Bacterial responses to plastic vary considerably depending on polymer composition, plastic chemicals (e.g., addditives, NIAS), weathering conditions, and experimental parameters employed in toxicological assessments (Pencik et al. 2023). Size of the MNPs in an ionic environment can produce a significant different impact on bacteria colonies, with 0.02 µm MNPs being strongly adsorbed on cell surfaces, 0.2 µm ones being adsorbed but at a lesser quantity, while 2 µm ones did not show significant adsorption (He et al. 2018). Saygin and Baysal (2020) showed that degradation products of conventional and biodegradable plastic bags and food packaging had a significant impact on pathogenic bacteria (e.g., *Staphylococcus aureus, Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*). Specifically, exposure of *B. subtilis* to cryomilled MPs (<5 µm) resulted in a higher activation of the viability compared to controls. On the other hand, *P. aeruginosa* showed a slight to high inhibition when exposed to biodegradable plastics. With similar conclusions, Klein et al. (2021) highlighted polymer-specific toxicity profiles on *Aliivibrio fischeri* exposed to artificially-aged conventional and recycled (PET, PS, LDPE, PVC) and biodegradable (Bio-PBS and PBAT-PLA-starch) plastic extracts. Recycled LDPE, PBAT-PLA-starch and Bio-PBS triggered the highest toxicity for *Allivibrio fischeri*, whereas other plastics caused minimal to no effects (Klein et al., 2021). They also showed that weathering intensity increased the toxicity of the plastics, especially for the PBAT-PLA-starch and Bio-PBS. Testing 65 different plastic items (conventional ones, biobased/biodegradable ones, cellulosic ones) Zimmerman et al. (2020a, 2021), showed that most of them (47/61) induced a significant inhibition of the bioluminescence of *A. fischeri*. They specifically argued that this toxicity was probably due to the 17 to 20,965 chemical compounds released by plastics. The PBS plastic bar and food tray tested released 3,864 and 10,959 chemical compounds, respectively. For a PBAT waste bag and pellet 15,843 and 9,161 chemicals were identified (Zimmermann et al. 2020a). In comparison, HPDE bin liners and PP yogurt cups released 1,401 and 7,036 chemical compounds (Zimmermann et al. 2021). To the best of our knowledge, only one work was published concerning the effects of fishing gear degradation products on marine bacteria. Thus, Gambardella et al. (2024) exposed *A. fischeri* to leachates of micronized ropes and aquaculture equipments (PE, PP, ethylene propylene diene monomer (EPDM)) sampled on

beaches of the Adriatic sea and the French beaches of the Bay of Biscay. After the exposure of *A. fischeri* to leachates at 1 g/L they found no significant toxicity compared to the controls. Given the limited number of studies available on this topic, it is essential to conduct further research on the toxicity of FNs used by fishermen, particularly those made of PA, in order to more accurately assess the risks associated with their degradation products on marine bacteria.

1.2.3.2.2 Effects of MPs, NPs and plastic chemicals on phytoplankton

Microalgae can also serve as ecotoxicological models due to their foundational role as primary producers in aquatic ecosystems, contributing approximately to 50% of global primary production (Prata et al. 2019). Additionally, their ecological significance, widespread distribution, sensitivity to contaminants, and established testing protocols (e.g., OECD Guideline 201) make them valuable bioindicators for assessing the impact of plastic degradation products on aquatic ecosystems (Prata et al., 2019; Parsai et al., 2022). Multiple toxicity mechanisms have been documented in microalgae exposed to environmentally degraded plastics (Figure 1.21).

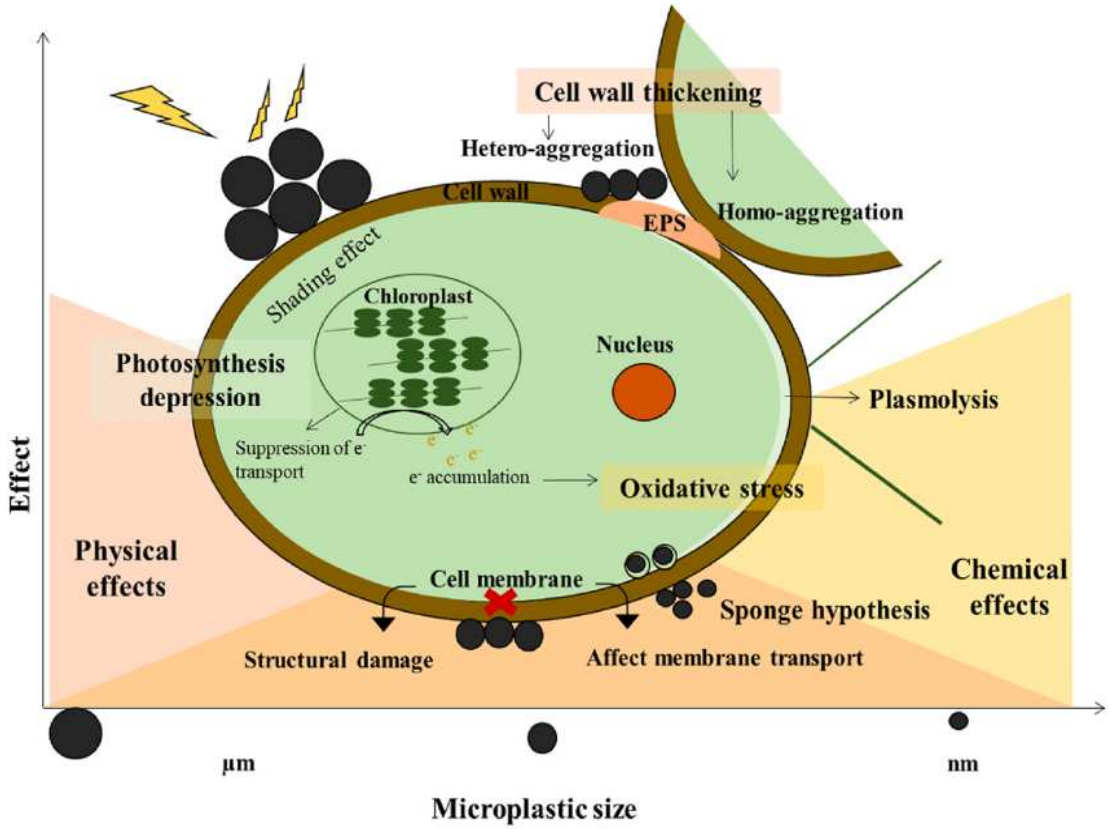

Figure 1.21: Illustration of the suggested mechanisms of toxicity of microplastics on microalgae.
Taken from Parsai et al. (2022)

Physical adsorption of MPs and NPs to microalgae cell surfaces was shown to be the initial factor of toxicity (Prata et al. 2019; Parsai et al. 2022). First, it may diminish the fluidity of cell membranes, thereby impacting membrane transport and altering the exchange of substances between the cell and its environment. This alteration can consequently affect overall cellular

metabolism and potentially result in growth inhibition. Furthermore, the adsorption of MNPs might exert adverse effects on cellular structures resulting in plasmolysis, vacuolation, and distortion of membrane structures within the cell (Figure 1.21) (Parsai et al. 2022). Elevated production of reactive oxygen species (ROS) due to exposure to MPs may lead to oxidative stress and damage the cells (Meng et al. 2024) (Figure 1.21). Natarajan et al. (2022) showed that for the microalga *Scenedesmus obliquus*, an increase in ROS production was correlated with both a higher concentration of MPs and a reduction in MP sizes. The smallest MPs (1 µm PS MPs) caused higher decrease of photosynthetic effectiveness, membrane integrity and esterase activity of the microalgae *S. obliquus* compared to bigger MPs (12 µm PS MPs) (Natarajan et al. 2022). In addition, the adsorption of MPs and NPs to microalgae cells may induce the secretion of extracellular polymeric substances (EPS). The subsequent secretion of EPS may facilitate hetero-aggregation (microalgae-MPs) and lead to the depletion of nutrients and multivalent metals. Therefore, EPS secretion could play a partial role in the cytotoxic effects and photosynthesis inhibition observed in microalgae (Besseling et al. 2014; Lagarde et al. 2016; Meng et al. 2024). The adsorption of MPs and NPs on cell surfaces was also reported to impair photosynthesis by limiting light availability and gas exchange and impacting their growth (Zhang et al., 2017). However, two studies excluded that a layer of MNPs on the ocean's surface or in the water column could have a shading effect on microalgae, reducing their photosynthetic capacity (Schwab et al. 2011; Zhang et al. 2017). Energy metabolism disruption represents another significant impact mechanism. Indeed, Meng et al. (2024) investigated the effects of aged MPs on ATPase activity in *Isochrysis galbana* and observed that Na^+/K^+ and Ca^{2+}/Mg^{2+} ATPase activities were significantly reduced or increased depending on the plastic polymer in microalgae exposed to aged PS, PVC, and polymethyl methacrylate MPs. This disruption in ion transport was reported to affect cellular homeostasis and energy allocation. The same study also reported a substantial increase in EPS concentration in microalgae exposed to aged MPs. Additionally, there was a significant decrease in non-esterified fatty acid content. According to the authors, these effects indicated altered carbon metabolism and energy storage (Meng et al. 2024). This energy metabolism disruption was hypothesized to be the main driver of growth inhibition and reduced photosynthetic efficiency. Ultimately, the higher concentrations of phthalates (diisobutyl phthalate, di-2-methoxyethyl phthalate, di(2-ethylhexyl) phthalate) released by new plastics, in comparison to aged plastics, were interpreted as a contributing factor to the higher toxicity exhibited on microalgae (Parsai et al. 2022; Meng et al. 2024). Schiavo et al. (2021) further underscored that the growth inhibition, oxidative stress (DCFH-DA), and DNA damage observed in the marine microalgae *Dunaliella tertiolecta* were attributed to the synergistic effect of polymeric matrices and trace metals present in the PP, PS, and PE leachates.

Considering that even minimal impacts on phytoplankton can have substantial consequences for the broader marine ecosystem as well as terrestrial life, it is noteworthy to mention that multiple studies have indicated that MPs (usually pristine PS MPs) provoke no significant acute toxicity on microalgae (Zhang et al. 2017; Prata et al. 2019; Meng et al. 2024). Nonetheless, a comprehensive understanding of the effects of environmentally realistic MNPs on microalgae remains necessary. To the best of our knowledge, the impacts of degradation products from FNs on microalgae have not yet been examined. Thus, it is essential to conduct investigations

to comprehensively assess the potential environmental risks posed by FNs on marine microalgae.

1.2.3.2.3 Effects of MPs, NPs and plastic chemicals on zooplankton

Alongside phytoplankton, zooplankton are crucial organisms for ecosystem finctioning. Indeed, they constitute the most extensive animal group within oceanic ecosystems. Moreover, they serve as a pivotal ecological group in facilitating the transfer of energy to higher trophic levels and play a crucial role in the biological carbon cycle of the ocean (Bonnet et al. 2005; Borja et al. 2019; Rodríguez-Torres et al. 2024). Their sensitivity to pollutants, their short life cycle, high reproductive rates, and standardized testing protocols make them valuable for assessing both acute and chronic effects of pollutants (Cajaraville et al. 2016; Botterell et al. 2019; Hafez 2021).

Field studies have confirmed microplastic ingestion by zooplankton in natural environments for a wide number of species but with high variations between taxonomic groups (Botterell et al. 2019; Rodríguez-Torres et al. 2024). The bioavailability of MNPs is mainly driven by 4 families of factors: abundance/co-occurrence, characteristics of plastic (size, shape, colour, polymers, chemical composition), transformation (weathering, biofouling, aggregation) and selectivity of zooplankton (Botterell et al. 2019). This selectivity varies considerably among zooplankton life stages and taxa due to differences in feeding modes. Filter-feeding species tend to ingest substantially more MPs and NPs than selective grazers and more than raptorial (active feeders) species that require a physical prey stimulus (Cole et al. 2013; Botterell et al. 2019; Malinowski et al. 2023). It has also been shown that up to 80% of ingested MPs and NPS were excreted, regardless of polymer type, shape, biofilm presence, or sorbed pollutants (Cole et al. 2015; Xu et al. 2022).

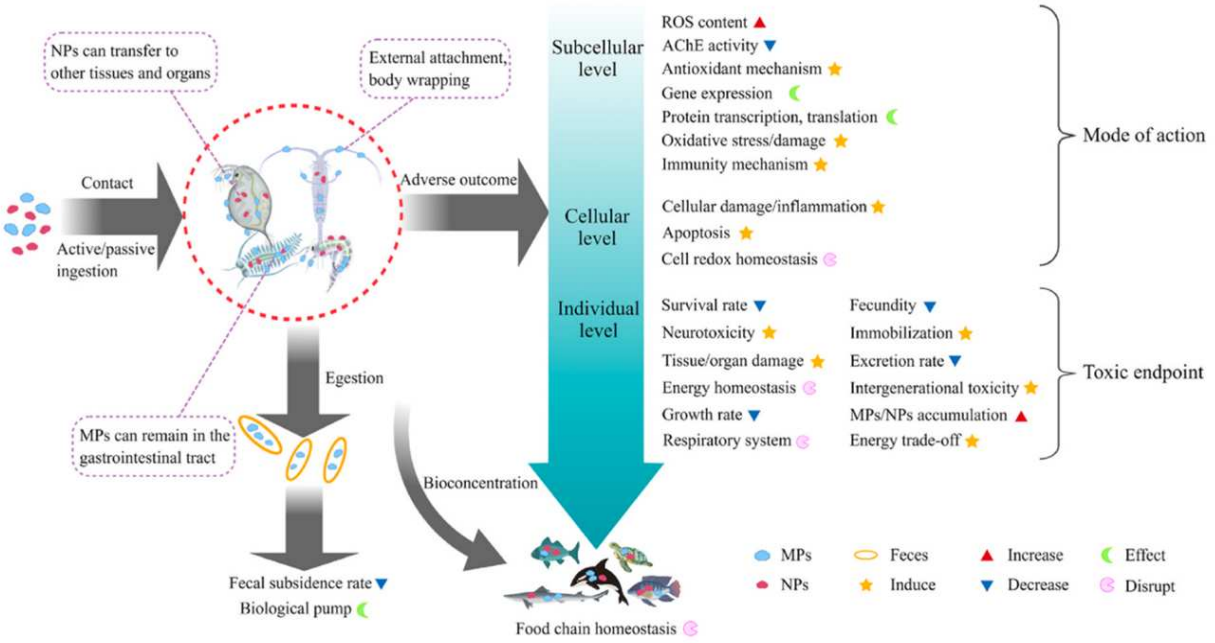

Figure 1.22: Overview of the ecotoxicological pathways and effects of microplastics MNPs on zooplankton. Symbols meaning are defined at the bottom right. Sourced from Zhao et al. (2024).

Exposure to MNPs and plastic chemicals caused multiple adverse effects on zooplankton. Zhao et al. (2024) recently summarized most of them in a diagram (Figure 1.22). In addition to the mode of action and toxicological endpoints of MNPs for zooplankton reviewed by Zhao et al. (2024) (Figure 1.22), other observed effects have been described. Particularly, reproductive impairments have been documented in zooplankton exposed to MNPs and plastic chemicals. Heindler et al. (2017) showed that there was a negative correlation between egg production by female and concentration of MPs for the copepod *Parvocalanus crassirostris* exposed to MPs derived from PET plastic bottles (5-10 µm). At concentrations of 10,000, 20,000 and 40,000 MPs/mL the decrease of egg production by female was of 10%, 18% and 48%, respectively, but was not significant compared to the controls. The decrease of egg production by female was significant only at 80,000 MPs/mL (-88%), i.e., a MPs:zooplankton ratio of 4:1 (Heindler et al. 2017). Concerning the zooplankton population size, a significant decrease occurred both after 6 and 24 days of exposure (25 to 40% comparing to the control) (Heindler et al. 2017). Furthermore, the authors observed that the proportion of adults and copepodites relative to nauplii was higher in the control group compared to those exposed to MPs for a duration of 24 days. Langenfeld et al. (2024) have shown similar results using a large-scale mesocosm experiment conducted over 10 weeks. Indeed, they highlighted temporary changes in zooplankton communities exposed to PE, PS and PET MPs (37-1086 µm). The authors reported a significant positive relationship between calanoid copepod abundance and MP concentrations (6, 24, 100, 414, 1,710, 7,071 and 29,240 MPs/L) after 19 and 33 days of exposure. This finding highlighted that MP pollution could cause temporary stress on copepods leading to increase of the population abundance. Moreover, the authors observed two independent short-term significant negative correlations between the reduction in egg production by female and the number of nauplii of the copepod *Tropocyclops extensus* and the concentration of MPs. Populations of the zooplankton species *Diaphanosoma birgei*, *Eubosmina sp.*, *Mesocyclops edax* and *Mesocyclops edax* were not significantly impacted by MPs (Langenfeld et al. 2024). Koski et al. (2021) reported no significant effects of new and aged tire wear particles (8-20 µm) between 10 and 10,000 MPs/L, on *Acartia tonsa* egg production by female at high (480 ± 55 µg C/L) and low (43 ± 5 µg C/L) food concentrations. However, the authors reported significant effects of new wear particles at 10, 1,000 and 10,000 MPs/L on *A. tonsa* faecal pellet production at low food concentration. The aged wear particles caused a significant increase of faecal pellets produced by *A. tonsa* when exposed at 100, 1,000 and 10,000 MPs/L with low concentration food supply. Botterel et al. (2025) recently reported that after 72 hours of exposure to a blend of PP, PA, and LDPE microplastics (5-100 µm), *A. tonsa* exhibited an LC50 of 182 µg of MPs per liter. The authors also observed a significant increase of the egg size of *A. tonsa* following a 6-day exposure to concentrations of 10 and 100 µg of MPs per liter. Globally, using ocean circulation and biogeochemical models (NEMO/PISCES-PLASTIC), Richon et al. (2024) estimated that in the most contaminated regions of the oceans, MP pollution substantially reduced the grazing of zooplankton. Short-term simulation (10 years) mainy emphized grazing impacts on coastal and upwelling regions, while after long-term simulation (100 years) the impacts of MP pollution spread over intertropical regions, especially aroud in highly contaminated areas (oceans gyres). The authors emphasized that a reduction in zooplankton grazing in regions with high levels of contamination over extended periods could potentially reduce up to 50% the annual primary

production, carbon export flux, as well as nutrient recycling and remineralization (Richon et al. 2024).

Concerning the effects of plastic chemical degradation products on zooplankton, Zimmerman et al (2020b), showed ambivalent effects depending on the polymers. For PVC MPs, toxicity was driven by the extractable chemicals rather than the particles themselves, while for PUR and PLA MPs, the particle characteristics were responsible for adverse effects. In another study, the mobility inhibition of *Daphnia magna* due to PE cosmetic microbeads was reported to be enhanced by their aging and adsorption of silver in the biofilm (Kalčíková et al. 2020). The di(2-ethylhexyl) phthalate often used as plasticizer had more effects on the copepod *P. crassirostris* than the MPs by themselves (Heindler et al. 2017). Other studies also claimed that at currently measured environmental concentrations, particles by themselves may pose limited risks to zooplankton populations, whereas the opposite is true for chemical degradation products (Beiras et al. 2018; Gunaalan et al. 2020).

Table 1.5: Leachate toxicity of micronized FG (< 250 µm) coming from beaches of the Italian Adriatic sea and of the French part of the Bay of Biscay.

Percentage effects represents the toxicity of the leachates at a concentration of 1 g/L. The astherisks indicate a significant difference compared to the control ($p < 0.05$). EC50 and LC50 mean the effective or lethal concentration of leachate that caused 50% effects on the tested species. Units of EC50 and LC50 are g/L. "SE" means standard error and "CI" means confidence interval at 95%. Modified from Gambardella et al. 2024.

Zooplankton	Endpoints	FG Adriatic		FG Bay of Biscay	
		Effect±SE (%)	EC50 or LC50 (g/L) (95% CI)	Effect±SE (%)	EC50 or LC50 (g/L) (95% CI)
Sea urchin pluteus	Developmental anomalies	87±2 *	0.23 (0.16 – 0.32)	100 *	0.43 (0.37 – 0.54)
	Behavioral diruption	5±2	> 1	35±2 *	> 1
Barnacles nauplii	Immobility	9±1	> 1	18±2	> 1
	Behavioral diruption	44±3 *	> 1	42±1 *	> 1
Rotifer nauplii	Immobility	29±8	> 1	10±2	> 1
	Behavioral diruption	41±2 *	> 1	26±3 *	> 1
Copepod nauplii	Mortality	59±6 *	0.90 (0.80 – 0.99)	8±3	> 1

Once again, concerning effects of plastic fishing gear on zooplankton and more specifically on copepods, very few published data were found. The only study identified was conducted by Gambardella et al. (2024), in which exposed holoplankton species (*Acartia tonsa* and *Brachionus plicatilis*) and two meroplankton species (*P. lividus* and *A. amphitrite*) were exposed to leachates coming from ropes and aquaculture equipments collected from the Adriatic beaches and the French beaches of the Bay of Biscay (Table 1.5). Based on the environmental risk assessment approach called "Weight of Evidence" (Regoli et al. 2019), the authors highlighted that FG from the beaches of the Adriatic sea had a higher level of ecotoxicological hazard (moderate) compared to those of the beaches of the French part of the Bay of Biscay (absent) (Gambardella et al. 2024).

1.2.3.2.4 Effects of MPs, NPs and plastic chemicals on fishes

In vivo or in vitro bioassays in fishes are now well-recognized as powerful ecotoxicological tools and ocean health indicators. Numerous fish species can be selected to study the toxicity of pollutants. Toxic effects can be addressed at different biological levels, from molecular the one to decipher the toxicity mechanisms of pollutants to the ecological one to highlight the potential effects of pollutants on fish populations (Cajaraville et al. 2003; Goksøyr et al. 2003; Melvin & Wilson 2013; Ford et al. 2021). In vitro assays with fish cell lines provide a robust and efficient screening of the toxicity of MNPs and plastic chemicals, while reducing the use of adults or embyos of fish (Pannetier 2018). Using different rainbow trout cell lines, Pannetier et al. (2019) and Pinto et al. (2024) showed tissue-specific sensitivities to secondary MPs exposure. Indeed, the first work showed that organic extracts from environmental MPs (<600 µm, 1 mg/mL to 30 ng/mL of plastic) collected from beaches induced significant DNA damage in Rainbow Trout liver cells (RTL-W1) after 24 h exposure. Extracts also modulated phase I biotransformation ethoxyresorufin-O-deethylase (EROD) activity. In this study extracts from marine litter from North Atlantic beaches induced stronger responses compared to marine litter from South Pacific and North Pacific beaches. On the contrary, Pinto et al. (2024) reported no significant cytotoxicity (metabolic activity, plasma membrane and lysosomal integrity) in the Rainbow Trout intestinal epithelium cell line (RTgutGC) exposed to artificially aged HDPE MPs (2-15 µm) and MNPs (0.2-9.9 µm) after 24 h exposure to concentrations from 12.5 to 200 mg/L. In addition to differences in the origins of MNPs studied and in exposure concentrations, Pannetier at al. (2019) used DMSO extracts that extracted more chemicals than direct exposure of MPs and NPs in cell line medium. Using the same RTgutGC cell line and also RTgill-W1 cell line, Dudefoi et al. (2024) revealed that the EC_{50} of tire and road wear particles (TRWP) was higher than the concentration of TRWP found in rivers. They also showed that while leaching chemicals were the primary drivers of toxicity, the presence of particles also contributed to the toxicity through continuous chemical release during exposure. In a review focused on fish cell lines to assess the toxicity of MNPs, Revel et al. (2021) described that exposure fibroblast cells of *Sparus prata*, neural cells of *Dicentrarchus labrax* and brain cell lines of *Fundulus heteroclitus* to NPs (pristine PS from $1x10^6$ to 1 mg/L) can induce changes in phase II biotransformation glutathione S-transferase activity. Additionally, PS NPs can modulate catalase activity, indicating the disruption of cellular antioxidant systems. Furthermore, research has shown that plastic chemicals leached from PE MPs lead to a reduction in EROD activity in RTL-W1 cells, a metabolism which is especially involved in the detoxification of organic pollutants (Heinrich & Braunbeck 2019).

The toxicological effects of MNPs on fish have been thoroughly documented through biomarker analyses on tissues of fishes sampled in the environment. Based on a battery of biomarkers (general conditions, neurotoxicity, biotransformation, oxidative stress) Lourenço et al. (2025) calculated higher integrated biomarker response (IBR) index for two wild Atlantic fish species contaminated by MPs compared to individuals without MPs. The IBR index reached 0.317 for *Merluccius merluccius* and 0.2 for *Trisopterus luscus*, whereas fish without MPs exhibited values of 0 and 0.062, respectively. MPs fragments (< 150 µm) (PA, polyacrylonitrile, PE, PES, PET, polymethyl methacrylate, PP, and PS), were identified in the gastrointestinal tracts, gills, livers, brains, and muscles of contaminated fish (Lourenço et al.

2025). Using a similar battery of biomarkers, Martins et al. (2025) reported results for euryhaline species (*Mugil cephalus*, *Platichthys flesus*) and a freshwater species (*Cyprinus carpio*). The fish were sampled from the Minho River estuary (Iberian Peninsula) during the winter and summer of 2023. In the summer, fish individuals contaminated with more than 0.1 MP/g of tissue exhibited IBR indices that were 1.2 to 1.8 times higher than those recorded in fish with contamination levels below 0.1 MP/g of tissue. For fish sampled during the winter, the increase in the IBR index was generally consistent with that observed in summer, except for flounders. In flounders, individuals with more than 0.1 MP/g of tissue exhibited an IBR index that was 0.8 times lower than those with contamination levels of 0.1 MP/g or less (Martins et al. 2025). In Mediterannean sea between 1.78±0.26 and 6.11±0.48 g of MP (Polyethylene-co-vinyl acetate, HDPE, LDPE, PA, Polyethyl methacrylate) ranging from 0.45 µm and over 3 µm were identified by gram of gastro-intestinal tract and muscle tissue in the commercial fish *Serranus scriba* (Zitouni et al. 2020). Variability in MP concentrations within fish tissues depended on the sampling sites along the Tunisian coast. Interestingly, several plastic additives such as processing aids, lubricants, stabilizers, and emulsifiers were also detected in the tissues of *Serranus scriba*. The presence of MPs in the tissues of this fish species was associated with significant oxidative stress. The authors reported a significant negative correlation between acetylcholinesterase (AChE) activity and the quantity of the smallest MPs detected in fish tissues (ranging from <1.2 to 0.45 µm). Variations in contamination levels (MP concentration, MP sizes, and plastic chemical composition) across the different sampling sites, resulted in significant differences in biomarker responses (Zitouni et al. 2020).

Regarding experimental exposures of fish, a study with *D. rerio* demonstrated that pristine polystyrene NPs (50 nm) penetrated intestinal barriers and primarily modulated antioxidant defenses. In contrast, MPs of 4.5 µm remained within the gut lumen and induced phase I/II biotransformation enzymes, accompanied by hepatocellular vacuolization (Martínez-Álvarez et al. 2024). The authors further reported that PS MNPs did not increase polycyclic aromatic hydrocarbon (PAH) levels in zebrafish. Plastic additives, and especially the photoinitiator 1-hydroxy-cyclohexyl-phenyl-ketone contained in leachates derived from 3D-printed acrylate plastics (60 mg/L), led to strong ROS production in *D. rerio*. The leachates of this 3D-printed plastic also induced caspase-3-dependent apoptosis and a 44% brain AChE reduction within 48 h in Zebrafish (Walpitagama et al. 2019). Working with larvae of the marine fish *Cyprinodon variegatus*, Moreno Abril et al. (2024) found that leachates from commercial plastic bags, including industrial and compostable (PBAT), home-compostable (PLA-based blends), LDPE, and recycled PE at both 1 and 10 g/L concentrations, altered gene expression biomarkers related to steroidogenesis and detoxification. At 10 g/L, home-compostable (PLA blends), LDPE, and recycled PE bag leachates significantly altered estrogenic biomarkers (notably vitellogenin ab and zona pellucida glycoprotein 2). At the same concentration (10 g/L) the industrial-compostable bag leachate (PBAT) induced strong upregulation of steroidogenic biomarkers (steroidogenic acute regulatory: 2.16-fold and Cytochrome P450 19a6: 3.08-fold) at 1 g/L compared to control. Notably, most of these commercial and alternative plastic bags released quantifiable levels of phthalates. Recycled and oxo-degradable bags contained approximately twice as much di(2-ethylhexyl) phthalate as conventional LDPE bags (Moreno Abril et al. 2024).

Table 1.6: Summary of several studies investigating the acute, sub-chronic and chronic effects of microplastics and nanoplastics on various fish species.
"mpf" = months post fertilization. "E3 medium" = zebrafish medium composed of NaCl, KCl, CaCl2, H2O and MgSO4. *A. polyacanthus = Acanthochromis polyacanthus. C. variegatus = Cyprinodon variegatus*

Fish Species	MPs origin	Polymer	Size	MP preparation	Method	Endpoint	Effects	Reference
D. rerio (larvae)	Beaches from Guadeloupe	PE, PP, PS	Mean: 87.1 µm	Cryomilling MPs and extraction in E3 medium	Acute waterborne exposure at 10,000 and 50,000 mg/L	Survival	No larval mortality	Cormier et al. (2021a)
						Development	No significant developmental anomalies	
						Growth	No significant effects on larval size	
D. rerio, O. melastigma (larvae)	Commercial powder	PE, PVC	PE: 5 µm PVC 105 µm	Spiked with PFOS, BP3 and BaP	Chronic foodborne exposure at 1% wet weight in food	Survival	No significant mortality observed in any treatment group	Cormier et al. (2021b)
						Growth	After 2 months no effect on growth. After 4 months: significant decrease of body weight and length; stronger effect in females (20-35% decrease in body weight) than males; similar effects for PE and PVC regardless of spiking	
						Reproduction	Zebrafish: Spawning attempts reduced for PE-BP3 (48%) and all PVC treatments (12.1-24.2%). *O. latipes*: Significant delay in spawning onset; reduced reproductive output for PE treatments (31-56%); for PVC treatments (63-86%), increase in reproductive output of 148 % for PVC-BaP.	
D. rerio (juveniles)	Artificial plastic plants	PS	Mean: 17 µm Min - Max: 2 - 135 µm	Environmental weathering (5 years)	Acute waterborne exposure at 4×10^4 and 4×10^6 MPs/m^3	Feeding	No significant changes in nutritional status parameters	Guimarães et al. (2021)
D. rerio (embryos)	Beaches from Guadeloupe	PE, PP, PS	53-100 µm	Cryomilled MPs and leachates preparation in E3 medium	Acute waterborne exposure at 1 or 10 mg/L	Development	No significant developmental anomalies; no difference in head or total length	Cormier et al. (2022)
						Reproduction	No change in egg hatching time or rate	
D. rerio, O. melastigma (embryos to adults)	Beaches from Guadeloupe	PE, PP, PS	53-100 µm	Cryomilling MPs and extraction in E3 medium and food contamination with MPs	F1 larvae from parents exposed to MPs through diet (1% wet weight)	Survival	Zebrafish: premature mortality in larvae exposed-parents. F1 larvae decreased 2% in length. Marine medaka F1 increased 5% in length versus controls.	Cormier et al. (2022)
					Chronic foodborne exposure at 1% wet weight in food	Growth	Zebrafish: no differences at 2 mpf, reduced weight in females and males at 4 mpf, and decreased female weight at 6 mpf. Marine medaka exhibited reduced length and weight in both sexes at 6 mpf.	
					Chronic foodborne exposure at 1% wet weight in food	Reproduction	Zebrafish: 70% decrease in reproductive success; no changes in egg number or fertilization rate. Marine medaka: 42% decrease in reproductive output; fertilization rate unchanged	
				Cryomilling MPs and extraction in E3 medium	Acute waterborne exposure at 1 or 10 mg/L	Transgenerational effects	Acute exposure: No embryonic mortality (<5%); Chronic exposure: No significant mortality in adults.	

Continuation Table 1.6

Fish Species	MPs origin	Polymer	Size	MP preparation	Method	Endpoint	Effects	Reference
D. rerio (embryos & larvae)	Food container	PP	Median: 116 µm	Grinded food container and UV irrradiation (20 days) and then mixed with exposure medium	Acute waterborne exposure at 2000 and 20,000 MP/L	Survival	No significant mortality (0-20%)	Prata et al., (2022)
						Heart rate	Significant decrease in heart rate	
						Development	Significant effect on swimming bladder inflation at 50 mg/L (88% non-inflated).	
						Growth	Significant decrease in body length (3.5-3.8 mm vs. 4.0 mm in control)	
						Reproduction	No significant effect on hatching rates.	
D. rerio (embryos & larvae)	Commercial powder	PA	5 - 50 µm	Xenon irradiation of MPs in humic acid and fulvic acid	Sub-chronic waterborne exposure at 1, 10, and 20 mg/L	Survival	No mortality observed in all exposure groups, including pristine and aged microplastics	Zhang et al. (2022)
						Feeding	Aged MPs more difficult to depurate by zebrafish than pristine MPs. Intestinal damage observed: disappearance of intestinal folding, shedding of enterocytes, and emaciation of intestinal microvilli.	
						Growth	Body length decreased when exposed to aged MPs (- 0.5 mm to - 0.7 mm). Body size significantly decrease when exposed to virgin and aged MPs (12.8% - 29.8%)	
						Reproduction	Significant hatching rate decreased when exposed to high-dose aged MPs (-7 to - 11 %)	
D. rerio (embryos)	Commercial powder	PS	NPs: 50 nm, 500 nm and MPs: 4.5 µm	MNPs spiking with BaP	Acute waterborne MNPs exposure at six different concentrations (0 to 50.1 mg/L)	Survival	No significant mortality (0-13.3%)	(Martínez-Álvarez et al. 2022)
						Hatching time	No significant effects	
						Development	Significant malfromations (56.7 %) when exposed to 50.1 mg/L of MP-BaP.	
D. rerio (embryos & larvae)	Secondary NPs derived from pristine pellets	PLA, PP, PS, LDPE	91-712 nm	Grinded pellets	Acute waterborne exposure at 0.001, 0.01, 0.1, 1, 10 mg/L	Survival	No significant mortality	(Tamayo-Belda et al. 2023)
						Development	No significant malformations of the head, tail or spine.	
						Hatching rate	No significant effect	
						Heart rate	PLA NPs significant increase at 0.1 and 10 mg/L. PP NPs significant decrease at 1 mg/L. PS NPs significant increase at 0.01 and 10 mg/L. LDPE NPs no significant effect.	
D. rerio (adults)	Disposable facial masks	PP	Virgin: ⌀80 ± 101 µm Aged: ⌀18 ± 16 µm	Natural weathering in a lake (56 days)	Chronic waterborne exposure at 50 mg/L (~ 2.06×10^5 MPs/L)	Survival	No significant differences in survival	Kim et al. (2023b)
						Development	Liver: Significant increase in damaged nuclei; Gills: Hypertrophy, proliferation, lamellar fusion, dilated and clubbed tips in secondary lamella; Intestine: Damaged goblet cells and microvilli	
						Growth	No significant decrease in growth parameters (wet weight, total body length, Fulton's condition factor); Significant decrease (6.38%) in skin pigment chroma saturation	

Continuation Table 1.6

Fish Species	MPs origin	Polymer	Size	MP preparation	Method	Endpoint	Effects	Reference
D. rerio (embryos & larvae)	Secondary MNPs derived from water bottles	PET	0.07-2032 µm	Degradation of mesopieces using trifluoroacetic acid	Acute waterborne exposure at 0.5, 1, 5, 10 and 20 mg/L.	Survival	No significant mortality (0-5%) (embryos)	(de Souza Teodoro et al. 2024)
						Hatching rate	No significant effect (embryos)	
						Spontaneous tail coiling	Significant decrease at 20 mg/L.	
						Heart rate	Significant increase at 1, 5, 10 and 20 mg/L (embryos).	
						Development	No significant difference of larvae body length or eye area. Significant decrease of the distance between the eyes at 0.5, 1, 5, 10 and 20 mg/L.	
O. latipes (larvae and juveniles)	MPs from beaches in Easter Island, Guam Island and Hawaii	PE, PP, PS	16 - 962 µm	Grinded MPs and food contamination with MPs	Chronic foodborne exposure at 0.1 % of MPs (700 MPs/L)	Survival	Significant increase in larvae mortality (27.8 ± 3.9%)	Pannetier et al. (2020)
		PE, PP	9 - 862 µm		Chronic foodborne exposure at 0.01 % of MPs (70 MPs/L)	Development	Significant increase in head/body length ratio for larvae exposed to Easter Island	
		PE, PP, PS	16 - 962 µm		Chronic foodborne exposure at 0.01 - 1 % of MPs (70-700 MPs/L)	Development	No significant effects on biometry parameters (weight, length, Fulton's condition factor) in juveniles	
		PE, PP, PS	24 - 777 µm		Chronic foodborne exposure at 0.1 - 1 % of MPs (700-7000 MPs/L)	Growth	Significant reduction in body length and/or head length depending on the island of origin of MPs	
O. melastigma (embryos & larvae)	Virgin powder	PE	4-6 µm	MPs Spiked with: PFOS (low concentration: 0.12 µg/g, high: 55.65 µg/g); BP3 (low: 0.14 ng/g, high: 24 ng/g); BaP BP3 (low: 0.01 µg/g, high: 16.64 *g/g)	Acute waterborne exposure at 10 mg/L	Survival	No effect on survival of virgin MPs; MP-PFOS and MP-BP3 significantly reduced embryonic survival (-28 %, -48 %)	Le Bihanic et al. (2020)
						Development	No significant effect of virgin MP; MP-BaP and MP-BP3 induced morphological abnormalities	
						Growth	No significant effect on growth of virgin MPs; MP-BaP low reduced total length; MP-BP3 high reduced head length	
O. melastigma (embryos & larvae)	15 Plastic bags	PE, PP, PE-PA		UV irradiation, leachates in seawater and fish digests	Acute waterborne exposure at 0.01 g/L without MPs	Survival	Only 1/15 plastic bag leachate caused significant mortality.	Qiu et al., (2022b)
						Heart rate	Heart rate significantly increased	
						Development	Developmental retardations, reduction in eye pigmentation, and abnormal oil droplet fusion inside embryos	
A. polyacanthus (juveniles)	Recycled plastic pellets	PET	125 - 5000 µm	Cryomilling and seawater aging (14 days)	Acute waterborne exposure at 0, 0.021, 0.044, 0.067, 0.86 mg/L	Growth	Negative effect on growth and body condition, especially at higher plastic concentrations	Critchell & Hoogenboom (2018)
				Cryomilling and seawater aging (14 days) and food contamination with MPs	Chronic foodborne exposure at 0, 0.025, 0.055, 0.083, 0.1 mg/L	Growth	No significant effect on growth and body condition	

Continuation Table 1.6

Fish Species	MPs origin	Polymer	Size	MP preparation	Method	Endpoint	Effects	Reference
C. variegatus (larvae)	Commercial resin	PHB and PHBv	< 250 µm	Leachates of grinded MPs	Acute waterborne exposure at 1 and 10 g/L	Survival	100 % of mortality for leachates of PHB and PHBv at 10 g/L. 0% and 4% mortality at 1 g/L for PHB and PHBv leachates respectively.	(Moreno Abril et al. 2024)
S. solea (juveniles)	Environmental MPs from Seine estuary	PE, PP, PVC, PET	<100 µm	Grinded MPs and food contamination with MPs	Sub-chronic foodborne exposure through sediment exposure at 1 or 100 mg of MP/kg of sediment	Survival	No significant mortality	(Pannetier et al. 2025)
						Development	Significant skin color change of the fish for the exposure with 100 MP/kg	
S. solea (juveniles)	Commercial powder	PVC	125-250 µm	MPs spiking with BaP or BP3 and food contamination with MPs	Sub-chronic foodborne exposure through sediment exposure at 1 or 100 mg of MP/kg of sediment	Survival	No significant mortality.	
						Development	Significant increase of chroma number when exposed to BP3-MPs	

In the case of fish in vivo experiments, various endpoints can be investigated, with the most commonly examined being survival, heart rate, growth, development, feeding and reproduction (Table 1.6). Results are heterogeneous as most of the plastics studied and the methods used are different. Several studies highlighted no significant lethal effects when the fish (embryos, larvae and adults) were exposed to low MP or leachate concentrations (Table 1.6). However, sublethal effects were often observed, especially when the fish were exposed to leachates, extracts containing plastic chemicals or fish exposed to MPs spiked with pollutants. Moreover, chronic exposures tend to cause more effects than acute ones (Table 1.6).

Studying the behavior of fishes was also reported as a suitable tool to reveal sublethal effects that traditional lethality-based assessments might miss (Jacquin et al. 2020). Indeed, these assays enable the detection of subtle behavioral alterations, such as disrupted exploratory patterns, vertical migrations, social interactions, or stress responses, through quantification of changes in phototaxis, thigmotaxis, rheotaxis, and chemical avoidance behaviors (Table 1.6). Behavior disruption can also serve as an early indicator of neurotoxic or endocrine-disrupting effects at environmentally relevant concentrations of pollutants (Melvin & Wilson 2013; Ford et al. 2021). Studies investigating the effects of leachates from MPs and NPs and plastic chemicals coming from weathered plastics have demonstrated various changes in the behavior of fish species. Qiu et al. (2022a) reported that 15 plastics items (mulch and bags made of PA/PE, PE, PP) weathered in seawater and fish digestive medium caused either hyperactivity or hypoactivity in marine medaka larvae (*Oryzias melastigma*). The authors mainly linked these behavioral disruptions to the 240 organic compounds they quantified in the leachates, which included antioxidants, degradation by-products, dyes and flame retardants. The toxicity mechanism was partially explained by the alterations in glial fibrillary acidic protein and myelin basic protein mRNA levels, which indicated potential neurotoxic effects. Enhanced mRNA levels of *O. melastigma* telomerase reverse transcriptase, tumor necrosis factor alpha, and cytochrome p450 26b1 genes suggested increased cell proliferation, immune dysfunction and stimulation of ROS production (Qiu et al. 2022a). Additionaly, they highlighted a significant inhibition of *p53* mRNA expression which indicated a disruption in the initiation of DNA repair in normal cells and a potential alteration of the apoptosis mechanism of abnormal cells (Qiu et al. 2022a). Other studies also related the disruption of fish behavior to plastic additives. Indeed, relevant concentrations (20-200 µg/L) of di(2-ethylhexyl)-phthalate (DEHP), a common plastic additive, was shown to increase maximum velocity and absolute turn angle of Japanese medaka larvae (*Oryzias latipes*) (Yang et al. 2018). Specifically, the developmental and behavioral disruptions were explained as resulting from a combined impact on oxidative stress, neurotoxicity, and cell apoptosis mecahnisms. Shi et al. (2022) highlithed that synthetic phenolic antioxidants used in plastic manufacturing (butylated hydroxytoluene (BHT), 2.4-di-tert-butylphenol (2,4-DTVP) and 4-tert-octylphenol (4-t-OP)) exhibited compound-specific neurobehavioral effects in zebrafish larvae. The 2,4-DTBP increased locomotor activity while BHT and 4-t-OP decreased it. Moreover, the exposure to 2,4-DTBP and BHT caused anxiety-like behavior and conversely, exposure to 4-t-OP caused anxiolytic-like responses. These behavior disruptions were notably linked to the dysregulation of neurotransmitter-related pathways, highlighting the neurotoxicity of these plastic

additives. In addition to the possibility of studying sublethal effects that cannot be detected with lethal toxicity tests, behavioral alterations observed in laboratory experiments have potential environmental implications. These implications are particularly relevant concerning fish populations and fish interactions with their environment and their predators (Table 1.7).

Table 1.7: Overview of the main studied disrupted behavioral responses in fish, their definitions, organism-level implications and population and trophic-level impacts.

Disrupted behavior	Behavior definition	Organism implications	Population & Trophic implications	References
Basal swimming activity	Distance swum or swimming velocity of the fish without stimuli	Energy over-consumption Foraging or feeding efficiency decrease Reproduction alteration Survival decrease	Population renewal, Sociality, Predator avoidance	(Jacquin et al. 2020) (Brodin et al. 2013)
Rheotaxis	Ability to orientate in a current	Neuromasts alteration Lateral line impairment Disorientation	Mate-finding decrease, Predator avoidance	(Johnson et al. 2007) (Froehlicher et al. 2009)
Phototaxis	Locomotion response to light, causing fish to approach (positive) or move away from (negative) it	Disorientation, Anxiety Foraging or feeding efficiency decrease Survival decrease	Inapropriate habitat selection and use Migration impairment Predator avoidance	(Easter & Nicola 1996) (Schnörr et al. 2012) (Sandoval-Herrera et al. 2019) (Shi et al. 2022)
Thigmotaxis	Alteration of the time spent by a fish close to solid structure versus an open area	Anxiety increase	Sociality Spatial usage patterns Predator avoidance	(Treit & Fundytus 1988) (Champagne et al. 2010) (Schnörr et al. 2012) (Jacquin et al. 2020)
Habituation	Fish behavioral response reduced across time when exposed to a continuous stimulus	Energy over-consumption Foraging or feeding efficiency decrease Survival decrease	Fail to recognize actual dangers, Predator avoidance	(Best et al. 2008) (Roberts et al. 2016) (Colwill 2019)
Vibrational response	Disruption of the fish reaction to a vribrational stimulus	Mauthner cell deficiency Neuromasts alteration Lateral line impairment Foraging or feeding efficiency decrease	Migratory cues undetectable Inapropriate habitat selection and use Population distribution Predator avoidance	(Carlson et al. 1998) (Baker & Montgomery 2001) (Faucher et al. 2006) (Froehlicher et al. 2009)

Concerning fishing gear, Gambardella et al. (2024) exposed *O. latipes* larvae to leachates of ropes and aquaculture equipments sample on beaches of the Adriatic sea (PP, PE, EPDM) and Bay of Biscay (PE, PP). Larvae exposed to leachates of Adriatic sea's FG at 1 g/L caused no significant effects (survival, heart rate, development), while those of the Bay of Biscay caused a significant increase of the heart rate of the larvae. Another study exposed *D. rerio* embryos and larvae to leachates of artificially aged PE bag, twisted trawl net, and floating foam and also to octylphenol polyethoxylates ($OPEO_n$) (Chen et al. 2023). Their findings highlighted that leachates from twisted trawl net significantly impacted embryos heartbeat at the lowest (0.625 g/L) and highest concentrations (10 g/L). Most notably, the leachates from the plastic packaging bags and the foams appeared more toxic than those from twisted trawl nets. Moreover, they found that direct exposure to OPEOn (0.1 to 2.5 mg/L) caused higher effects on survival rates, heart rate and hatching rates than plastic leachates. This last observation

emphasized that the mixture of MPs, NPs, additives and NIAS produced by degradation of larger plastics is a complex medium to be considered as a whole when studying its toxicity (Chen et al. 2023). Therefore, as recommended in this last study, and according to the gaps in knowledge presented in the above sub-sections, further investigations have to be carried out on the toxicity of fishing gear. Notably, focus needs to be made on the MPs, NPs, additives, NIAS and by-products coming from their degradation, as the plastic chemicals seemed to be the main source of plastic toxicity. Moreover, other species and different fishing gear must be studied and compared to get a comprehensive picture of the contribution of fishing gear to plastic pollution of the aquatic environments. With the development of biobased and biodegradable fishing gear intended to substitute existing oil-based gear, it is essential to intensify efforts on evaluating their potential toxicity. This would facilitate the determination of whether the current composition of biobased and biodegradable fishing gear is environmentally safe or if modifications are required to mitigate any potential environmental risks posed by these novel FG.

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1.3 Highlights and conclusions

Highligths of the chapter:

- Plastic fishing nets were introduced in the fishing sector in the 1950s.
- The SE BoB is an area with substantial fishing effort.
- Abandonned and lost or otherwised discarded fishing gear (ALDFG) were found in most of the aquatic compartments of the BoB.
- ALDFG are among the top three causes of mortality for macrofauna in oceans.
- Very little knowledge exists on the contribution of the fishing nets to the MP, NP and plastic chemical pollution of the oceans.

Conclusions:

The state of the art concerning the fate and effects of MPs, NPs, and plastic chemicals resulting from the degradation of FNs is still very limited in the scientific literature. When focusing on the SE BoB, the available knowledge is even more restricted. There are also few detailed historical resources on the transition from FNs made of natural fibers to plastic materials, and subsequently on their evolution. Nevertheless, over the past decade, several studies have been published regarding the transition from oil-based and non-biodegradable FNs to biobased and biodegradable ones. Studies have primarily focused on the synthesis of materials, their efficiency in fishing activities, and their degradation, with an emphasis on mechanical properties. Concerning the degradation of FNs into MPs, NPs, and plastic chemicals, a substantial part of the existing knowledge is based on assumptions derived from observations of the weathering of other plastics. Regarding the ecotoxicity of MPs, NPs, and plastic chemicals, the extent of knowledge is even more limited. Of the few studies that exist, most address the toxicity of gear from aquaculture, ropes, or ALDFG stranded on beaches. Consequently, there is a substantial need for experimental studies to accurately assess the contribution of FNs to MP, NP and plastic chemical pollution of the marine ecosystems and to estimate their environmental risk.

Chapter 2 From sea to shore: estimating fishing gear loss in the Southeastern Bay of Biscay through models and a novel gear identification key

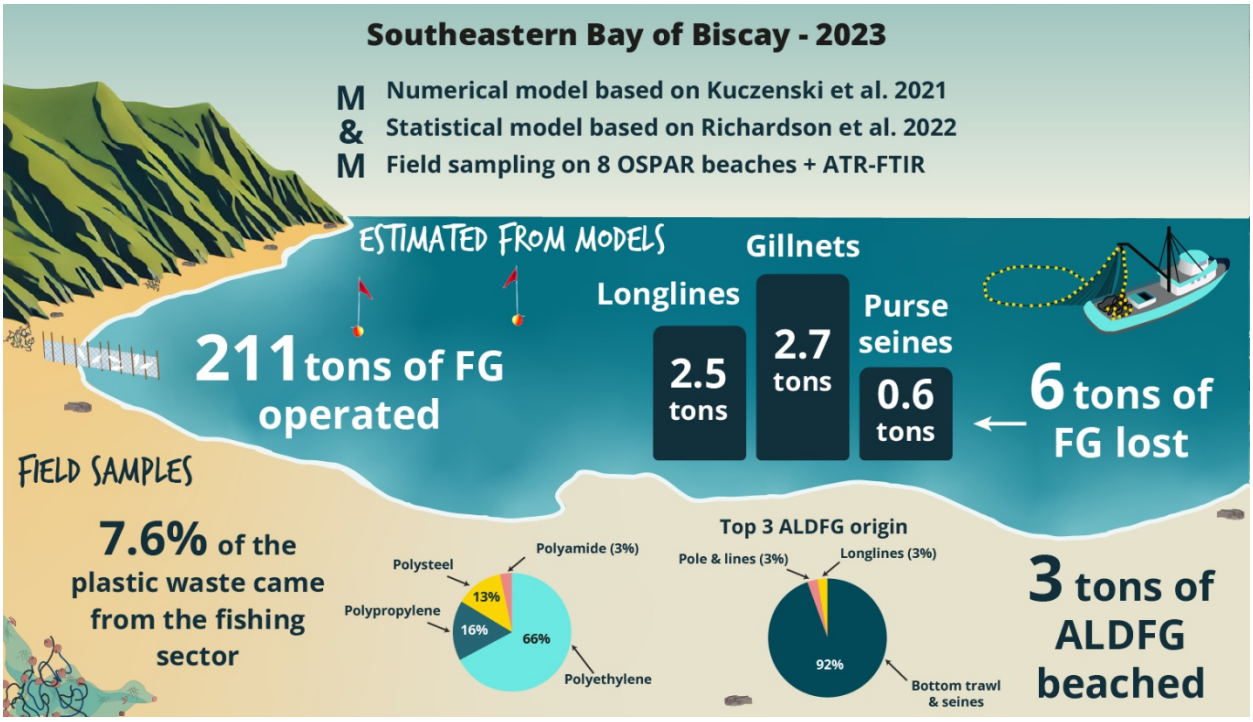
Context:

Abandoned, lost, or otherwise discarded fishing gear (ALDFG) is recognized as a major threat to the marine environment. Their origins are various, but their loss is mainly due to conflicts between fishing *métiers* and difficult meteorological and sea floor conditions. ALDFG is often among the top plastic items found in beach or ocean cleanups. However, detailed categorization of these items is often lacking, which makes it impossible to mitigate their loss according to specific fishing *métiers*. This lack represents a substantial issue, as the nature of ALDFG differs between fishing *métiers* such as trawlers and gillnetters. Consequently, the locations where these nets are lost vary according to differences in buoyancy and transport characteristics. Furthermore, although global estimations of used and lost fishing gear by fishing *métier* exist, data are frequently unavailable at the regional level and for specific fishing fleets. This necessitates labor-intensive investigations and sociological surveys for their acquisition. Therefore, these knowledge insufficiencies represent a substantial gap in evaluating the environmental risks associated with used and lost fishing gear in the SE BoB and other regions around the world.

Objective:

To characterize the efficient fishing activity of the Southeastern Bay of Biscay, to be able to document the main fishing nets used and to determine their polymer composition, and to estimate the quantity of fishing nets used, lost and washed up on selected beaches of the Southeastern Bay of Biscay.

The chapter in one illustration:



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Abstract

Annual plastic pollution from lost fishing gear (FG) ranges from 48.4 Kt to 100 Kt. To estimate the quantity of used and lost FG in the Southeastern Bay of Biscay (SE BoB), we downscaled existing models. To correlate these estimates with field data, we developed an identification key (ID-K) based on marine litter monitoring. The ID-K complements existing plastic litter sorting classifications and makes it possible to identify the FG origins. Eight beaches in the SE BoB were sampled in the 4 seasons of 2023. The ID-K was used to identify the Abandoned, Lost, or Discarded Fishing Gear (ALDFG) collected and their polymer composition was determined by ATR-FTIR. In total, we estimated that 211 tons of FG were used and 6 tons were lost. Gillnets (2.7 tons), and longlines (2.5 tons), were the most lost FG. Of the 14,662 macroplastics collected at all sites and seasons, 7.6% (number of items) were identified as coming from the fishing sector. Of these, 89.8% of the items were mending pieces, 2.6% lines, and 2.5% longlines. In total, we estimated that 3 tons of ALDFG were beached in the SE BoB coastline during the studied period. Regarding polymer composition, polyethylene (PE) accounted for 66.4% of the FG, polypropylene (PP) 15.7%, PE-PP 13.0%, and polyamide 3.3%. Beached ALDFG originated mainly from bottom trawl/bottom seine nets (92.2%). These novel results provided key data that can enable fishing industry stakeholders to take action to reduce pollution from plastic FG, particularly with regard to mending pieces.

2.1 Introduction

The fishing sector is crucial for the economy of many countries and for food supply (FAO 2024c). Following demographic growth that occurred after the 1950s, global fish captures and production surged from 19 million tons in 1950 to 92 million tons in 2022 (FAO 2024b). One significant improvement of the fishing sector to explain this rapid growth was the exponential increase in the use of plastic in fishing gear (FG). This cheaper material brought more resistance and durability to the fishing nets (FNs) and ropes and allowed enhanced fishing efficiency (Sahrhage & Lundbeck 1992; Matsushita et al. 2008; Niaounakis 2017). However, as an unintended consequence, the use of plastic FG produced a significant quantity of mishandled wastes. These wastes are still poorly recycled due to high variability in polymer composition, structure, and degradation rate, as well as the high costs for the collection and recycling processes. Additionally, the lack of waste recovery facilities/sectors led to tons of end-of-life fishing gear (EOL FG) accumulated in sheds and harbors. Therefore, these wastes also fed landfills, open dumps and waste incinerators (Mengo et al. 2023; Su et al. 2023). Further, plastic FG can be abandoned, lost, or otherwise discarded (ALDFG) during their use or their repair and contribute to aquatic plastic pollution (Macfadyen et al. 2009; Galgani et al. 2015; Gilman et al. 2016). These ALDFG can have direct impacts on marine life by ghost fishing mammals, fish, crustaceans etc. and by damaging marine habitats (García-Barón et al. 2019; Lively & Good 2019; Ostle et al. 2019). ALDFG can also contribute to the pollution by microplastics, nanoplastics, and plastic chemicals through their degradation (Wright et al. 2021; Napper et al. 2022; Dusacre et al. 2025).

However, the annual quantity of EOLFG accumulated and ALDFG released into seas and oceans is still under debate, even if the last few years have brought some reasonable estimates. The EOLFG quantities are mainly known at local scales and for a few countries' fishing fleets or territories. In Norway, it was estimated that 2,700 tons of fishing rope wastes entered the category of EOLFG annually (Deshpande et al. 2023), while in Taiwan, approximately 4,271 tons of mixed FG reached the EOLFG status in 2020 (Su et al. 2023). In the case of Spain, Basurko et al. (2023) and Andrés et al. (2022) estimated that approximately 1,643 tons of EOLFG were annually accumulated in mainland ports. Additionally, 900 tons of EOL Spanish tuna purse seine, deployed in the Indian, Atlantic, and Pacific Oceans, are mismanaged annually in various ports worldwide. Nevertheless, to our knowledge, no data exists on a global scale nor at a regional scale, for instance at the Southeastern Bay of Biscay (SE BoB). For the ALDFG, it is quite the opposite, as worldwide estimates are available. Based on a numerical model, Kuczenski et al. (2021a) estimated that between 1.5 Mt and 2.09 Mt of plastic FG are used yearly of which 28.4 to 99.5 Kt are lost in seas and oceans. Data are also available per fishing fleet and fishing *métier*. A worldwide sociological study estimated that 2% of the FG were lost yearly, which represented 2,962 km^2 of gillnet, 75,048 km^2 of purse seine, 217 km^2 of all trawl nets, 755,133 km of mainline and branch longlines and finally 16,198,663 pots and traps (Richardson et al. 2022).

At the local scale, the ALDFG were mainly estimated by sampling and quantification or through life cycle assessment. In sixteen non-exhaustive studies at local scale, the estimated amount of ALDFG at sea surface ranged from 1.9% to 78.5% in terms of the number of items and from 21.2% to 86% in terms of weight (Chambault et al. 2018; Ruiz et al. 2020; Gago et al. 2021; Goodman et al. 2021; Basurko et al. 2022; Lebreton et al. 2022; Royer et al. 2023). In the sea floor, the number of ALDFG items ranged from 16.2% to 67% (Van den Beld et al. 2017; Gerigny et al. 2023). Finally, on different beaches of the globe, fishing-related items represented 3% to 38% of the total waste (Gago et al. 2014; MSFD Technical Group on Marine Litter et al. 2017; Nelms et al. 2020; Lacroix et al. 2022; Muñoz-Pérez et al. 2023). Nevertheless, these sixteen studies used ten different classifications to sort the ALDFG and only three were designed to standardize litter monitoring (OSPAR Commission 2010; Hanke et al. 2013; Schuyler et al. 2020). Results were reported as "fishing items", "FISH", "fishing and aquaculture sectors", "Fishing litter", "maritime-related plastic items" or with more detailed categories. Several units were used (items/m^2, items/100m, number of items, weight of the items). Nevertheless, most data were also presented as proportions of the specific category relative to the total weight or number of items collected. Some focused on particular fisheries (Richardson et al. 2017; Goodman et al. 2021), and others classified the ALDFG based on polymer, color or structure (Corniuk et al. 2023; Vilke et al. 2024).

However, none of these classifications indicated the particular fishing *métier* of the ALDFG. Moreover, due to the lack of standardization, publications refer to identical items with distinct names. Therefore, existing marine litter classifications do not allow identifying specific fisheries, which is crucial for settling ALDFG mitigation policies. In fact, each regional fishery is specific, and requires an adapted management approach. Moreover, each regional fishery can use specific gears at specific fishing periods and contribute to plastic pollution differentially. For example, the recommendations to reduce trawl net losses could not be the same as those

to reduce gillnet losses, and according to the region, some fisheries are to be prioritized over others. For all these reasons, the first aim of our study was to estimate the weight and size of the FG used, lost and EOL in the SE BoB, for each main fishing *métier* (e.g., trawl net, purse seine, gillnet, longline, and pot and trap). The second objective was to study the correlation between lost FG estimations and beached FG. For this purpose, we sampled the anthropogenic litter on eight not cleaned beaches of the SE BoB once per season in 2023. We then quantified and identified ALDFG items and characterized their polymer composition using an universal identification key (ID-K) we developed to quantify and characterize the ALDFG according to the fishing *métier*.

2.2 Material and Methods

2.2.1 Studied area

The monitoring of beach litter and the analysis of efficient fishing activity were conducted within a defined region of 70,030 km^2 and 71,422 km^3 situated in the Southeastern part of the Bay of Biscay (North East Atlantic) (EMODnet Digital Bathymetry 2024). The study area extended from coordinates 46.30°N, 4.00°W (point at top left of the studied area) to 46.30°N, 1.00°W (point at top right of the studied area), and from 43.20°N, 4.00°W (point at bottom left of the studied area) to 43.20°N, 1.00°W (point at bottom right of the studied area) (Figure 2.1). This area belongs to the FAO fishing Area 27 and more specifically to the 27.8.b and part of the 27.8.c (FAO 2025). The total length of the coastline studied (Figure 2.1) was estimated with a resolution of 1 m (McNamara & Da Silva 2022) using the R packages “rnaturalearth”, “terra”, “geodata”, and “sf” (Massicotte et al. 2023; Hijmans et al. 2024, 2025; Pebesma et al. 2024). The method, GeoPackages and rasters are available in supplementary material (Appendix 2.A).

2.2.2 Efficient fishing activity and fishing fleet

The efficient fishing activity (hours of fishing) by fishing fleet according to fishing *métier* within the studied region for the period from November 1, 2022, to October 31, 2023, was obtained from the open-access Global Fishing Watch (GFW) database (GFW 2024). The efficient fishing activity is estimated by GFW thanks to a deep learning algorithm that uses the automatic identification system of the ships (AIS) and the changes in the speed and direction of the vessel (Kroodsma et al. 2018).

Data were grouped by vessel flag and gear, with a daily temporal resolution and a 10th degree geographic resolution. These data represented the fishing efficient activity of boats larger than 15 m and those <15 m equipped with AIS system (European Commission 2021). For confidentiality purposes, details of the fishing vessels (main engine power, auxiliary engine power, overall length, second FG, tonnage) were not included in the public database of GFW. Therefore, the European Union and national fishing registers (MTE France 2019; CIDPMEM 64-40 2021; European Commission 2024; MAPA Spain 2024; DAFM Ireland 2025) were used to complete the GFW database and to add the information of the small-scale professional fishing fleet (boat <15 m without AIS). Finally, the database was carefully checked to ensure

that the list of ships included were fishing in the area and that there were no duplicates or errors in the GFW dataset due to slow speeds (e.g., shipyard).

2.2.3 Used and lost FG estimations

2.2.3.1 Used and lost FG weight estimations using a numerical model

The weight of the different FG used by the fleet active in 2023 in the studied area (Figure 2.1) was estimated by using the numerical model reported by Kuczenski et al. (2021b). This model aimed to evaluate the FG used and lost by each fishing *métier*. Kuczenski et al. (2021a) used it for fishing gear estimations at a global scale. This model is based on four main variables: vessel characteristics (engine power, ship tonnage and overall length), efficient fishing activity (fishing hours), fishing effort (KW/hours fished) and fishery catch and landings (tons of fish). The efficient fishing activity was extracted as explained in Section 2.2. Catch and landings of 2023 were obtained from the Eurostat catches database (European Commission & Eurostat 2024). All these data were introduced in the software released by Kuczenski et al. (2021a). The FG taken onboard and lost were estimated for two active gears, trawls and purse seines, and two passive gears, gillnets and longlines. The software developed by Kuczenski et al. (2021a) with some modifications made to fit with our data are available in the Appendix 2.A.

2.2.3.2 Used and lost FG size estimations using a sociological and statistical method

Based on the global sociological study conducted by Richardson et al. (2022) to estimate the quantity of fishing gear lost at global scale, we estimated the used and lost surface area of trawl nets, purse seines, gillnets (in km²), the length of longlines (in km), and the number of pots and traps in our study area (Figure 2.1). Our methodology comprised several steps:

1) We developed statistical models (linear models or generalized additive models) for each FG, based on the relationship between the size or number of FG used and the fishing effort (Richardson et al. 2022) (detailed statistical models are presented in Appendix 2.B, Table 2.B1).
2) These statistical models were then applied to predict the quantity of FG used, using the known fishing effort of each active fishing vessel in our study area in 2023. The engine power taken into account to calculate the fishing effort was the sum of the main engine and the auxiliary engine power for each active fishing boat in our studied area and period (European Commission 2007).
3) To estimate the size or number of FG lost by each fishing vessel in our study area, we applied the percentages of FG lost by each FG (bottom trawl nets and bottom seines, purse seines, gillnets, and longlines) as reported by Richardson et al. (2022).
4) The quantity of used and lost FG for each fishing vessel was then summed by fishing *métier*. Finally, for each fishing *métier*, we performed 5,000 bootstrap iterations (Davison & Hinkley 1997) to estimate the median and confidence interval of the used and lost FG quantity.

This methodology was applied exclusively to the primary FG, as the ratio of fishing activity between primary and secondary FG for each vessel was unknown. It is important to note

that using the efficient fishing activity variable excluded the fishing vessels without AIS, i.e., boats <15 m (European Commission 2021). To obtain an estimation for the small-scale fishery, we repeated the process using only the engine power in kW as the fishing effort variable in the statistical models to predict the FG used and lost by the small-scale fishing fleet. The two values are reported independently in the results section.

2.2.4 Development of the Identification Key for ALDFG

The identification key (ID-K) was developed to identify the fishing *métier* originating a given sampled ALDFG. The development of the ID-K was made in several steps. First, a beach litter sampling following the NOAA monitoring protocol (2021) was made on 02/05/2022 on a non-cleaned beach on the Southwest French Atlantic coast (45°19'38.8"N, 1°09'34.7"W, Montalivet, New Aquitaine, France). A total of 1146 items were sampled, including 179 fishing-related items. Based on these 179 fishing-related items, we distinguished 23 different categories to classify the ALDFG according to the fishing *métier* they are used for (Figure 2.2). This was achieved with the help of a literature review on the specific aspects and structures of the different FG (Percier 1958; McKenna et al. 2004; Díaz et al. 2006; Weissenberger 2015; ICCAT 2016; He et al. 2021; INdiGO Project 2021; Sauer et al. 2021; FAO 2024a). Afterwards, the 23 categories were checked and refined independently by two fishermen, a gillnetter and a trawler. Using this final classification, the first version of the ID-K was developed to easily and methodically recognize the different ALDFG based on their differences in shape, structure, and texture. During two months, this first version was tested by 16 different citizens with the same ALDFG set. These 16 citizens included students (9), one scientific communication officer (1), and research technicians and engineers (6). Finally, participants were interviewed to ask them about the positive and negative aspects suggestions for improving clarity and accessibility of the ID-K. A last refinement of the ID-K was made during the sorting of 7 beach litter sampled in 2022 in 3 sites of the Arcachon Bay (New Aquitaine, France) and one in the South of Les Landes (New Aquitaine, France) using the OSPAR Marine Litter Monitoring protocol (OSPAR Commission 2010), resulting in 260 fishing-related items. Ultimately, the ID-K was finalized and amended with a guide presenting the ALDFG categories of the ID-K with illustrated descriptions, the methodology to use it, the ID-K, and the classification tables (Appendix 2.A). A bank of images accompanies this guide too.

2.2.5 Beach sampling

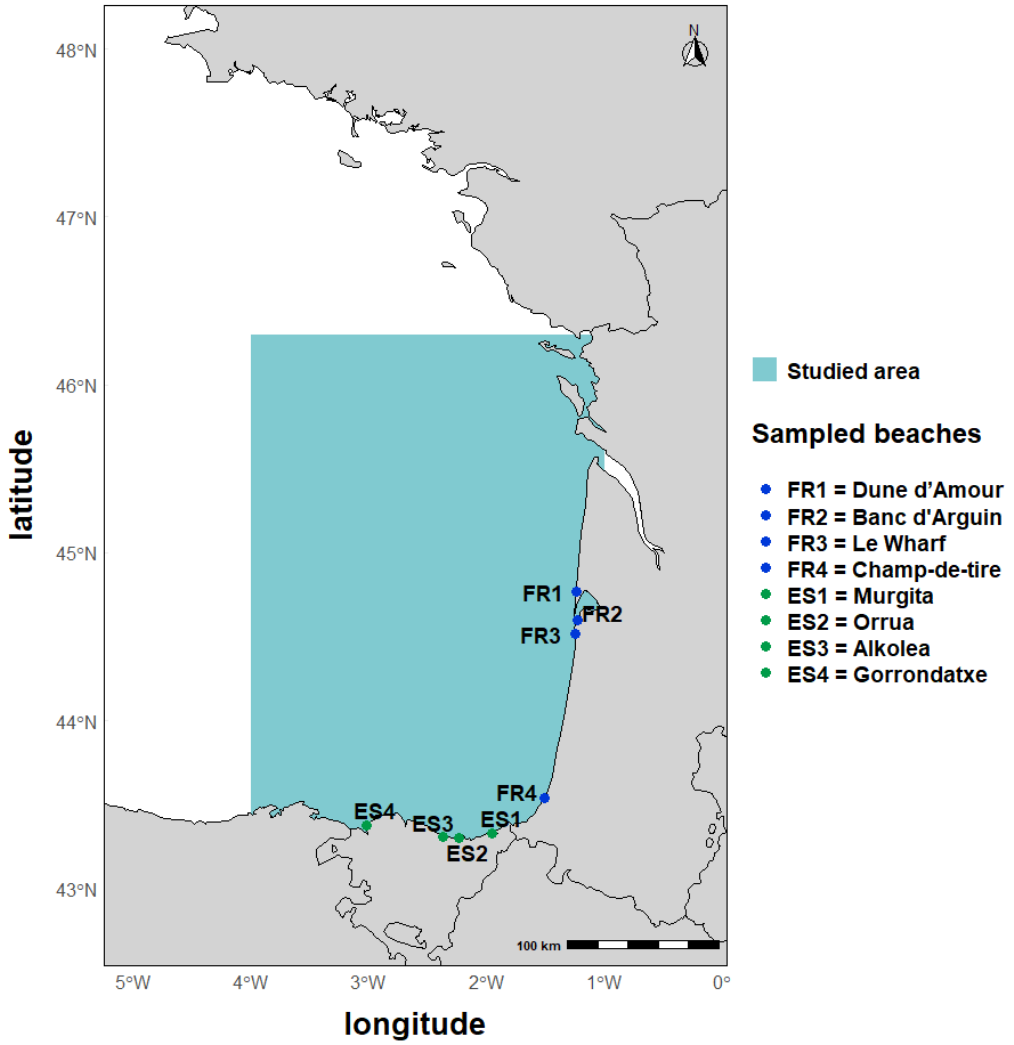

Figure 2.1: Studied area and sampled beaches.
Coordinates of the studied area 46.30°N, 4.00°W to 46.30°N, 1.00°W, and 43.20°N, 4.00°W to 43.20°N, 1.00°W. Coordinates of sampled beaches are detailed in the Appendix 2.B Table 2.B.2.

The ID-K was applied to identify ALDFG in stranded marine litter from eight beaches of the SE BoB between 46° 19' 12.0" N, 1° 15' 36.0" W and 43° 26' 24.0" N, 4° 1' 48.0" W (Figure 2.1). Among them, 4 beaches were located in the region Nouvelle-Aquitaine (France) and 4 in the autonomous community of the Basque country (Spain). These sites are part of the marine litter monitoring program of the Marine Strategy Framework Directive of the OSPAR Convention that is coordinated by "Le CEDRE" (Lacroix et al. 2022). The sites were sampled following the same OSPAR protocol (2010): FR1 (Dune d'Amour), FR2 (Banc d'Arguin), FR3 (Le Wharf) in the Marine Nature Park of the Arcachon Bay, and FR4 (Champ-de-tire), ES1 (Murgita), ES2 (Orrua), ES3 (Alkolea), ES4 (Gorrondatxe) by the NGO Surfrider Europe (Figure 2.1). Details of each beach are described in the Appendix 2.B, Table 2.B.2. The samples of the winter season were collected between 1 and 25 January (precise dates available in Appendix 2.B, Table 2.B.2). Those of the spring season between 4 and 27 April, those of summer season between 1 and 27 July and those of autumn season between 10 and 28 October. Unfortunately, the ES1 beach could not be sampled in autumn due to poor weather conditions during the sampling period. Beaches were sampled every three months and they were not

cleaned between the samplings, therefore the samples represented the accumulated litter of the past three months. Beach transects of 100 meters in length were sampled, with the width extending from the upper boundary of the shore (base of a dune, cliff, or man-made barrier), to the water's edge. Since beach widths were not measured during the samplings, they were estimated using satellite images from the software Google Earth pro (version 7.3.6.10201). For each available satellite image, the distance between the beach slope and the water edge was measured, and the image time was registered. Finally, tide times and tide ranges, as well as the date and time of the samplings were used to estimate the beach width using linear regressions. The mean percentage coverage of each beach at high tide was also determined. The method is described in the Appendix 2.A.

2.2.6 Quantification and characterization of beached ALDFG

As a first step, the marine litter collected was classified using the survey form of the OSPAR marine litter monitoring (OSPAR Commission 2010). This form comprises 116 subcategories divided into 12 categories, plus pellets (nurdles). Finally, 12 subcategories of litter related to FG (Crates; Crab/lobster pots; Octopus pots; String and cord, diameter >1 cm; String and cord, diameter <1 cm; Tangled nets, cord, rope and string; Fish boxes; Fishing line, angling; Floats/Buoys; Lobster and fish tags; Nets and pieces of net <50 cm; Nets and pieces of net >50 cm) were selected and sorted using the ID-K (Figure 2.2) into 23 FG categories (Appendix 2.A). The fishing-related items of each category were quantified both as number of items and weight (in grams) (OSPAR Commission 2010; Lacroix et al. 2022) and also as number of items per square meter. The polymer of each item was identified through Attenuated-Total-Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy (Nicolet spectrometer, Nexus 870 with MIRacle diamond crystal). One ATR-FTIR spectrum was acquired (OMNIC software V9.2.98, Thermo Fisher) for each item on a tiny, clean, and minimally degraded part with a spectral range of 400 to 4,000 cm^{-1}, a spectral resolution of 4 cm^{-1}, an acquisition of 20 scans and an automatic baseline correction. To reduce the analysis time, a random subsampling of categories was carried out based on the subsampling method of Lefebvre et al. (2023). In total, 1,165 fishing-related items were analyzed by ATR-FTIR spectroscopy, which represented 48% of the total number of collected ALDFG items, a much higher percentage than the minimal 10% recommended by the guide to the ”Monitoring of Marine Litter in European Seas” (Hanke et al. 2013). The color of each item was also registered. In case an item had various colors, materials, or parts, one piece of each color and material was cut and analyzed separately.

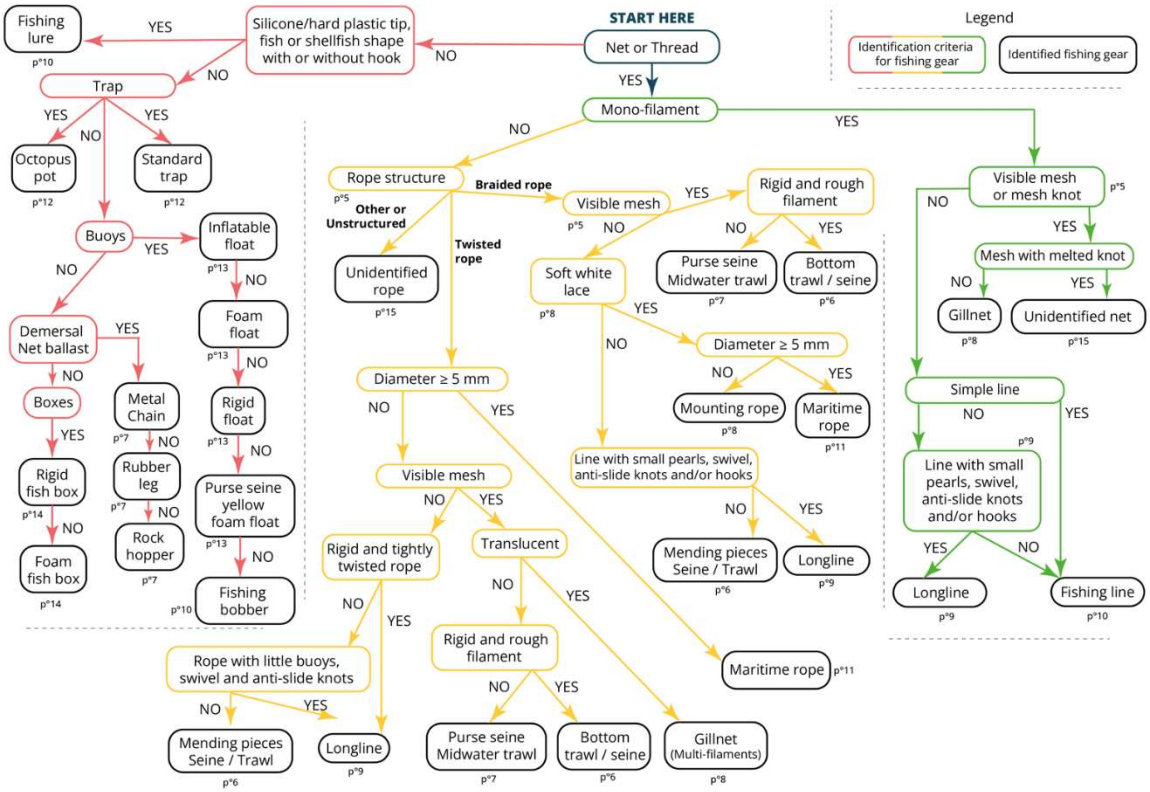

Figure 2.2: Identification key of the fishing gear lost in the ocean and washed up in beaches.
Black boxes represent the 23 categories of FG. The colored boxes indicate the identification criteria to identify the final FG. The page numbers below some boxes (p°x) refer to the page number of the document named "Guide to identify fishing gear lost in the ocean and washed up in beaches", which guides and assists users of the identification key (Appendix 2.A).

To investigate the relationships between the fishing effort, the quantity of FG used, the quantity of FG lost by the fishing fleet studied and the beached ALDFG beached that we sampled, we conducted a series of correlation analyses using R version 4.3.1 and Rstudio RStudio version 2024.04.0+735. To facilitate comparison across different scales and units, all variables were standardized by subtracting the mean from each value and then dividing by the standard deviation, prior to correlation analysis. Due to the non-normal distribution of the data, the small amount of the data (n=16) and the presence of ties, we used the non-parametric correlation test of Spearman for data comparisons between conditions. We examined the associations between:

1) The quantity of beached plastics and beached ALDFG on the coastline, considering all seasons and sites;
2) The weight of ALDFG and their abundance in number, considering all seasons and sites;
3) The sum of fishing effort and the sum of ALDFG beached on the coastline across four different FG categories (Bottom Trawl nets and bottom seines, purse seines, gillnets and longlines) across seasons;
4) The sum of FG used by the fishing fleet studied and the sum of ALDFG beached on the coastline for the four different FG categories across seasons;
5) The sum of lost FG by the fishing fleet studied and the sum of ALDFG beached on the coastline for the four different FG categories across seasons. For this fifth correlation, we additionally conducted Kendall's rank correlation test to validate the results. This

test was chosen as it provides a different approach to handling ties in the data compared to Spearman's method.

2.3 Results

2.3.1 Efficient fishing activity

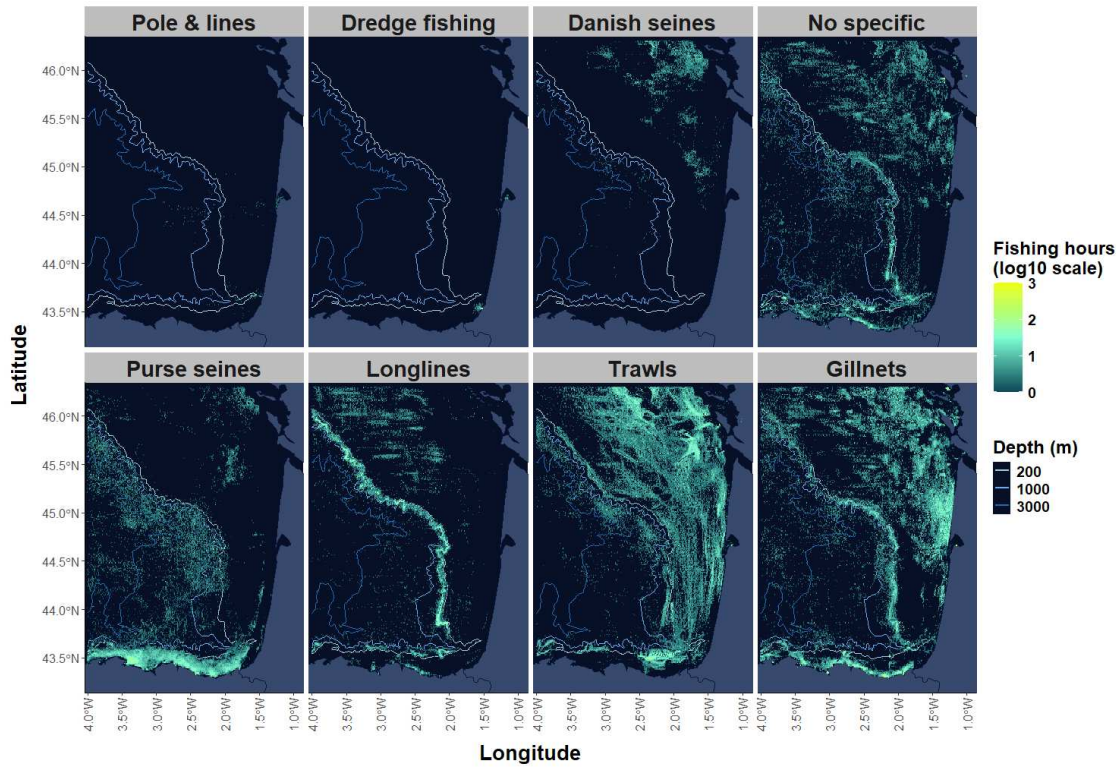

Figure 2.3: Efficient fishing activity for each fishing gear used in 2023.
FG used in hours are sorted in ascending order from top left to bottom right. Fishing gear categories are those of GFW (2024). Sub-categories grouping inside are explained in SM (Appendix 2.B, Table 2.B3).

The fishing fleet used in the SE BoB in 2023 consisted of 1,041 vessels (670 vessels equipped with AIS (64%) and 371 without it (36%)). Thirteen different fisheries using distinct primary FG were referenced. Set gillnets accounted for 35.4% of the vessels (20.1% of the total engine power (kW)), bottom trawls for 18.4% (26.4% of the tot. kW), purse seines for 15.8% (24.9% of the tot. kW), set longlines for 15.7% (17.5% of the tot. kW), pole and lines for 3.9% (2.30% of the tot. kW), and the other seven for 10.9% (dredges, lift nets, midwater trawls, no specific, pots and traps, Danish seines, drifting longlines, drifting gillnets) (8.79% of the tot. KW). Six countries were represented. France represented 50.8% of the fleet (40.8% of the kW), Spain 46.8% (52.7% of the kW) and Belgium, Ireland, United Kingdom and Portugal represented 2.4% (6.5% of the kW).

In terms of apparent efficient fishing activity, the vessels equipped with AIS (670) accounted for 401,839 hours of fishing in the study area. Gillnets were the preponderant used gears (119,484 fished hours, 29.7%) followed by the trawl nets (113,639 fished hours, 28.3%), the longlines (69,143 fished hours, 17.2%) and the purse seines (57,027 fished hours, 14.2%) (Figure 2.3). Purse seines were used at 99.9% by the Spanish fleet and were therefore mainly

used on the North continental shelf of the coast of the Basque Country (Spain). Similarly, 79.9% of longlines were used by the Spanish fleet. However, this gear was used mainly in the upwelling zones along the continental slope between 200 and 1,000 m of depth (Figure 2.3). In contrast, French fishing fleet mainly used gillnets (67.7%), trawls (60.8%), Danish seines (100.0%) and pole and lines (96.3%). These gears were used mostly in the continental shelf with extensive geographical coverage (Figure 2.3).

2.3.2 Used and lost fishing gear

By combining the sociological study with the numerical model, it was possible to estimate the volume of plastic fishing gear in terms of gear size, number of items, and weight in 2023. For the AIS-equipped fleet and the median estimate (tau = 0.5), gillnets were the FG used the most in weight with 92 tons (95% CI: 10-656) of plastic gear (6.8 km^2, 95% CI: 5.7-7.8)) (Table 2.1 and Table 2.2). The loss of these gillnets represented 2.97% in weight during the annual fishing activity which represented 2.75 tons (95% CI: 0.2-4) (Table 2.1). Expressed in surface, the lost percentage was lower with 0.81% of gillnets lost (Table 2.1 and Table 2.2). Longlines were the second most used and lost FG with 64 tons (95% CI: 29-141) of longlines used and 2.5 tons (95% CI: 0.8-6.27) lost (Table 2.2). In length, 4,265 km (95% CI: 3,655-4,933) of longlines (sum of mainlines and branchlines) were used, and a similar percentage was lost (3.45%) (Table 2.1). Although the trawl was the gear used by the second fishing fleet in efficient fishing activity, trawl nets accounted for the lowest used weight with 11 tons (95% CI: 7-16) of plastic gear and 0.42 km^2 (95% CI: 0.38-0.46) (Table 2.1 and Table 2.2). In 2023, a total mass of 211 tons of plastic gear was used and 3% were lost (6.3 tons) by the four largest fisheries in the SE BoB (Table 2.2). The total surface area and length of used FG were 14.12 km^2 and 4,265 km, from which 0.17 km^2 (1.20%) and 146 km (3.42%) were lost (Table 2.1).

Estimates of FG size and number of items of the small-scale fisheries (vessels not equipped with AIS) showed a non-negligible volume of plastic FG used, especially for gillnets (2.5 km^2 (95% CI: 2.1-3.0)) and longlines (7,536 km (95% CI: 1,094-20,307)). Regarding main polymers of FG (Appendix 2.B, Table 2.B.3), polyamide represented 97.5% of the used gear weight (200 tons). In contrast, polyethylene or co-polyethylene-polypropylene (PE-PP) accounted for only 2.5% of the weight (11 tons).

The results showed that both models gave similar percentages of FG loss (Table 2.1 and Table 2.2). Although similar surface area was used in gillnets (6.8 km^2) and purse seines (6.9 km^2), the loss of the purse seines was 1.9 times greater than that of the gillnets.

Table 2.1: Gear operations and losses in surface area and length by fishing gear for the fishing fleet operating in the Southeastern Bay of Biscay in 2023.
The presence of AIS (Automatic Identification System) indicates the ability to measure fishing activity in hours for the equipped vessels. CI means "Confidence interval". Based on Richardson et al. (2022).

					Gear operation/year			Gear lost/year		
Fishing gear	Units	Vessel number	AIS	Average percent lost	Median	CI 0.05	CI 0.95	Median	CI 0.05	CI 0.95
Gillnets	m^2	219	Yes	0.81%	6,768,415	5,725,633	7,809,455	54,824	46,378	63,257
		167	No		2,539,790	2,136,183	2,964,327	20,572	17,303	24,011
Purse seines	m^2	160	Yes	1.51%	6,923,671	6,664,235	7,188,507	104,547	100,630	108,546
		4	No		160,507	160,123	160,941	2,424	2,418	2,430
Trawls	m^2	174	Yes	3.57%	417,791	376,167	459,544	14,915	13,429	16,406
		62	No		17,812	16,079	19,918	636	574	711
Longlines: mainlines	m	104	Yes	3.33%	2,683,536	2,392,496	2,992,071	89,362	79,670	99,636
		70	No		860,070	770,802	944,584	28,640	25,668	31,455
Longlines: branchlines	m	104	Yes	3.58%	1,582,233	1,263,007	1,941,421	56,644	45,216	69,503
		70	No		6,675,662	322,999	19,363,028	238,989	11,563	693,196
Pots & Traps	Items	25	No	0.74%	3,447	2,586	4,550	26	19	34

Table 2.2: Gear operations and losses in weight by fishing gear for fishing fleet equipped with AIS and operating in the Southeastern Bay of Biscay in 2023.
The tau values (0.5, 0.7, and 0.9) are parameters used in quantile regression models to adjust the sensitivity of the analysis to different parts of the data distribution. NA means that the model was not based on quantile regression using tau parameter. CI means "Confidence interval". Based on Kuczenski et al. (2021a).

			Gear operation/year (kg/year)			Gear lost/year (kg/year)		
Fishing gear	tau	Median percent lost	Median	CI 0.05	CI 0.95	Median	CI 0.05	CI 0.95
Gillnets	NA	2.97%	$9.27x10^4$	$1.03x10^4$	$6.56x10^5$	$2.75x10^3$	216	$4.06x10^4$
Purse seines	0.5	1.48%	$4.41x10^4$	$8.78x10^3$	$1.45x10^5$	651	94.6	$5.42x10^3$
	0.7	1.47%	$1.90x10^5$	$3.69x10^4$	$5.85x10^5$	$2.79x10^3$	408	$2.13x10^4$
	0.9	1.42%	$8.97x10^5$	$1.82x10^5$	$2.64x10^6$	$1.27x10^4$	$2.06x10^3$	$9.41x10^4$
Trawls	0.5	3.30%	$1.08x10^4$	$7.15x10^3$	$1.62x10^4$	356	221	582
	0.7	3.28%	$3.26x10^4$	$2.09x10^4$	$5.11x10^5$	$1.07x10^3$	641	$1.79x10^3$
	0.9	3.25%	$1.84x10^5$	$8.52x10^4$	$4.19x10^5$	$5.98x10^3$	$2.70x10^3$	$1.42x10^4$
Longlines	0.5	3.92%	$6.35x10^4$	$2.94x10^4$	$1.41x10^5$	$2.49x10^3$	840	$6.27x10^3$
	0.7	4.03%	$1.30x10^5$	$5.63x10^4$	$3.10x10^5$	$5.24x10^3$	$1.7x10^3$	$1.39x10^4$
	0.9	3.96%	$4.45x10^5$	$2.07x10^5$	$9.86x10^5$	$1.76x10^4$	$6.13x10^3$	$4.45x10^4$

2.3.3 Quantification and characterization of beached ALDFG according to seasons

The OSPAR monitoring carried out in the eight beaches at four seasons enabled the sampling of 16,563 items, from which 96% (15,907) were plastic items. On average, by site and season, a median of 200 plastic items/100 m (95% CI: 6-1,880) or a number of $6.09x10^{-2}$±$6.34x10^{-2}$ (SD) plastic items/m^2, were found. From these plastic wastes, the maritime-related items of the OSPAR classification accounted for 15.1% of the plastic litter sampled (17 FG/100 m (95% CI: 0-439) or $8.86x10^{-3}$±$1.26x10^{-2}$ (SD) FG/m^2). Using the novel ID-K developed, we focused on fishing gear. Thus, unidentified nets and ropes not originating from the fishing or maritime sector were excluded and therefore the maritime-related items represented 9.3% of the plastic litter (12 FG/100 m (95% CI: 0-231) or $5.74x10^{-3}$±$7.18x10^{-3}$ (SD) FG/m^2). Finally, excluding the maritime ropes which cannot be 100% attributed to the fishing industry, the FG items coming from the fishing sector represented 7.6% of the plastic litter (10 FG/100 m (95% CI: 0-196) or $4.68x10^{-3}$±$6.1x10^{-3}$ (SD) FG/m^2). Comparing by seasons, in 2023, there was a positive and significant correlation between the quantity of beached plastics and ALDFG (ρ = 0.87, $p < 0.001$, n = 31). In addition, the ID-K made it possible to identify the fishery in which the ALDFG were used. In the eight beaches sampled, 14 categories of ALDFG and two categories of unidentified items were found among the 23 categories of the ID-K (Figure 2.4).

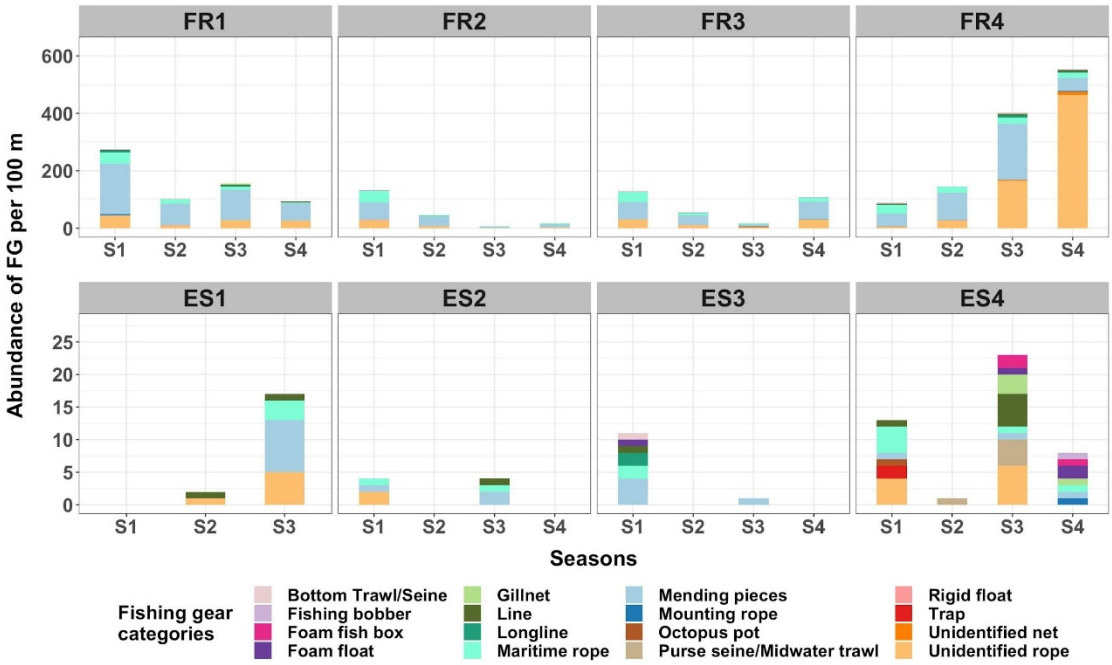

Figure 2.4: Total amount of fishing gear items collected per 100 m beach and season.
S1 = winter 2022-2023; S2 = spring 2023; S3 = summer 2023; S4 = autumn 2023. ES1 S4 was not sampled due to bad weather conditions. Categories according to the identification key (ID-K).

Overall, the "mending pieces" was the most abundant category with 45.1% of the ALDFG (1,086 items). It was followed by the "unidentified rope" representing 37.6% of the ALDFG (906 items) and the "maritime rope" 11.5% of the ALDFG (276 items). In weight, "maritime ropes" were the most abundant (58.0% of the ALDFG, 7.8 kg) and the "mending pieces" were

the second most abundant (9.50% of the ALDFG, 1.3 kg). The unidentified ropes accounted for 6.5% of the ALDFG in weight, the foam float 6.4% of the ALDFG and the bottom Trawl/Seine 6.4% of the ALDFG. Focusing only on fishing related items ("unidentified ropes", "unidentified nets" and "maritime ropes" excluded) the "mending pieces" represented 89.8% of the ALDFG in number of items, the "lines" 2.6% and the "longlines" 2.5%. In the studied period, the four French beaches accumulated 86.4% of the total ALDFG weight collected in the study area (96.5% of the abundance in items/100 m) with a mean of 0.7±0.9 (SD) kg/100 m (145±148 (SD) items/100 m). As for the beaches of the Basque Country (Spain) 0.1±0.2 kg/100 m; (5.6±7.3 items/100 m) of the ALDFG were collected. Since there was a positive correlation between the mass of gears and their abundance in numbers ($\rho = 0.80$, $p < 0.001$, $n = 31$), the rest of the analyses were based on the mass values. Comparisons between seasons and between sites showed a wide heterogeneity of beached ALDFG. The "Banc d'Arguin" (FR2) (Figure 2.5) accumulated 34.2% in weight of the beached ALDFG during the year, followed by the "Dune d'Amour" (FR1) (26.4%), "Le Wharf" (FR3) (16.8%) and "Gorrondatxe" (ES4) (10.5%).

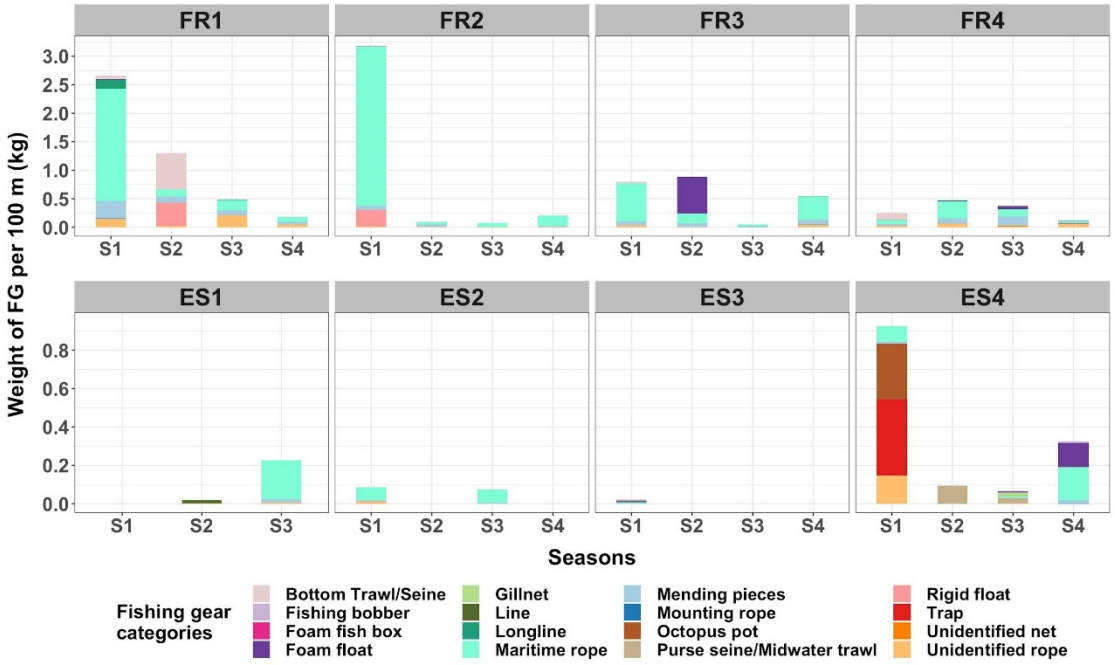

Figure 2.5: Total amount of fishing gear in weight collected per 100 m beach and season.
S1 = winter 2022-2023; S2 = spring 2023; S3 = summer 2023; S4 = autumn 2023. ES1 S4 was not sampled due to bad weather conditions. Categories according to the identification key (ID-K).

Across all sites, no correlation was found between season and gear accumulation ($\rho = -0.21$, $p = 0,25$, $n = 31$). Upon examining the hydrodynamics of the different beaches, the width estimation of the sampled beaches of the Basque country (Spain) had a higher beach coverage at high tide (58.7±20.3 (SD) % of beach coverage) than the French beaches (31.5±15.7 (SD) % of the beach coverage). When grouping the beached ALDFG by major fishing *métier* categories (Appendix 2.B, Table 2.B.3), the bottom trawl and bottom seine nets accounted for 66.6% of the weight of the beached ALDFG (92% in number of ALDFG), the pots and traps 21.4% (0.3% in number of items), the longlines 6% (2.5% in number of items), the purse seines 3.8% (0.4% in number of items), the pole and lines 1.1% (2.8% in number of items) and the gillnets 1.1%

(1.8% in number of items). The total mass of ALDFG washed up in the Southeastern coastline of the Bay of Biscay could be extrapolated from the ALDFG average (0.4 kg/100 m, 95% CI: 0-1.83, excluding the unidentified items) and the coastline linear size (747 Km). Therefore, in 2023, it can be estimated that 3,036 kg of ALDFG (95% CI: 1,662-5,798 kg) were beached on the shore of the studied area (Figure 2.1).

Concerning the color of the ALDFG, 49.2% of the ALDFG were green, 12.2% were blue and 11.3% were white (Figure 2.6). 83.1% of the ALDFG had only one color, while 14.2% had two and 2.7% had three colors.

Most of the items were made of polyethylene (PE 66.4%), followed by 15.7% made of polypropylene (PP), and 13.0% made of PE-PP (Figure 2.7). Polyamide (PA), accounted for only 3.4% which includes 100% of the gillnets found. The mending pieces and maritime ropes accounted for 67.1% and 12.8% of the PE, respectively. The unidentified ropes, although predominantly made of PE, PP, and PE-PP, also contained PA, polyethylene terephthalate (PET), and cellulose. 97.6% of the ALDFG were composed of a single polymer, while the remaining items were made of two polymers.

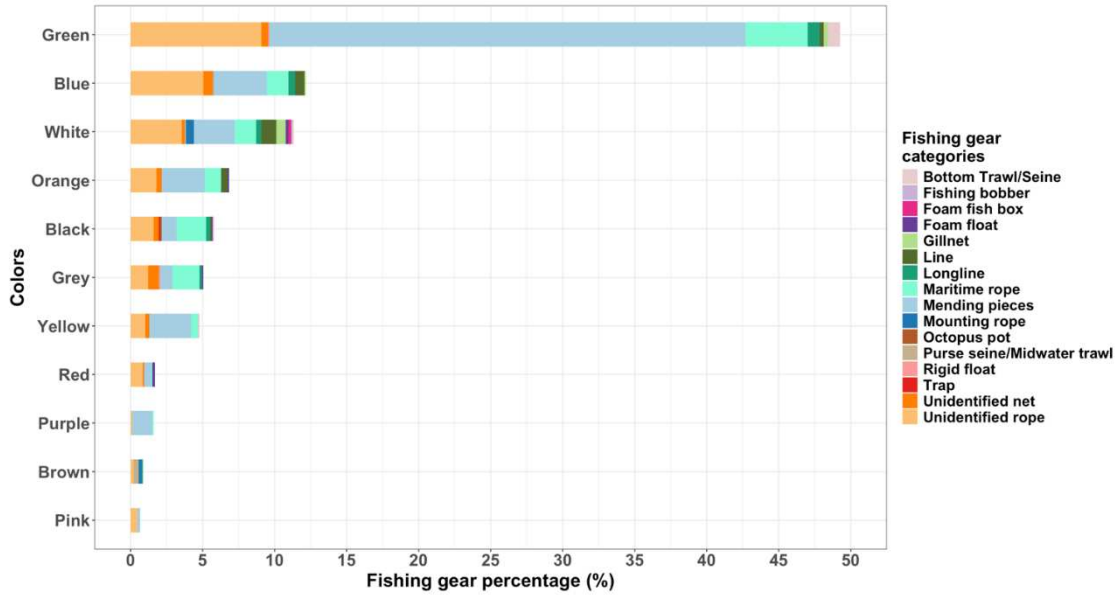

Figure 2.6: Color (%) of the different fishing gear items collected in this study.
Categories according to the identification key (ID-K).

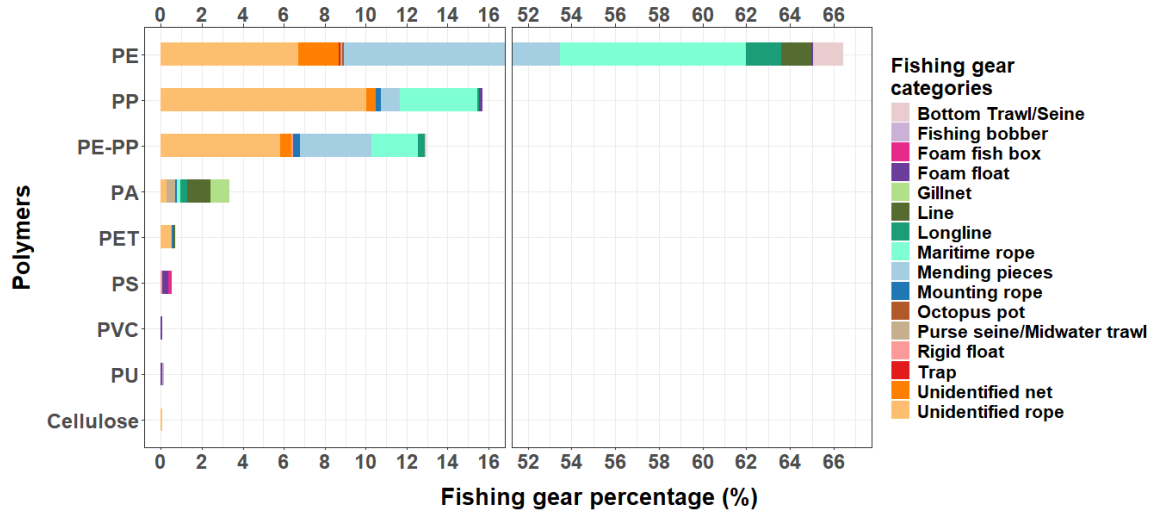

Figure 2.7: Polymer composition (%) of the different fishing gear items collected in this study.
The plot is cut between 17% and 51% to show the polymers of categories of the identification key (ID-K) with few items. PE = polyethylene; PP = polypropylene; PE-PP = polyethylene-polypropylene; PA = polyamide; PET = polyethylene terephthalate; PS = polystyrene; PVC = polyvinyl chloride; PU = polyurethane.

2.3.4 Correlations between beached ALDFG, efficient fishing activity and lost FG

Grouping beached ALDFG by major fishing *métier* (Appendix 2.B, Table 2.B.3) allowed the study of the relationship between the volume of ALDFG beached seasonally and the efficient fishing activity (fished hours) by season. In terms of mass or number of ALDFG, there was no significant correlation between these variables when all the fishing *métier* were considered together ($p > 0.05$, $n = 20$). Secondly, when considering all sites and FG together, we found no correlation between the seasonal number of beached ALDFG items and the estimated surface area and length of FG used seasonally. However, we found a significant and negative correlation between the quantity of ALDFG in number of items beached seasonally and the estimated surface area of FG (Spearman's: $\rho = -0.71$, p-value < 0.01, $n = 16$) and length of FG (Kendall's: $\tau = -0.52$, p-value < 0.01, $n = 16$) lost in the SE BoB.

2.4 Discussion

In 2022, the fishing and aquaculture sector generated USD 195 billion, directly employed 62 million people, and approximately 550 million were partially dependent on it for their livelihoods (FAO 2022, 2024c). Aquatic food provided 17% of animal proteins and provides 20% of the average per capita animal proteins intake for 3.3 billion people in 2019. This makes fishing and aquaculture an essential activity for humankind. However, its contribution to pollution by microplastics, nanoplastics, and additives (Montarsolo et al. 2018; Napper et al. 2022; Chen et al. 2023; Dusacre et al. 2025), along with its involvement in habitat destruction and depletion of marine biodiversity, makes it a significant threat to the marine environment (Pauly et al. 2005; Macfadyen et al. 2009; Pitcher & Cheung 2013; Boonstra & Österblom 2014). The primary challenges in implementing mitigation measures to address these impacts

lie on the diversity of impacts across the different fishing *métier* and on the limited knowledge of the quantities of FG used and lost on a local scale. Therefore, this study contributed to fill this knowledge gap by estimating the amount of FG used and lost by the different fishing *métier* in the SE BoB.

Using GFW (2024), it was possible to estimate that in 2023, 163,140 fishing boats equipped with AIS fished for 130,000,000 hours at the global scale. In comparison, the SE BoB fishing fleet equipped with AIS represented 0.3% of this effort (401,839 h). Comparing the last worldwide estimate of FG used in 2018 (2.1 Mt, tau = 0.9) (Kuczenski et al. 2021a) and the data of our study, the SE BoB fishery represented 0.1% of the gear used worldwide. In addition, our estimate of the total quantity of lost FG in the SE BoB represented 0.07% of the quantity of FG lost worldwide. However, the efficient fishing activity in 2018 was approximately 50% of the one recorded during the period analyzed in this study. Consequently, only trend comparisons could be considered, with the understanding that absolute values may not be directly comparable due to this substantial difference in efficient fishing activity. At the European scale, it was estimated that 25,710 tons of FNs were purchased in the European Union (Viool et al. 2018). Therefore, based on our estimations, the FG quantities used in the Bay of Biscay in 2023 accounted for 0.8% (tau = 0.5) to 6.3% (tau = 0.9) of this amount. These proportions seemed a reasonable estimate, given that the studied area represented 4.2% of the efficient fishing activity of the European Union fishing fleet between November 2002 and November 2023 (GFW 2024). Comparing the loss rate at the European scale was more challenging because studies are still scarce and the units used (Rijkure et al. 2024) were often not the ones recommended by Richardson et al. (2017, 2019, 2022) or Kuczenski et al. (2021a). However, for comparable fisheries (similar species and fishing effort), our loss rates for longlines (3.3% - 4.0%) were consistent with those estimated for Norway or Croatia longline fishing fleets, with a probability of loss of 4.7% (3.8 - 5.5) in Norwegian waters and 3.0% (1.0-5.9) in Croatian waters (Cerbule et al. 2022, 2023). Nonetheless, in Turkey one study reported a longline loss of 79.2%, which was 20 times higher than our estimate (Ayaz et al. 2010). The authors explained this high longline loss rate by the presence of a small-scale fishing fleet fishing on reef sea floor, which increased the snagging of longlines (35% of the longline loss reason) compared to sandy sea floor. Moreover, they estimated that 41% of this longline loss rate was due to vandalism and fishing conflict. Concerning gillnets, Dagtekin et al. (2018) documented a 1.5% loss rate for gillnets, while Ayaz et al. (2010) reported a 0.8% loss rate for gillnets and a 3.4% loss rate for trammel nets in Turkey's marine waters. These loss rates were comparable to our findings for gillnets, which ranged from 0.8% to 3%. In 2004, the European Fantared Partnership (MacMullen et al. 2004) estimated slightly lower lost rates in Norway with a loss of 0.1% to 0.6% considering all FNs together. This last study also highlighted the possible reduction of the final losses thanks to the recovery of the FG after their loss by fishermen. The recovery rates estimated for their different studied sites went from 4.6% to 47%, considering all FG. The recovery rate of FG was not included in our estimations because this variable was not included in the models used and because there was no data available within our studied area.

The difference between the used and lost quantity of FG estimated in this work made it possible to look at the amount of FG that reached their end-of-life by the end of 2023. Relying

on oral communication from the local fishermen and on the lifespan estimates of gillnets and longlines (Appendix 2.B, Table 2.B.3), we estimated that these FG are renewed every fishing season, i.e., after 8 to 10 months of use. Therefore, for gillnets and longlines, 90 tons (95% CI: 10-615) and 61 tons (95% CI: 29-135) respectively, were brought back to the ports and therefore ended up as end-of-life FG. Considering the 5 main ports of the Basque Country and Cantabria coastline (Spain) (Hondarribia, Pasaia, Bermeo, Ondarroa, Santander), Basurko et al. (2023) estimated, through a sociological survey, the quantity of annual end-of-life gillnets to be 37.5 tons/year, which was inside the confidence interval of our work. The similarity of these estimates validated our method and could enable stakeholders in the fishing sector to use it for other geographical areas.

Concerning the direct impact of ALDFG, several studies highlighted a positive correlation between lost FG, in net surface area or length, and ghost fishing and habitat destruction rates (Erzini et al. 1997; Lively & Good 2019; Thomas et al. 2019; Perroca et al. 2024). In our case, we estimated an annual loss of 0.17 km^2 of FN and 146 km of longline within an area of 81,836 km^2. Considering that our studied area contained 20 marine protected areas (Alloncle et al. 2019; Ruiz et al. 2022) with biodiversity hotspots and vulnerable habitats (Borja et al. 2019), our loss estimates of FG represented a non-negligible threat for marine life and habitats (Van den Beld et al. 2017; Gerigny et al. 2023). Indeed, the regions surrounding the Cantabrian and Aquitaine slopes have been recognized as fishing hotspots. The issue, however, lies on the fact that these regions are also acknowledged as containing vulnerable marine ecosystems, which include habitats of cold-water corals, along with spawning, feeding, and nursery areas for various species of fish and invertebrates (Van Rooij et al. 2010; Davies et al. 2017; Van den Beld et al. 2020). In addition, the Capbreton Canyon, a well-known cetacean hotspot, is also a recognized fishing hotspot. Moreover, an important fishing activity was observed on some of the estuaries of the Basque Country and their coastal waters (first nautical miles), where macroalgae habitats of high biological value were identified (Pascual et al. 2011). Furthermore, through degradation of key habitats for fish and invertebrate populations, the FG lost during fishing activity could contribute to the decrease of marine ecosystem health (Costello et al. 2005; Roberts et al. 2006; Williams et al. 2010) and therefore could impact the fish stock and the fishing yield in the SE BoB (Macfadyen et al. 2009; FAO 2022; ICES 2022). However, we cannot assume that all the FG lost stayed in the SE BoB. Indeed, as with other macroplastics in the ocean, a part of the lost FG can be transported by currents outside the studied area (Ruiz et al. 2020). The SE BoB was reported as an accumulation area for marine litter (Galgani et al. 1995; Gago et al. 2015; Pereiro et al. 2019) and Ruiz et al. (2022) estimated that approximately 20% of the FG lost in the Bay of Biscay escape from this area, especially in winter and spring. Weaker currents and winds in summer tend to cause a higher beaching of floating plastic litter in the Basque Country (Spain), New-Aquitaine (France) and Brittany (France) coastlines (Mendoza et al. 2020; Ruiz et al. 2022).

It was estimated that fishing-related items represented 3 to 15% of the plastic waste stranded on European beaches (MSFD Technical Group on Marine Litter et al. 2017). On the OSPAR beaches sampled between 2018 and 2020, the “maritime-related plastic items” had a median of 36 items/100 m in global. For the “Bay of Biscay and Iberian Coast” the median concentration was 51 items/100 m and 19% of the plastic litter in the Spanish shores was also

"maritime-related plastic items" (Lacroix et al. 2022). In our studied area in 2023, using the novel ID-K, we estimated that the abundance of ALDFG (maritime ropes and FG) represented 9.3% of the plastic wastes, which was within the range of Addamo et al. (2017). However, we found that our estimation was 4.25 times lower (12 items/100 m vs. 51 items/100 m) than the estimation of Lacroix et al. (2022). This difference can be explained by the fact that the work of Lacroix et al. (2022) was not designed to focus specifically on FG and therefore did not distinguish "maritime-related plastic items" from the FG. Moreover, ropes and gear from marine farms and from terrestrial origins were often not clearly distinguished and were thus included in this category. Therefore, they included "unidentified ropes" and "unidentified nets" in their estimates, resulting in an overestimation when compared with our data.

The use of the ID-K to identify the origin of the ALDFG highlighted that different fishing *métier* do not contribute at the same level to the stranded beach plastic litter pollution. Bottom trawl and bottom seines represented 66.9% of the weight and 93% in number of items of beached FG. According to our results, the amount of bottom trawl and seine items on beaches was not proportional to the fishing effort, as we found no significant correlation between these two variables. Conversely, our analysis revealed a significant negative correlation between the use of bottom trawl and seine and the estimated quantity of lost FG. This finding suggested that an increase in the loss of these FG did not necessarily correspond to a higher beaching of these FG. This significant negative correlation also indicates that gear items related to bottom trawl and seines had more probability to be beached than the other FG. Two main hypotheses can explain this phenomenon. First, most of the bottom trawls and seines related items were mending pieces that float due to the positive buoyancy of PE and PE-PP, and can be easily transported by currents and beached. They originate from the repair of nets onboard or at the harbors (Viool et al. 2018; Gilman et al. 2023). Second, the entire bottom trawl and seines are rarely lost and it is more likely that larger or smaller pieces of net get torn off during a hooking event on the sea floor (Richardson et al. 2022). These pieces of net are generally unleaded and can rise to the surface and then be beached.

On the other hand, while we might have expected a large number of gillnets, purse seines and longlines to be beached, only a few items were found on the beaches. One main reason could explain this discrepancy. Indeed, these three FG types are mainly made of polyamide (Appendix 2.B, Table 2.B.3) and are often leaded when lost. These two factors result in a negative buoyancy of the nets favoring their sink and their deposition on the sea floor (MacMullen et al. 2004; Macfadyen et al. 2009). When lost FNs sank to the bottom, they tend not to move too much, and are either spread out or curl up, giving rise to ghost fishing (Macfadyen et al. 2009; Thomas et al. 2019; Apete et al. 2024). Eventually, they will be colonized by macrofoulers and will finally accumulate on sediment (Saldanha et al. 2003). The other hypothesis to explain the lower rate of pieces or full nets of gillnets, purse seines and longlines on beaches could be the recovery operations undertaken by fishermen when they lose their FG. This curative solution has already been shown to play a significant role in reducing ALDFG marine pollution (MacMullen et al. 2004; Richardson et al. 2021). However, to date and to the best of our knowledge no publication has qualitatively or quantitatively evaluated the fishermen efforts to retrieve lost FG in the SE BoB. On the contrary, there are evidences of longlines and gillnets in sea floor and especially in some of the canyons in the

area studied in this work (canyons of Saint-Nazaire, Ars and Arcachon), particularly in areas of high fishing activity (Van den Beld et al. 2017). All these results highlighted that the novel ID-K is a very useful tool to complement existing marine litter classifications when estimating the amount of fishing gear beached or lost at sea. The ID-K can be implemented across diverse sampling protocols, including experimental methodologies or citizen science initiatives.

Finally, we would like to emphasize the issue of mending which contributes significantly to plastic pollution on beaches and ocean surfaces, as discussed above. This pollution resulting mainly from bad habits and inadequate facilities (few specialized garbage containers, no collection equipment, work area without protection against rain runoff), seems to be one of the simplest sources of ALDFG to be avoided. To this end, a number of reports and initiatives have already come up with solutions that seem likely to be effective (Fish & Click 2021; OCEANOPLASTIC 2023). Fishermen, fisheries committees, marine natural parks, and other stakeholders are encouraged to utilize the knowledge and tools provided by our study to better implement and evaluate the efficiency of management initiatives and policies at a local scale to effectively reduce the ALDFG pollution that threatens marine ecosystems.

2.5 Conclusions

This study provides a detailed estimation of the used, lost and end-of-life management of fishing gear in the SE BoB. We estimated a loss of 6 tons of fishing gear (0.17 km^2 of nets, and 146 km of longlines) in 2023, highlighting potential environmental impacts in an area with numerous protected marine areas and biodiversity hotspots. However, these estimations did not include the small-scale fisheries, which leads to an underestimation of the FG used and lost, especially for gillnets and longlines. The novel identification key developed highlighted the relevance of examining the fate of fishing gear in relation with their specific fishing *métier*. Coupled with beach litter monitoring, this approach revealed that 3 tons of fishing gear were beached in the SE BoB shores in 2023. The fishing gear maritime-related items represented 9.3% of the plastic waste found on beaches, while fishing-related items accounted for 7.6%. Bottom trawl and bottom seine nets constituted 66.9% of the weight and 93% of the number of items recorded. Nevertheless, the negative correlation between the loss of bottom trawl/bottom seine nets and the volume of lost fishing gear indicated that the high incidence of beaching observed for bottom trawl/ bottom seine nets was not attributed to a higher loss rate but rather to their positive buoyancy and surface transport capacity, which facilitated their accumulation on beaches. Conversely, gillnets, longlines, and purse seines appear to predominantly settle on the sea floor upon loss, contributing to ghost fishing. To address the issue of ALDFG, we recommend to focus on preventive measures, such as improving gear management practices onboard and in ports, and to enhance retrieval efforts for lost fishing gear, particularly in areas of high fishing activity and areas with vulnerable ecosystems, such as the SE BoB.

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2.6 Supplementary materials

2.6.1 Appendix 2.A

GeoPackages, rasters and the QGIS project used to estimate the surface and the volume of the studied area are available in this following GitHub repository: https://github.com/EdgarDusacre/Plasfito_public/tree/964f4d365b4ddcef0281b5ef7fc4d1f94918aeaf/Plasfito%20-%20Studied%20area .

All data utilized in the paper for the estimations of used and lost fishing gear, along with the R code, are accessible through this GitHub repository: https://github.com/EdgarDusacre/Plasfito_public/tree/60b1030207b5a6fd1c7dcfab848471983c9dc347/FG_operated_lost .

All documents related to the identification key can be found in a separate GitHub repository: https://github.com/EdgarDusacre/Plasfito_public/tree/60b1030207b5a6fd1c7dcfab848471983c9dc347/FG_Identification_Key .

Table 2.B.1: Statistical relation between each fishing métier size and the corresponding fishing effort in kW per day fished.

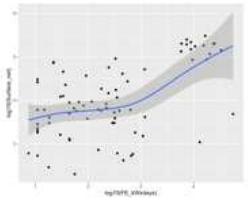
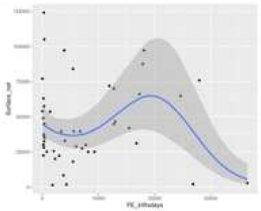
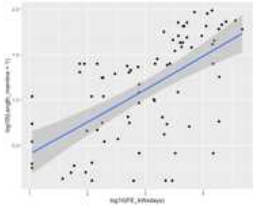
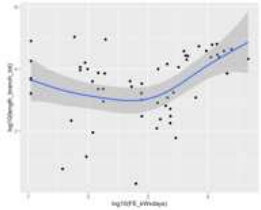
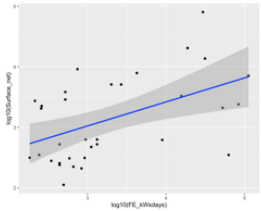
		Parametric coefficient				Smooth term					
Fishing gear	Statistical model	Estimate	SE	t-value	p-value	edf	F-value	p-value	Deviance explained	Effectif	Plot correlation
Gillnets	GAM inverse gaussian. (k-value = 15)	3.99	0.08	52.8	<0.0001	5.8	7.16	<0.001	37.3%	79	
Purse seines	GAM Gamma (link = log) (k-value = 9)	10.63	0.09	120.9	<0.0001	3.43	4.19	<0.01	17.0%	52	
Longline: mainlines	GAM Gaussian (k-value = 9)	1.12	0.05	22.13	<0.0001	1.08	39.7	<0.0001	37.2%	82	
Longline: branclines	GAM Gaussian (k-value = 9)	3.47	0.12	28.28	<0.0001	4.12	3.92	<0.01	28.2%	60	
Bottom trawls & seines	Linear	1.83	0.42	4.36	<0.001	0.40	0.13	3.18	<0.01	0.22	34	

Table 2.B.2: Detailed description of the sampled beaches.

Beach name (ID)	Coordinates	City, Country	Type of shore	Hydrodynamism	Sampling dates (m/d/y)
Dune d'Amour (FR1)	44°45'578"N 1°14'25.5"W	Lège-Cap-Ferret, France	Sandy	Strong tidal currents, Atlantic swells	01/24/2023 04/04/2023 07/27/2023 10/16/2023
Banc d'Arguin (FR2)	44°35'53.3"N 1°13'36.6"W	La Teste-de-Buch, France	Sandy	Highly dynamic, Significant sediment migration	01/23/2023 04/03/2023 07/18/2023 10/11/2023
Le Wharf (FR3)	44°30'536"N 1°15'14.1"W	La Teste-de-Buch, France	Sandy	Strong tidal currents, Atlantic swells	01/25/2023 04/05/2023 07/18/2023 10/18/2023
Champ-de-tire (FR4)	43°32'27.8"N 1°30'48.7"W	Tarnos, France	Sandy	River influence, Strong tidal currents, Atlantic swells	01/21/2023 04/24/2023 07/18/2023 10/18/2023
Murgita (ES1)	43°19'58.9"N1°5 7'1.8"W	Donostia, Spain	Rocky	Sheltered bay, complex currents, Atlantic swells	01/24/2023 04/20/2023 07/27/2023
Orrua (ES2)	43°18'14.4"N 2°14'22.8"W	Getaria, Spain	Rocky	Moderate-energy infralittoral rock	01/24/2023 04/20/2023 07/27/2023 10/05/2023
Alkolea (ES3)	43°18'26.6"N 2°22'14.5"W	Mutriku, Spain	Rocky	Moderate-energy infralittoral rock	01/25/2023 04/13/2023 07/19/2023 10/28/2023
Gorrondatxe (ES4)	43°22'42.6"N 3°1'15.1"W	Getxo, Spain	Sandy/Rocky	Low energy infralittoral rock	01/25/2023 04/20/2023 07/15/2023 10/27/2023

Table 2.B.3: Overview of fishing gear categories, sub-categories, and sub-sub-categories, including International Standard Statistical Classification of Fishing Gear codes (ISSCFG), polymers used in nets or lines, net lifespans by country, net repair practices, the categories of beached ALDFG coming from the identification key (ID-K) and references.

Fishing gear categories	Fishing gear sub-categories	Fishing gear sub-sub-categories	ISSCFG Code	Polymers of the gear	Net lifespans: Country	Net repair	Beached ALDFG categories (ID-K)	References
Trawls	Demersal (bottom) trawls and seines	Beam trawl	03.11	Polyethylene (HDPE) Polysteel (PE-PP)	0,5 years : UK	Continuously	Trawl nets Mending pieces Bottom trawl Bottom seine Rock hopper ballast Rubber leg ballast Metal chain ballast Midwater trawl	(Chambers et al. 2021)
		Pair trawl	03.15		3-5 years: UK 2,8±1,8 years: Norway 10 years: France			(Basurko et al. 2023) (Chambers et al. 2021) (Deshpande et al. 2019) (DLAL Project 2020) This work
		Single, twin or multiple otter trawl	03.12, 03.13, 03.14					
		Semipelagic trawls	03.3					
		Danish seine	02.2		3 years: UK 1,5-3,9±1,8 years: Norway			(Chambers et al. 2021) (Deshpande et al. 2019) (Syversen et al. 2022) This work
	Pelagic (midwater) trawls	Single trawl	03.29	Polyethylene (HDPE) Polysteel (PE-PP) Polyamide	8 years: UK 2,8 years: China			(Chambers et al. 2021) (Nyatchouba Nsangue et al. 2023) This work
		Pair trawl	03.22					
		Single otter trawl	03.21					
Purse seines			01.1	Polyamide	Decades: Spain (small scale) 3,2±0,8 years: Spain (tropical tuna) 10,2±5,3 years: Norway	Continuously	Purse seine Purse seine foam float	(Andrés et al. 2022) (Basurko et al. 2023) (Deshpande et al. 2019) This work

Cintinuation Table 2.B.3

Gillnets	Demersal (Anchored)	Set gillnet	07.1	Polyamide	1 year: France 0,5 years: UK 2,1±1,1 years: Norway (all species) 0,2-0,3 years: Norway (Atlantic cod) 0,25-1 year: Korea (yellow croaker)	Rarely: France, Spain & Norway Continuously: UK	Gillnet Mounting rope	(DG MARE 2021) (Basurko et al. 2023) (Chambers et al. 2021) (Deshpande et al. 2019) (Grimaldo et al. 2020) (Kim et al. 2016) This work
		Trammel net	07.5					
		Combined gillnet-trammel net	07.6					
	Pelagic	Drift gillnet	07.2					
		Encircling gillnet	07.3					
Longlines	Demersal (Anchored)	Set longline	09.31	Polyamide	0,75 years: UK (mainline) 0,5 years: UK (branchline) 3,0±2,6 years: Norway	Few knowledge but appears to be continuous	Longline	(Basurko et al. 2023) (Chambers et al. 2021) (Deshpande et al. 2019) This work
	Pelagic	Drifting	09.32					
Pots & Traps			08.2	Polyethylene (HDPE) Polypropylene Polyamide	10-25 years: UK 6,1±4,6 years: Norway 2 years: Canada (Dungeness crab) 4 years : Global (King crab)	Standard pots: continuously Octopus pots: rarely	Standard trap Octopus trap	(Basurko et al. 2023) (Chambers et al. 2021) (Deshpande et al. 2019) (Breen 1987) (Stevens 2014)
Pole & Lines		Handlines and hand-operated pole-and-lines	09.1	Polyamide	Unknown	Unknown	Line Fishing bobber Lure	(Basurko et al. 2023)
		Mechanized lines and pole-and-lines	09.2					
		Trolling lines	09.5					
		Vertical lines	09.4					

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2.7 Highlights and conclusions

Highligths of the chapter:

- 211 t of fishing gear were used in 2023 in the SE BoB
- 6 t of fishing gear were lost in 2023 in the SE BoB
- 3 t of ALDFG were beached in 2023 on the SE BoB
- 7.6% of plastic beached on the SE BoB coastline were ALDFG
- 92.2% of the ALDFG beached were originated from bottom trawl and seine nets

Conclusions:

This chapter provides a comprehensive understanding of the state of the fishing sector in the SE BoB. The different fishing gear were neither used nor lost in the same quantities, nor did they appear to be transported similarly by marine currents. Consequently, their fate and impacts on the marine environment are likely to be distinct. In studying the effects of fishing gear from different fishing *métiers*, it is imperative to analyze each one independently or to consider their distinctive characteristics. The new data produced in the framework of this chapter can now be utilized to evaluate the environmental risks associated with FNs. However, to assess this environmental risk, there is still a lack of data regarding the potential toxicity of degradation products from FNs, including MPs, NPs, and plastic chemicals. Therefore, it was essential to select the most commonly used nets within the SE BoB, i.e., gillnets and trawls, and to conduct toxicity assessments on both new and end-of-life nets.

Chapter 3 Comparison of toxicity profiles of DMSO extracts of new and end-of-life oil-based and partially biobased and biodegradable fishing nets

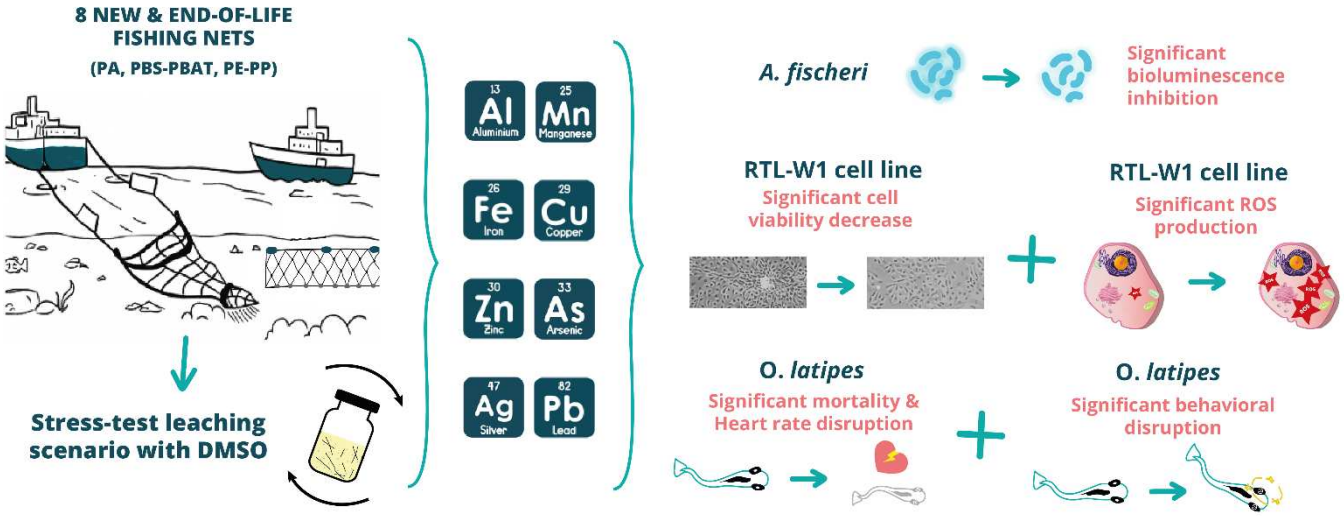
Context:

Plastic FNs, similar to other frequently utilized plastics, contain plastic chemicals. Among the 16,000+ known chemicals associated with plastics, 5,410 (32%) have been classified as either hazardous or less hazardous, while 10,726 (66%) lack hazard data. This indicates that at least 32% of FNs contain hazardous chemical compounds. Whether during operational use or after loss, the degradation of FNs likely results in the release of plastic additives, non-intentionally added substances (NIAS), or degradation by-products into the aquatic environment. Furthermore, the chemical hazard associated with FNs extends beyond their inherent composition, as plastics have the capacity to adsorb and desorb aquatic environmental pollutants during their submersion in water. Among these environmental pollutants, certain substances are recognized for their toxicity to numerous aquatic species, including trace metals (e.g., copper, zinc, lead, cadmium), as well as persistent organic compounds (e.g., polychlorinated biphenyls and organochlorine pesticides), in addition to polycyclic aromatic hydrocarbons. Consequently, to comprehensively understand the chemical risks posed by plastic fishing nets as sources of plastic chemicals or as vectors of chemical pollutants, it is imperative to characterize the chemicals released from FNs, as well as those they can adsorb. Subsequently, the toxicity of these chemical mixtures must be investigated.

Objectives:

To determine the toxicity of extracts of dimethyl sulfoxide containing plastic chemicals and environmental pollutants of several new and end-of-life oil-based fishing nets used in the SE BoB, as well as biobased and biodegradable fishing nets under development.

The chapter in one illustration:



The content of this chapter reflects a scientific publication that is under preparation to be submitted to the scientific journal "Journal of Hazardous Materials" as:

Dusacre, E., Gonzalez Soto, N., Combes, Y., Clérandeau, C., Dassié, E., Lecomte, S., Lagarde, F., Morin, B., Miren, P. C., & Cachot, J. n.d. "Comparison of toxicity profiles of DMSO extracts of new and end-of-life oil-based and partially biobased and biodegradable fishing nets*."*

Abstract

Millions of tons of fishing nets (FNs) are used, lost, or are at the end of life yearly (Kuczenski et al. 2021). These FNs pose a potential threat to aquatic environments, as they can release hazardous products. To better assess this risk, we conducted a study of the adverse effects of eight different new and end-of-life (EOL) FNs: one trawl net of oil-based polyethylene-polypropylene (PE-PP), five gillnets of oil-based polyamide 6 (PA6) and two of biobased and biodegradable poly-butylene-succinate-co-butyrate-co-adipate-co-terephthalate (PBS-PBAT). Dimethyl sulfoxide extracts of FNs were made to simulate a stress-test leaching scenario. Trace metals were quantified by ICP OES. The toxicity of the extracts was determined in *Aliivibrio fischeri*, RTL-W1 rainbow trout liver cell line, and *Oryzias latipes* larvae. Cu, Fe, Mn, and Zn were the trace metals found the most. The bioluminescence of *A. fischeri* was significantly affected by five extracts of FNs (new and EOL PA6 and PBS-PBAT FN). Most FNs reduced fish cell viability, three EOL PA6 FNs enhanced ROS production. Some FNs increased larval mortality (EOL PE-PP FN (18%) and PBS-PBAT FN (27%)) and altered heart rate (two EOL PA6). One EOL PBS-PBAT FN showed the most significant behavioral toxicity, with a behavioral stress index increasing 13.5 times from its new state. The integrated biological response index enabled to rank one EOL PBS-PBAT FN as the most toxic, followed by one new PA6 FN and another EOL PA6 FNs. FN toxicity came mainly from the chemical compounds they released, again pointing to the need for better control of additive use.

Introduction

The fishing industry is a complex sector, using a wide diversity of fishing gear depending on the fishing *métier* (Weissenberger 2015). However, whatever the fishing *métier*, the large majority of fishing net (FN) panels is made of plastic (Matsushita et al. 2008). Bottom trawl net panels and Danish/Scottish seine net panels are predominantly composed of either polyethylene or a blend of polyethylene and polypropylene. On the contrary, pelagic trawl net panels, purse seine net panels, gillnet panels, longlines, and pole lines are manufactured primarily from polyamide 6 (INdiGO Project 2021; Andrés et al. 2022; Basurko et al. 2023). Plastic FN panels contained also a large diversity of additives and synthesis by-products (Vilke et al. 2024; Wagner et al. 2024; Dusacre et al. 2025). FNs represented approximately 0.5% (2.15 million tons) of plastics produced worldwide in 2017 (Geyer 2020; Kuczenski et al. 2021), and could be considered a negligible source of plastic pollution. Nonetheless, due to their direct utilization in aquatic environments and the fact that approximately 2% are lost on average annually (Richardson et al. 2022), the fishing gear may account for 6% of macroplastic emissions in the ocean each year (Schwarz et al. 2023). This statement is corroborated by numerous field studies that have documented the substantial presence of fishing gear in samples collected during oceanic and beach plastic monitoring campaigns (Lebreton et al. 2017; Lacroix et al. 2022; Muñoz-Pérez et al. 2023; Royer et al. 2023; Dusacre et al. 2025). Their impacts are now well known and include scraping the sea floor, prolonged capture of target and non-target species, entanglement of organisms, engine propeller jamming and collision. Therefore, they were considered one of the deadliest macroplastic in the oceans (Macfadyen et al. 2009; García-Barón et al. 2019; Ostle et al. 2019). Recent evidence highlighted that plastics are also an invisible threat to aquatic organisms, due to microplastics, nanoplastics (MNPs) and plastic chemicals. Additives, non-intentionally added substances (NIAS) and sorbed pollutants are becoming more and more suspected to be the main explanation of plastic toxicity (Beiras et al. 2019, 2021; Groh et al. 2019; Le Bihanic et al. 2020; Cormier et al. 2021; Quade et al. 2023). It was estimated that more than 16,000 plastic chemicals can be found in plastics and that at least 26% are of concern due to their environmental persistence, bioaccumulation, mobility, and toxicity (Wagner et al. 2024). Furthermore, the hazard of 10,000 (62.5%) of these chemicals remains unknown (Wagner et al. 2024). The lack of strong chemical bonds of additives and NIAS to the polymeric matrix primarily accounts for their tendency to leach easily, contributing to their ubiquity, bioavailability, and mobility (Barrick et al. 2021). Regarding the FNs, to the best of our knowledge only three studies (Brakstad et al. 2022; Chen et al. 2023; Dusacre et al. 2025) described the leaching of some additives and NIAS (26 and 27 respectively) from artificially aged new FNs. Only one investigated the toxicity of the mixture of MNPs and plastic chemicals (Dusacre et al. 2025). Plastic toxicity and chemical hazards may also result from the "Trojan horse" effect, in which plastics in the environment absorb or adsorb environmental contaminants, which can be later released into organisms after ingestion. Barrick et al. (2021) and Vilke et al. (2024) investigated this phenomenon with fishing gear by studying the toxicity of lines and ropes abandoned, lost or otherwise discarded coming from beaches. They found that tens of different plastic chemicals and adsorbed/absorbed environmental pollutants, were released from gear and caused genotoxicity and oxidative damage in the marine mussels

M. galloprovincialis. However, to the best of our knowledge, no studies have made a comparison of leaching and toxicity between several identical new and EOL FNs. Furthermore, no studies have compared various polyamide gillnets from different sellers, nor have they compared oil-based nets versus biobased/biodegradable nets. In an effort to address these research gaps, we simulated a stress-test leaching scenario by extracting additives, NIAS, or environmental pollutants with the aprotic organosulfur polar solvent dimethyl sulfoxide (DMSO). This was accomplished on eight new and the corresponding eight EOL FNs. The toxicity of DMSO extracts of FNs was then evaluated on different aquatic models: marine bacterium *Aliivibrio fischeri*, rainbow trout liver waterloo-1 cell line and the larvae of the fish *Oryzias latipes*. Finally, we compiled all the data and calculated the integrated biomarker response (IBR) index for each FN.

3.1 Materials and Methods

3.1.1 Fishing nets

FNs were selected based on the two fishing *métiers* mainly used in the SE BoB (SE BoB): trawls and gillnets (GFW 2024). The eight FNs studied were described (identification, type of FN, polymer, structure, color, diameter, origin and state) in the Table 3.1.

Table 3.1:: Description of the eight fishing nets (FNs) studied.
The FN indentifications (ID) are the names utilized in this study to distinguish among the various fishing nets (FNs). « PNM-BA (OFB) » means « Arcachon Bay Marine Natural Park (Office Français de la Biodiversité) ». « FN » means fishing net. "EOL" means end-of-life. Pictures are available in Appendix A, figure 3.A.1.

FN ID	Type of FN	Polymer	Characteristics	Origin	State
PE-PP ε4.25	Cod end trawl net	Mix PE and PP	Braided multifilament. Color: Green & yellow. Diameter 4.25 mm	Naberan company, Basque Country, Spain	New
				Fisherman, Saint-Jean-de-Luz, France	EOL: 12,700 h
PA α35	Straight gillnet	PA6	Monofilament. Color: Green. Diameter 0.35 mm	Fisherman Pasaia, Basque Country Spain	New
					EOL: 650 h
PA β33	Inner panel trammel net	PA6	Monofilament. Color: Purple. Diameter 0.33 mm	ETS Armand Mondiet, France	New
				Fisherman, Arcachon, France	EOL: 2,700 h
PA β95	Outer panel trammel net	PA6	Twisted multifilament. Color: Purple. Diameter 0.95 mm	ETS Armand Mondiet, France	New
				Fisherman, Arcachon, France	EOL: 2,700 h
PA γ33	Inner panel trammel net	PA6	Monofilament. Color: green. Diameter 0.33 mm	Alprech Filets, France	New
				PNM BA (OFB)	EOL: 624 h
PA γ60	Outer panel trammel net	PA6	Monofilament. Color: no color. Diameter 0.33 mm	Alprech Filets, France	New
				PNM-BA (OFB)	EOL: 624 h
PBS δ35	Inner panel trammel net	35% biobased & biodegradable PBS-PBAT	Monofilament. Color: white. Diameter 0.35 mm	PNM-BA (OFB)	New
					EOL: 624 h
PBS δ60	Inner panel trammel net	50% biobased & biodegradable PBS-PBAT	Monofilament. Color: white. Diameter 0.60 mm	PNM-BA (OFB)	New
					EOL : 624 h

3.1.2 SEM analysis

The surface state of the new and EOL FN pieces was analyzed by scanning electron microscopy (SEM). FN pieces were randomly selected, thinly coated with platinum using a platinum coater (JEOL JFC-1300) and placed in the SEM (JEOL, JSM 6510 LV, IMMM, Le Mans, France). The images were taken at four different magnifications (x50, x100, x250, x500) with an accelerating voltage of 20 kV, a working distance of 20 mm, and a spot size of 30 nm.

3.1.3 DMSO extraction of fishing nets

FNs were cut into pieces of 2 to 2.5 cm and 1 g was introduce in 4 mL amber glass vials (final concentration of mesoplastic: 330 mg/mL). Before use, the vials were rinsed three times with dimethyl sulfoxide (DMSO) (99.9% anhydrous, maximum 0.005% H2O, CAS [67-68-5], Scharlau S.L., Spain) and dried for 24 h. Subsequently, 3 mL of DMSO were added to the vials. Vials were covered with aluminum foil. The extraction was carried out using an orbital shaker (Ika KS 501) set at 175 rpm with vials on their sides for 24 h at 22°C (Le Bihanic et al. 2025). After 24 h, FN pieces were removed with a stainless squeezer and the DMSO extracts aliquoted in 1 mL amber glass vials and stored at -20°C. Three independent extractions were realized for each FN type (Table 3.1). Three control extractions consisting solely of DMSO contained within amber glass vials were also prepared.

3.1.4 Trace metals in DMSO extracts

Before trace metal analysis, DMSO extracts were diluted in MilliQ water to reduce matrix viscosity and then acidified to 2% with purified acid HNO_3 to put the metals in suspension. Metal quantification was performed using a 720-ES ICP-OES (Varian, Agilent Technologies Inc, USA) system. The instrument was calibrated using multielement standard solutions (SCP sciences, S210323034), and quality control samples at 65 ppb and 220 ppb were analyzed to ensure accuracy. Recovery rates for quality controls ranged from 93.34% to 102.20% for all measured elements (Ag, Al, As, Cu, Fe, Mn and Zn). Limit of quantification (LQ) for each element was determined and ranged from 1.45 µg/L for Mn to 13.98 µg/L for Al. Detailed LQ, quality controls, recovery percentage and wavelengths used for each trace metal are available in supplementary material (Appendix A, Table A.1). Control samples were analyzed to assess potential contamination, with most elements below the LQ, except for trace amounts of Zn detected in some controls. Concentrations below the LQ were not taken into account, and the data were corrected by subtracting the mean concentration of the control extracts.

3.1.5 Bioassays

3.1.5.1 Microtox© assay using A. fischeri

The acute toxicity of the DMSO extracts was first analysed through the exposure of the marine bacteria *Aliivibrio fischeri* using the Microtox© bioassay following the ISO 11348-3 guideline

(International Organization for Standardization 2007). Extracts of FNs and controls were diluted in 2% NaCl to reach extract concentrations of 3.3 mg/mL (1%), 1.65 mg/L (0.5%), 0.83 mg/mL (0.25%) and 0.41 mg/mL (0.12%). pH of the extracts were adjusted to 7.3±0.2 as recommended. Then, the marine bacteria *Aliivibrio fischeri* were exposed for 30 min to each extract. Bioluminescence values were normalized to the control (2% NaCl) containing bacteria, and bioluminescence inhibition was calculated for each sample. Finally, the data were statistically compared with DMSO controls.

3.1.5.2 Cell viability and ROS production of RTL-W1 cell line

The method used for the culture of RTL-W1 cell line and the preparation of the cytotoxicity assay and ROS production measure were previously described by Pannetier (2018). For both assay $2x10^5$ cells/ml per well were seed 24 h before the exposure (cytotoxicity: 96 wells micro-plate, 100 µl/well; ROS: 48 wells micro-plate, 500 µl/well).

For the cytotoxicity assay, all extracts were tested at a 1% dilution (3.3 mg/mL) i.e., the maximum concentration at which DMSO alone did not cause significant cell death. For samples having significant toxicity (above 15% of cytotoxicity), a dose response was carried out with five dilution points per FN's DMSO extract (0.01% = 0.03mg/L, 0.05% = 0.0165 mg/L, 0.1% = 0.3 mg/L, 0.5% = 1.65 mg/mL and 1% = 3 mg/mL). Intermediate dilutions were done in DMSO, thus 1% of DMSO was always present in all conditions as in the solvent control. For the ROS production assay, samples with cell mortality <15% were tested at maximum possible concentration (3.3 mg/mL). For samples with cell mortality above 15%, a concentration below the LC25 was selected for the ROS production assay (0.3 mg/mL). Each sample was run in triplicate, and all the analyses were repeated three times in independent plates using different cell flasks at seeding. Experiments were carried out with cells aged from the passage 10 to 12. In total, nine replicates per DMSO extract of FN were analyzed. Nine negative controls with only cells and complete medium were also run as an internal control of the test and nine controls with 1% DMSO and medium were also performed. Moreover, nine positive controls were also analyzed (2.5 mM H_2O_2, Scharlab, Barcelona, Spain).

The cytotoxicity of the DMSO extracts of FNs was quantified by fluorescence using the Alamar blue reduction assay (Ref: DAL1025, Thermo Fisher Scientific, Waltham, USA). Cell exposure was carried out for 24 h at 20°C. Then, the exposure medium was removed and the cells were washed with phosphate buffered saline (Ref: 20012-019; Gibco, Grand Island, USA). A 1/10 dilution of the stock Alamar Blue solution was prepared using complete L15 medium and was added to all the wells containing cells with FN conditions and 3 blanks (empty wells) per plate. Plates were incubated covered in aluminum foil inside an incubator (Brunswick galaxy 170 r) at 20°C for 2 hours. The fluorescence was then measured in a Fluostar Optima (BMG Labtech, Ortenberg, Germany) at Ex: 540 nm, Em: 590 nm.

For the ROS production measure the fluorometric probe chloromethyl-2,7-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA, Ref: C6827, Thermo Fisher Scientific, Waltham, USA) was used with an incubation of 20 min in darkness at 20°C. After being washed (L15 plain medium) cells were exposed 4 hours at 20°C. Afterward, the fluorescence was measured in a Fluostar Optima (BMG Labtech, Ortenberg, Germany) at 485 nm (excitation

light) and 520 nm (emission light). In both assays fluorescence data was normalized against the DMSO control of each plate and express as percentage as mean of the 3 plates.

3.1.5.3 Survival, heart rate and behaviour of O. latipes larvae

The embryos (Carbio strain) of Japanese medaka (*Oryzias latipes*) were purchased and received 24 hours after fertilization from the experimental facility PGP (Physiology Genomique Poissons, INRAE, Rennes, France). The embryo development process was similar to that described in Dusacre et al. (2025). Immediately after hatching (10 days after fertilization at 26°C), 18-20 larvae were exposed for 48 hours in a mix of deionised water and tap water (1/3 v/v) with DMSO extracts of FNs at a concentration of 0.33 mg/mL (0.1%). Half of the exposure medium was renewed daily (1/2 v/v). Triplicates were performed for each sample. Mortality was assessed daily using a binocular magnifier, and dead fish were removed. Larval mortality at 24 h and 48 h was cumulated to obtain the final mortality. Heart rate was measured for 16 larvae of each DMSO extract of FN and controls, at 26°C using the method described by Dusacre et al. (2025). The analysis of the behavior of the larvae was carried out using the DanioVision System (Noldus, Wageningen, The Netherlands) with a temperature of 26±0.5°C. 48 larvae were analyzed by condition. The behavior of the larvae was examined during 40 minutes, with 10 min of acclimatization without behavioral monitoring, 10 min of behavioral monitoring in the dark (LOFF1), 10 min of behavioral monitoring in an illuminated environment (LON), a multi-stress consisting of a vibratory stimulus and the extinction of the light, followed by behavioral monitoring for 10 minutes in the dark (LOFF2). Five behavioral endpoints were studied: 1) the basal locomotor activity (BLA) in distance swum (cm) during the LOFF1, LON and LOFF2 period; 2) the time spent (s) in the peripheral zone during the LOFF1, LON, and LOFF2; 3) the vibrational and light startle response (VLSR) when multi-stress occurred; 4) the vibrational and light motor response (VLMR) when multi-stress occurred; and 5) the acclimatization to sudden darkness during the final resting period (LOFF2). Finally, a behavioral stress index was calculated based on the method described in Dusacre et al. (2025).

3.1.6 Data and statistical analysis

RStudio (R version 4.3.1, RStudio 2024.04.0+735) was used for data analysis. Statistical test conditions were always checked before analysis by the means of density plots, Shapiro–Wilk, QQ-plots, Bartlett's, Levene's tests. Student's t-test was used for mean comparison between two groups if assumptions were met; otherwise, Welch's test was used for non-equal variances and Wilcoxon-Mann-Whitney for unmet requirements. Effective concentrations (ECx) were calculated using the "drm()" function from the "drc" R package (Ritz & Strebig 2016) with a four-parameter log-logistic model. The Spearman's method was used to assess relationships between FN's extract toxicity and chemical products.

The integrated biomarker response (IBR) index for each FN DMSO extract was based on bioluminescence inhibition of *A. fischeri*, cell viability of the RTL-W1 cell line, and the survival, heart rate and behavioral endpoints in Japanese medaka fish larvae, using the functions "ibr_std()" and "ibr_index()" from the R package "IBRtools" (Resende & Pereira 2024),

following the method of Devin et al. (2014). The IBR index was calculated based on all possible geometric permutations (5! = 120) of the five toxicity variables included.

3.2 Results

3.2.1 Surface condition of new and end-of-life fishing nets

The PE-PP ε4.25 trawl net used for 12,700 hours, was the most used and the most degraded net, showing fraying, fiber detachment, and fractures (Figure 3.1). Gillnets displayed less visible filament degradation but more biotic and abiotic material on their surface. The presence of a biofilm was highly suspected, especially on the PA γ33, PA γ60, PBS δ35 and PBS δ60, which tended to have a more covered surface and a thicker organic and inorganic film.

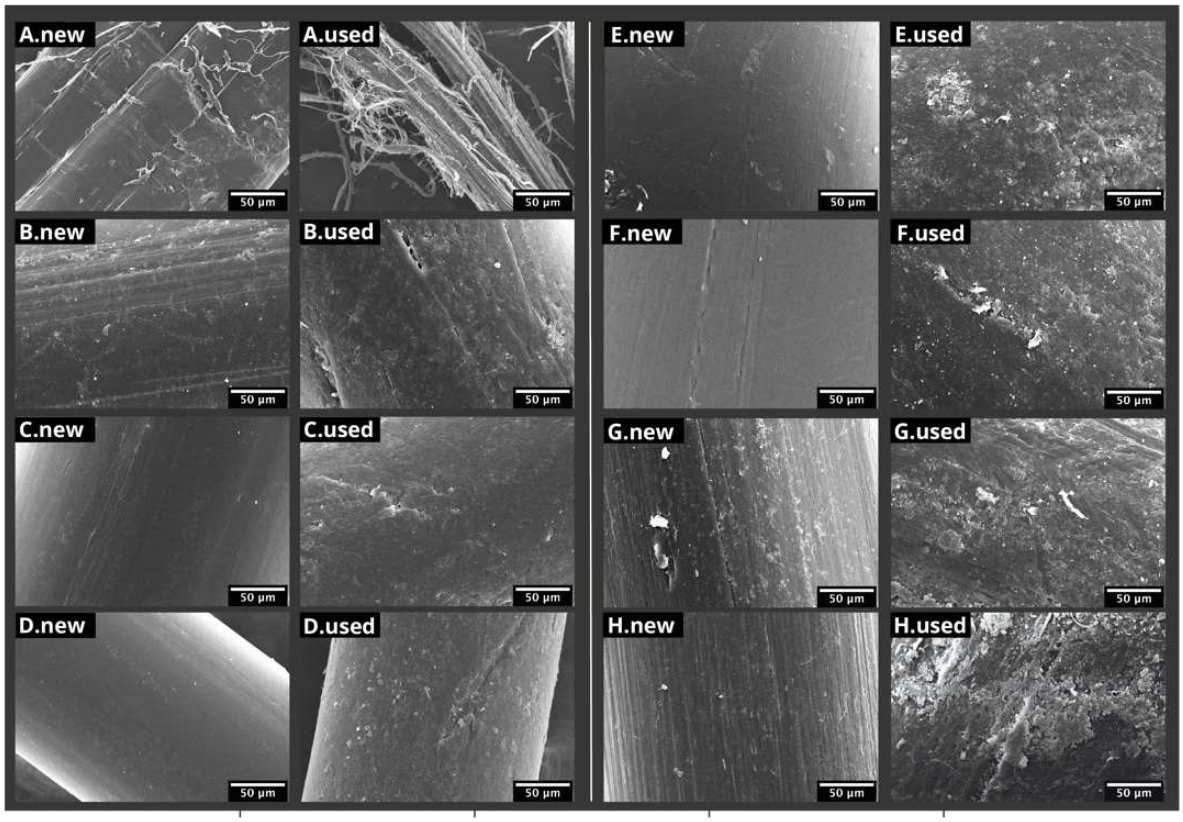


Figure 3.1: SEM images of the new and EOL filaments of FNs.
A.new and A.EOL are filaments of PE-PP ε4.25 FNs. B.new and B.EOL are filaments of PA α35 FNs. C.new and C.EOL are filament of PA β33 FNs. D.new and D.EOL are filaments of PA β95 FNs. E.new and E.EOL are filaments of PA γ33 FNs. F.new and F.EOL are filaments of PA γ60 FNs. G.new and G.EOL are filament pf PBS δ35 FNs. H.new and H.EOL are filaments of the PBS δ60 FNs. "new" indicates images of new FNs and "EOL" images of the end-of-life FNs after their operation by fishermen. Scale bars are 50 µm.

3.2.2 Trace metals in the DMSO extracts of FNs

Of the 14 targeted trace metals (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V, Zn), seven including Ag, Al, As, Cu, Fe, Mn, Zn were above the LQ in DMSO extracts of FNs (Figure 3.2). In extracts of PE-PP ε4.25 EOL FN, six metals exceeded control extract's levels but showed unreliable concentrations due to high variability. Extracts of new PA α35 FN showed reliable concentrations of Ag and As at 2.1±0.6 µg/L and 6.4±2 µg/L, respectively. Extracts of PA α35 EOL FN had four trace metals (Ag, Cu, Fe, Mn) between 13.67 µg/L and 87.07 µg/L, and Zn at 874±60.3 µg/L (Figure 3.2). The Zn was significantly higher in extracts of EOL FNs than in new one (df = 5, W = 30.2, $p < 0.0001$). Extracts of PA β33 and PA β95 new FNs consistently had higher trace metal concentrations than in extracts of EOL FNs. Specifically, Fe was significantly higher (df = 5, $p < 0.01$) in both new PA β extracts and Zn was significantly higher in the extracts of PA β95 new FN than in EOL one (df = 5, W = -6, $p < 0.05$). In extracts of PA γ new FNs, only As was reliably present (7±2.5 µg/L), while other metal concentrations were below control values or unreliable. Extracts of PBS δ FNs had the highest trace metal concentrations. Extracts of PBS δ35 and PBS δ60 new FNs had high Cu (194.6±12.1 µg/L; 251.6±50.2 µg/L) and Zn (278.5±34.8 µg/L; 197.7±7.04 µg/L) concentrations. In extracts of PBS δ35 FN, Cu decreased significantly while other metals increased between new and EOL extracts (df = 5, $p < 0.01$) (Figure 3.2). For PBS δ60, As, Cu, and Fe significantly decreased between extracts of new and EOL FNs, while Ag, Mn, and Zn significantly increased (df = 5, $p < 0.01$).

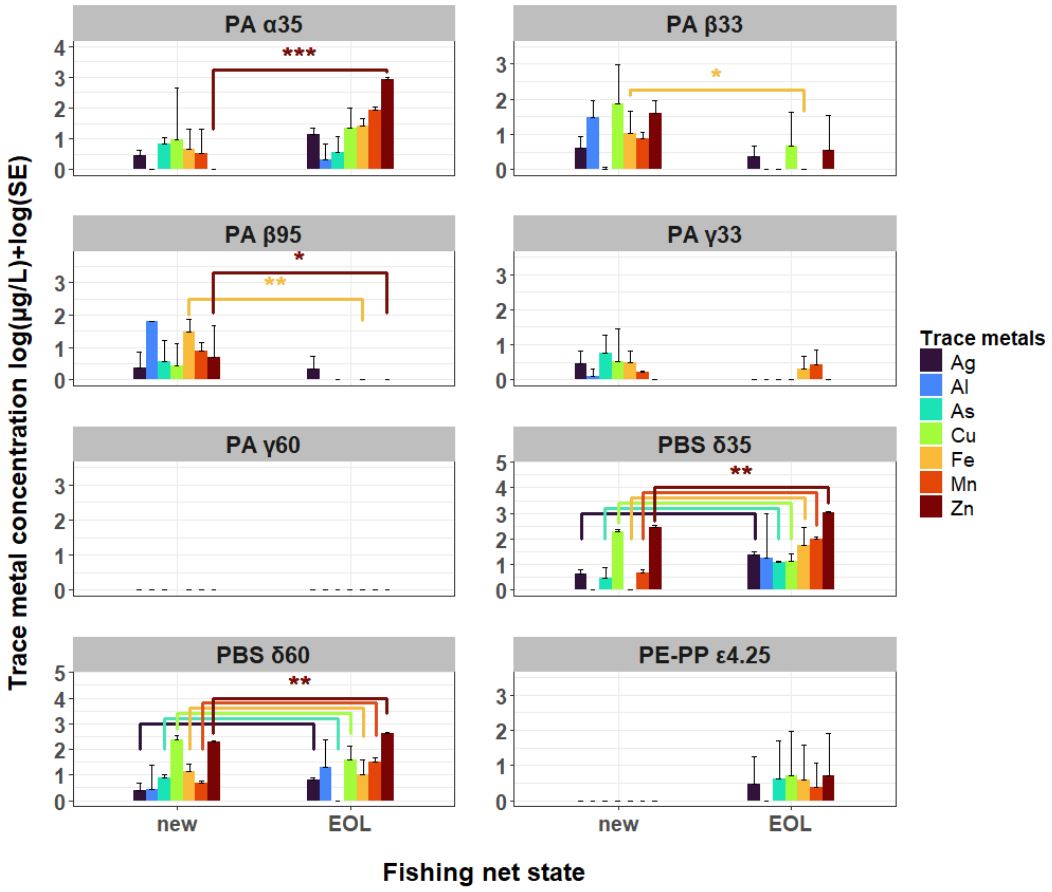


Figure 3.2: Concentrations of trace metals in DMSO extracts of new and EOL FNs.
Top of the colored bars are the metal average concentrations corrected with the DMSO control. Black lines at the top of the bars represent the standard deviations. Colored brackets indicate significant differences between concentrations of trace metal species in extracts of new and EOL FNs (Welch's test: "*" = p-values < 0.05 and "**" = p-values < 0.01). Sample size by FN state = 3.

Comparing the PA γ and PBS δ FNs, that were used for the same time in the same marine region, we highlighted that the PBS δ FNs released significant higher trace metal concentrations than the PA γ FNs (Appendix A, Table A.2). These higher concentrations were even more pronounced for EOL PBS δ FNs.

3.2.3 Toxicity of the DMSO extracts of FNs on the marine bacteria *A. fischeri*

At 3.33 mg/mL (1%), extracts of new and EOL FNs of PA α35, PBS δ35, and PBS δ60 significantly inhibited *A. fischeri* bioluminescence (Mann–Whitney U-test: $p < 0.05$) (Figure 3.3). Extracts of new FN of PA β33 and PA β95 induced significant inhibition of bioluminescence (df = 5, p-value < 0.01). In contrast, no significant inhibition occurred with PE-PP ε4.25, PA γ33, and PA γ60 extracts. Extracts of new PBS δ FNs showed the lowest EC_{20} (PBS δ35: 1.76±0.22 mg/mL; PBS δ 60: 1.38±0.7 mg/mL), indicating higher toxicity for *A. fischeri* (Appendix A, Table A.3). The third lowest EC_{20} was caused by the EOL PA α35 (1.99±0.21 mg/mL), followed by new PA β33 (2.55±0.29 mg/mL). Extracts of other FNs had higher EC_{20} than the maximum tested concentration (3.3 mg/mL). PBS δ35 extracts were significantly more toxic than PA γ33 extracts (df = 11, p-value < 0.01), but the inhibition effects of PBS δ60 and PA γ60 extracts were not statistically different.

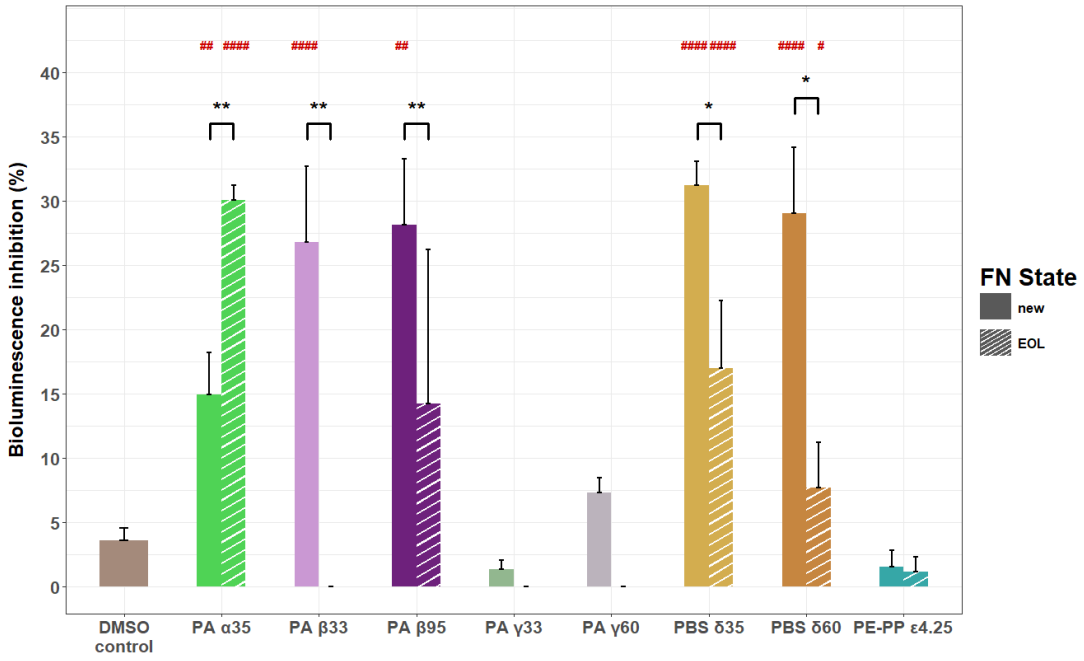


Figure 3.3: Bioluminescence inhibition of *A. fischeri* induced by DMSO extracts of FNs.
Top of the bars are the average bioluminescence inhibition (%). Full color bars show the new FNs, while the hatched bars represent the EOL ones. Black lines at the top of the bars represent the standard deviation. Red sharp signs indicate a significant difference of bioluminescence inhibition of the extracts of FNs compared to the DMSO control (Mann–Whitney U test: # = p-value < 0.05, ## = p-value < 0.01, #### = p-value < 0.0001). Black brackets highlight a significant difference between the extracts of new and EOL FNs (Mann–Whitney U test: * = p-value < 0.05, ** = p-value < 0.01).). Sample size by FN state: n = 6.

3.2.4 Effects of DMSO extracts of FNs on the RTL-W1's cell viability and ROS production

All DMSO extracts of FNs, except PBS δ60, significantly (Student's t-test: df = 17, p-value < 0.05) decreased the RTL-W1 cell viability at 3.33 mg/mL (1%) (Figure 3.4). Only the EOL PA α35 and PA γ60 extracts showed mortality above 15%, significantly higher than extracts of new FNs. Extracts of EOL PA α35 FN was the most toxic (EC_{20} = 0.07±0.05), followed by the extract of the PA γ60 FN (EC_{20} = 0.33±0.08 mg/mL).

At 3.3 mg/mL (1%), only the extract of PA γ33 EOL FN induced significant increase of ROS production (Figure 3.5). At 0.3 mg/mL (0.1%), only EOL PA α35 and PA γ60 induced significant increase of ROS production. Extracts of PA γ60 EOL FN induced higher mortality than PBS δ60 new FN (df = 17, T = -5, p-value < 0.001). Both extracts of PA γ EOL FNs caused more ROS production than extracts of PBS δ EOL FNs (df = 17, p-value < 0.01).

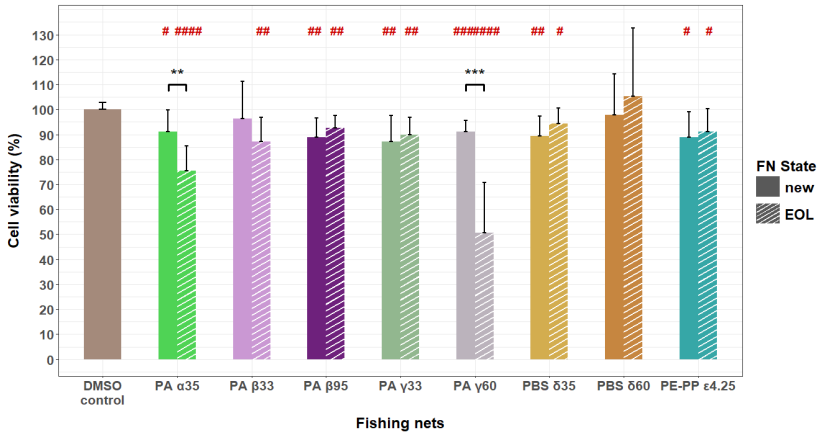


Figure 3.4: Cytotoxicity of the DMSO extracts of FNs on RTL-W1 cells.
Top of the bars are the average cell viability percentage (%) normalized according to the DMSO control. Full color bars are new FNs while hatched bars are EOL FNs. Black lines at the top of the bars represent the standard deviation. Red sharp signs indicate a significant difference of the cytotoxicity of the extracts of FNs compared to the DMSO control (Student's t-test: # = p-value < 0.05, ## = p-value < 0.01, ### = p-value < 0.001, #### = p-value < 0.0001). Black brackets highlight a significant difference between the extracts of new and the EOL FNs (Student's t-test: ** = p-value < 0.01, *** = p-value < 0.001). Negative and positive controls were also run (not shown). Sample size by FN and FN state: n = 9.

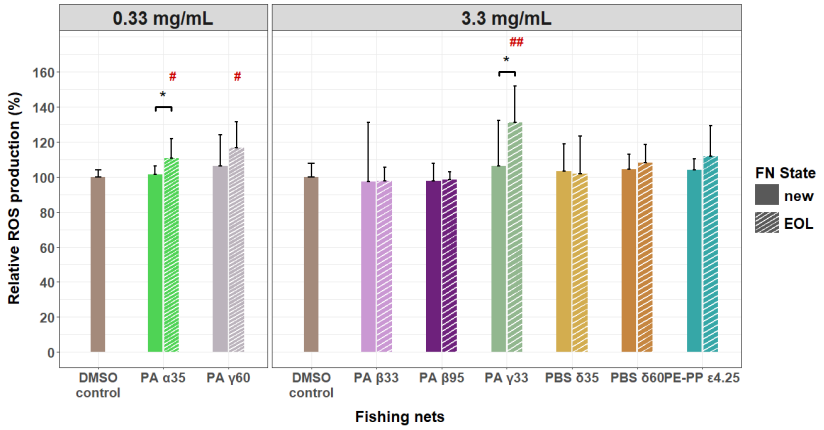


Figure 3.5: Effects of DMSO extracts of FNs on the ROS production of RTL-W1 cells.
The value indicated at the top of each panel is the concentration of DMSO extracts of FNs to which the cells were exposed to. Top of the bars are the relative average ROS production (%) normalized according to the DMSO control. Full color bars show the new FNs while the hatched bars represent the EOL FNs. Black lines at the top of the bars represent the standard deviation. Red sharp signs indicate a significant difference of ROS production by the extracts of FNs compared to the DMSO control (Student's t-test: # = p-value < 0.05, ## = p-value < 0.01). Black brackets highlight a significant difference between the extracts of new and EOL FNs (Student's t-test: * = p-value < 0.05). Negative and positive controls were also run (not shown). Sample size by FN and FN state: n = 9.

3.2.5 Effects of DMSO extracts of FNs on the survival, heart rate and behavior of the Japanese medaka larvae

At 0.33 mg/mL of DMSO extracts of FNs (0.1%), *O. latipes* larvae's survival was significantly reduced by the extracts of PE-PP ε4.25 and PBS δ60 EOL FNs compared to the DMSO control (Mann–Whitney U-test: $p < 0.05$) (Appendix 3.A, Figure 3.A.2). Extracts of PA β33 and β95 EOL FNs were the only ones to induce significant increase of the larvae's heart rate (21% and 23% increase, respectively) compared to DMSO control values (Mann–Whitney U-test: $df = 31$, $p < 0.05$) (Appendix 3.A, Figure 3.A.3).

Concerning the *O. latipes* larvae's behavior, most of the extracts of new FNs (PE-PP ε4.25, PA α35, PA β33, PA β95, PA γ33, PBS δ60) triggered a significant increase of the distance swum by the larvae during the period of light ON (LON) (Independent Dunn's test: $df = 47$, p-value < 0.05) (Appendix 3.A, Figure 3.A.4). Moreover, during the LON period four extracts of new FNs (PE-PP ε4.25, PA β33, PA β95, PBS δ35) led to a significant increase of the duration spent by the larvae in the peripheral zone. The larvae exposed to extracts of PE-PP ε4.25 EOL FN swum a significant lower distance during the multi-stressor stimuli and the recovery phase in the dark. Finally, extracts of PBS δ60 EOL FN were the ones that most disrupted the larvae's swimming behavior. It caused a significant (Independent Dunn's test: $df = 47$, p-value < 0.05) decrease of 6 behavioral endpoints including BLA_LOFF1, BLA_LON, TPZ_LON, VLSR, VLMR, ASD_LOFF2.

The calculation of the behavioral stress index (BSI), confirmed that extracts of the PBS δ60 EOL FN led to the highest sublethal effects to *O. latipes'* larvae with a BSI equal to 13.5, followed by the new PA β95 (BSI = 4.5) and the new PA β33 (BSI = 3.5) (Figure 3.6). However, the effects of these two latter decreased when the extracts were made with the EOL FNs. Furthermore, it can be emphasized that extracts of PA γ33, PA γ60, PBS δ35 new and EOL FNs and PBS δ60 new FN caused very few or did not cause any significant disruption in the swimming behavior of Japanese medaka fish larvae (Figure 3.6).

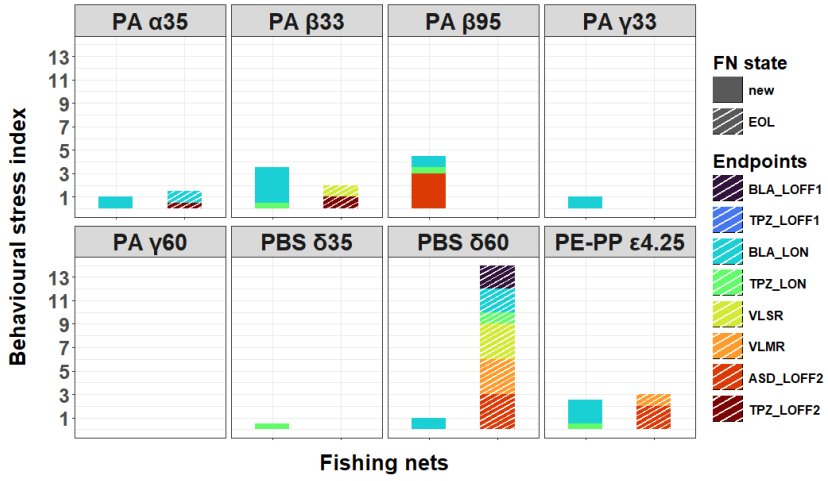


Figure 3.6: Behavioral stress index (BSI) of larvae of *Oryzias latipes*.
The stacked bars illustrate the cumulative behavioral stress indexes. Full color bars show the BSI of the extracts of new FNs, while the hatched bars represent the EOL ones. Each colored segment within the bars represents the relative contribution of the different behavioral endpoints, including Basal Locomotor Activity (BLA) and Time in Peripheral Zone (TPZ) during period with light off (LOFF1) and light on (LON), Vibrational and Light Startle Response (VLSR), Vibrational and Light Motor Response (VLMR), Acclimatization to Sudden Darkness (ASD) and TPZ during LOFF2.

3.2.6 Correlation between the toxicity of the DMSO extracts of FNs and the chemical compound concentrations

The trace metals and more especially the Ag, Cu, Fe, Mn and Zn were positively and significantly correlated with the bioluminescence inhibition of the marine bacteria *A. fischeri* (Figure 3.7). No other significant correlations were observed between toxicity response and trace metals concentrations.

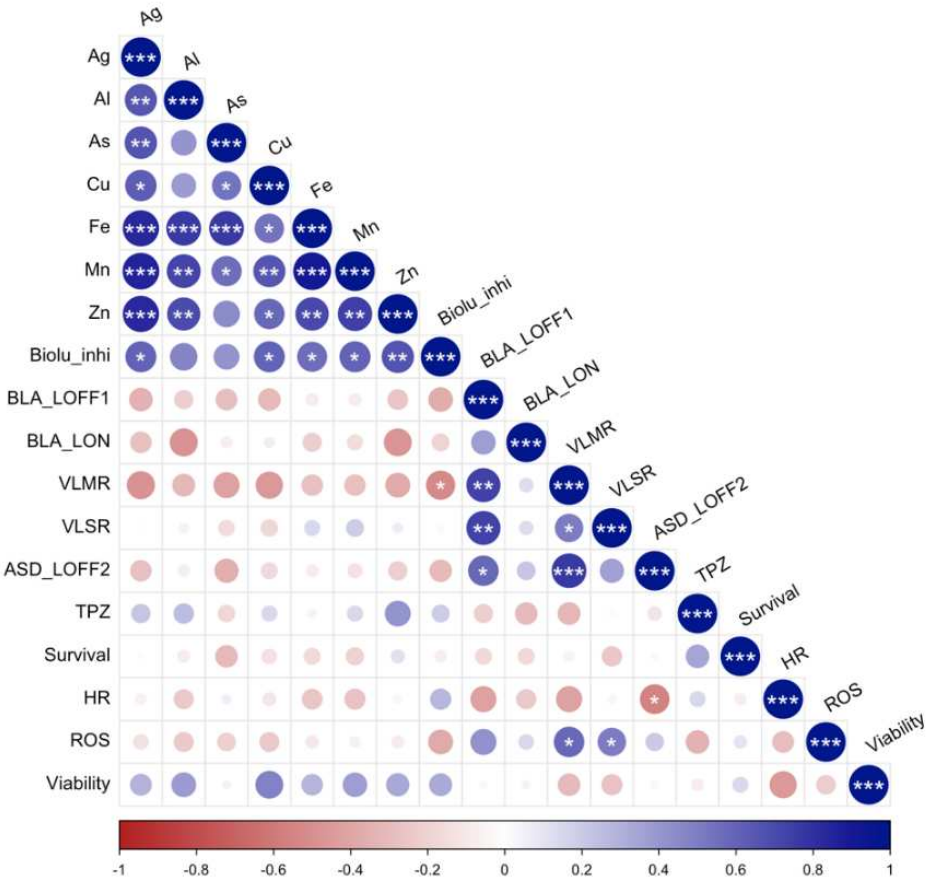


Figure 3.7: Correlation between all the toxicological endpoints and the concentrations of the trace metals considering the DMSO extracts of FNs all together. "Biolu_inhi" means the bioluminescence inhibition (*A. fischeri*).
BLA_LOFF1, BLA_LON, VLMR, VLSR, ASD_LOFF2 and TPZ are the Japanese medaka larvae (*O. latipes*) behaviour endpoints. Heart rate "HR" and "survival" concern the Japanese medaka and "ROS" and "Viability" the RTL-W1 cell line. The matrix uses color-coding to indicate correlation strength: blue for positive and red for negative correlations. The intensity of the color and the diameter of the circles reflect the strength of the correlation, with asterisks denoting statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001).

3.2.7 Integrated biomarker response index

The IBR showed that extracts of seven new FNs and of six EOL FNs caused significant global toxicity for the 3 species studied compared ton DMSO control (Figure 3.8). For the extracts of new FNs, three groups were identifiable, the extract of the new PA β95 (IBR = 3.75±0.16) was significantly more toxic than 6 of the other extracts of FNs. The extract of the new PBS δ35 (second highest IBR at 3.0±0.41) was the only one that did not show a significant difference from the new PA β95 extract. Among the EOL FNs, two groups of extracts were significantly

different in terms of IBR. The PBS δ35 and PA γ33 were the less toxic with an IBR index of 0.37±0.13 and 0.95±0.44, respectively, while the others had an IBR index between 2.58±0.55 (PA β95) and 6.95±3.55 (PBS δ60). The extract of the PBS δ60 EOL FN exhibited the most pronounced effects on the species exposed, and was found to be significantly more toxic than five other extracts of FNs (Figure 3.8).

In general, extracts of EOL FN were more toxic than extracts of new ones (PA α35, PA β33, PA γ60, PBS δ60, PE-PP ε4.25) (independent MWU test new vs. EOL FN extracts: W = 0, p-value < 0.00001). However, no significant evolution of toxicity was identified between the extracts of new PA γ33 and EOL PA γ33 (MWU test: W = 240, p-value = 0.33). The extracts of PA β95 and PBS δ35 new FNs were more toxic than the extracts of EOL FNs (MWU test: W = 576, p-value < 0.00001).

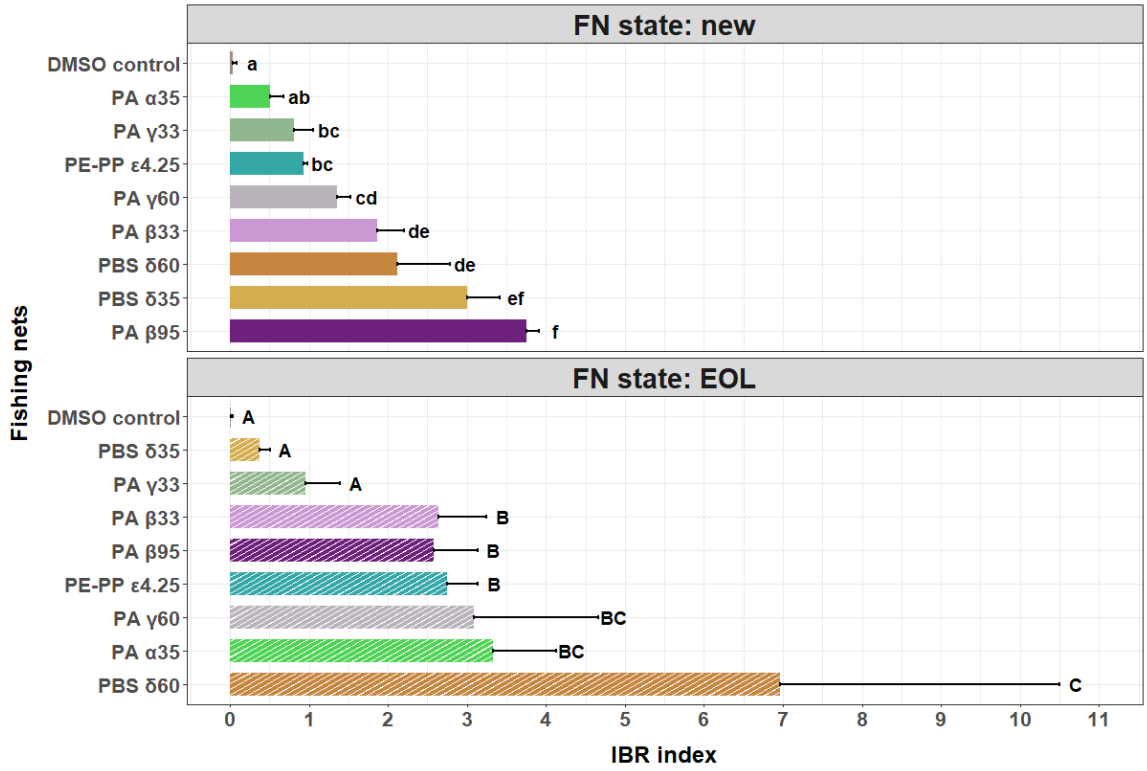


Figure 3.8: Integrated biomarker response (IBR) of the toxicity of DMSO extracts of new and EOL FNs.
Top of the bars represent the mean±SD calculated from 120 geometric permutations (5!). Tested species: *Aliivibrio fischeri*, *RTL-W1 cell line*, and *Oryzias latipes*. Statistics: independent Kruskal-Wallis's tests between artificial aging time (new FNs: n = 24, chi^2 = 203, df = 8, p-value < 0.00001; EOL FNs: n = 24, chi^2 = 167, df = 8, p-value < 0.00001). Post-hoc test executed with the Dunn's test with Holm correction. Different letters mean significant differences between extracts of FNs.

3.3 Discussion

3.3.1 Effects of the operation of FNs on their surface condition

During their operation, FNs deteriorate, starting with loss of physical properties, such as reduced tensile strength and extension, or reduced flexibility, eventually leading to structural modifications (Weller et al. 2015; Brakstad et al. 2022; Le Gué 2024). For bottom trawl nets, some parts, such as the ground gear, the footrope, the bottom panel and the cod end, can be

subject to severe abrasion due to contact with the sea floor during fishing activities (Weissenberger 2015; Bradshaw et al. 2021; Syversen et al. 2022). Regarding the cod end, the plastic filaments that constitute this part are additionally subjected to high tension forces while the trawl nets are being hauled back, due to the accumulation of captured species in it (Brinkhof et al. 2018). These degradation factors can explain the pronounced deterioration of the PE-PP ε4.25 cod end trawl that we studied. For gillnets used half or 1 full fishing season, the condition of the net surfaces was mostly similar to that of new ones and showed no major signs of degradation. After a weathering of 36 months at 20°C, Brakstad et al. 2022, observed similar results for a PA gillnet, while for a PBSAT gillnet they identified the first cracks and holes after 24 days of weathering. Le Gué et al. (2023) also showed comparable results for PBS-PBAT filaments with very low surface degradation after immersion in the marine environment for 18 months at 4°C and 15°C. However, at 25°C they showed a significant degradation with the presence of holes.

3.3.2 The ecotoxicological effects of DMSO extracts of FNs

The use of DMSO to reproduce a stress-test scenario of chemical compounds released from the plastic surface was already shown to be a good method to get a quick hazard assessment of newly studied plastics (Pannetier et al. 2019; Le Bihanic et al. 2020, 2025; Almeda et al. 2023). In our case, the knowledge about the toxicity of various new and EOL conventional and biobased/biodegradable FNs was still very scare. Concerning the marine bacterium *A. fischeri*, the higher toxicity of some extracts was significantly and positively correlated with the concentration of five trace metals and especially the zinc. This was the case for 5 new FNs that released enough trace metals to be toxic. This positive correlation indicated that new FNs can release toxic chemical compounds during their operation. Zimmermann et al. (2020) showed similar results through solid-phase extraction with methanol and concentration in DMSO of various household plastics. The authors calculated an EC_{20} for *A. fischeri* of 85.66±2 mg/mL for a new PBS plastic food tray and a EC_{20} > to 150 mg/mL for a new plastic bag made of PBS. For a waste bag and pellets made of polybutylene adipate terephthalate (PBAT), the same authors calculated EC_{20} of 37.9±4.6 mg/mL and > 150 mg/mL, respectively. Compared to our extracts of PBS-PBAT FNs, EC_{20} were lower (PBS δ35: 1.76±0.22 mg/mL; PBS δ 60: 1.38±0.7 mg/mL), showing higher FN toxicity than food tray, plastic bag, waste bag and pellets made of PBS or PBAT. The extract of the PA β33 new FN (EC_{20} = 2.55±0.29 mg/mL) had a higher toxicity than 100% of the methanol extracts of new household plastic products made of high density polyethylene, polypropylene, polystyrene and polyurethane (Zimmermann et al. 2019). However, one plastic wrap made of polychloride vinyl (1,53 mg/mL) and one shampoo bottle made of polylactic acid (0.07 mg/mL) had lower EC_{20} for the bacterium *A. fischeri* (Zimmermann et al. 2019).

Concerning the RTL-W1 cell viability, at equivalent concentration extracts of EOL FNs were more toxic than DMSO extracts of a mix of MPs (between 300 µm and 5 mm) collected on beaches of islands of the North Atlantic, North Pacific and South Pacific Oceans (Pannetier et al. 2019). Differences in toxicity between new and aged plastics can be explained by (i) a different additive composition depending on the plastic under consideration. (ii) Different additive release kinetics depending on the nature of the plastic and the degree of aging of the

plastic. (iii) More or less adsorption/desorption of pollutants depending on the nature of the material and its degree of aging. Therefore, the capacity of PA FNs to adsorb toxic pollutants during operation, and subsequently desorb them is of concern, given that tens of thousands of tons of PA gillnets were used and lost annually in oceans (Kuczenski et al. 2021). Moreover, it is known that marine organisms, including fishes, can ingest macro, meso, micro and nano pieces of plastic FNs that are lost, discarded or released in the oceans (Ramos et al. 2012; Grade et al. 2019).

The global impact of the FNs on TPZ and the rise of the BLA of the fish during photoperiods underscored that plastic chemicals leached from new FNs into the aquatic environment, possessed the potential to disrupt the basal movement patterns of fish and their stress level during daylight hours. This phenomenon often associated with an increase in fish anxiety may indicate a perturbation of certain neurotransmitters and hormonal systems (Peng et al. 2016; Shi et al. 2022). Moreover, such disruption may lead to an elevated energy consumption, which could impact other energy-demanding physiological processes such as growth, reproduction and feeding (Schreck 2010; Jacquin et al. 2020). This over-consumption of energy by fish larvae was also highlighted by the increased distance traveled during the rest phase after light and vibration stimulation when exposed to the extracts of PA β95 new FN. On the contrary, the decrease in VLMR and ASD response levels indicated that exposure of larvae to extracts of PE-PP ε4.25 and PBS δ60 FNs affected their swimming capacity (Qiu et al. 2022). According to Chen et al., (2017), this could be due to cellular oxidative damage and shorter body lengths.

The perturbation of the startle response of the fish larvae due to exposure to extracts of PA β33 and PBS δ60 EOL FNs highlighted that these EOL FNs can leached plastic chemicals and pollutants able to disrupt behavior. These pollutants can cause neurological and hormonal alterations, affecting the normal development of the visual system (Shi et al. 2022) and an alteration of the lateral line system of the larvae and therefore impacted the startle response (Faucher et al. 2006; Bleckmann & Zelick 2009; Faria et al. 2020). Such behavioral changes could lead to a decrease of the fish's ability to react and dodge predator attacks (Carlson et al. 1998; Weis et al. 2001). Concerning the extracts of the partially biobased and biodegradable PBS δ60 new FN, the significant behavioral disruption of *O. latipes* larvae is of concern as it was 3 to 13.5 times higher than the other extracts of FNs.

3.3.3 Summarized effects of DMSO extracts of FNs on the three aquatic species selected

The IBR index (Devin et al. 2014) was recognized as a useful tool to integrate toxicological data from multiple endpoints and multiple species (Sanchez et al. 2013; Resende & Pereira 2024). Overall, extracts of EOL FNs were more toxic than those of new FNs. However, it is essential to highlight that even if the extracts of EOL FNs were predominantly more toxic, the extracts of new FNs were not without impact, especially the oil-based PA β95 and the partially biobased and biodegradable PBS δ35, which were more toxic new than EOL. The main explanation would be that different kinetic of hazardous chemical release and sorption happened between the different FNs due to differences of polymer/water partition

coefficients (K_{pw}) (Lee et al. 2014; Xiao et al. 2020; Yu et al. 2021; Costigan et al. 2022). This would be particularly true for PA β95 FN in which a significant decrease in trace metals was observed in the extracts of EOL FN compared to the extracts of new FN. Bejgarn et al. (2015) highlighted a similar phenomenon by showing that leachates from four distinct household plastics (two packaging types, a DV case and a biodegradable bag) exerted a considerably greater effect on the survival of the copepod *N. spinipes* after exposure to artificial sunlight aging. On the contrary, artificial aging of four other plastics (garden hose, dashboard, computer, and phone cover) resulted in a reduction in the toxicity of their leachates. According to the authors' primary hypothesis, differences in chemical composition and crystallinity among the various plastics could explain these observed variations in toxicity. Biofilm colonization of plastics, including the EOL FNs, could be another explanation of the difference in chemical release. Indeed, biofilms were shown to change the kinetics of chemical desorption or adsorption by acting as a barrier between the aquatic environment and the plastic matrix (Rummel et al. 2017). However, concerning the sorption of environmental pollutants, biofilms could also act as enhancer of aquatic pollutant adsorption (Luo et al. 2022). Considering the SEM images of the EOL FNs, the PBS δ35 FN seemed to have more organic and inorganic materials than the other FNs but it was not the case for the PA β95 FN. However, based on weathering in two different ports during one year of the same FNs as those studied in this work, qualitative results highlighted a substantial colonization of PA β95 and PBS δ35 with extracellular matrix from the biofilm (Lefebvre et al. 2025).

Focusing on biobased and biodegradable plastics, recent studies of Quade et al., (2022, 2023) showed that two plastic bags made from polylactic acid-polybutylene adipate-co-terephthalate (PLA-PBAT) and maize starch-based polymer respectively, lost their toxicity after 7 days of aging in a marine mesocosm and then regain toxicity after 60 to 120 days. The authors explained that this observation may be due to a greater absorption of UV light causing a quicker but not linear release of toxic additives. These results are reminiscent of the striking increase of the IBR index between the extracts of the PBS δ60 new FN and the EOL one, which significantly rose by 3.2 after 640h of fishing operation over 4 months of fishing. However how to explain the difference between the toxicity of the EOL PBS δ35 and the EOL PBS δ60? In order to address this question, we formulated two primary possibilities. First, the PBS δ35 was less biobased (35%) than the PBS δ60 (50%) and thus, could photodegrade slowly and release fewer toxic additives. Second, these two nets being different panels of the same trammel net, the outer panels (PBS δ60) were potentially more susceptible to receive a higher UV dose that the inner panel (PBS δ35) and therefore leached more toxic additives. Nevertheless, these two last hypotheses remained to be verified.

3.4 Conclusions

This study highlighted differences in toxicity between new and EOL FNs in stress-test leaching scenario. First, we identified that cod end trawl net made of PE-PP was the most degraded FN after fishing activity, while gillnets were more embedded by organic and inorganic matter. Toxicity patterns of DMSO extracts of FNs varied among species, even with similar polymers. Only the effects observed in *A. fischeri* were correlated to trace metals. While the other effects observed were certainly more related to organic plastic chemical or organic environmental

pollutants. DMSO extracts of EOL FNs were generally more toxic than new ones, except for three FNs. In a comparative analysis of trammel nets that were used within the same geographical area and duration, it was observed that the EOL inner panels made of PA and PBS-PBAT exhibited lower levels of toxicity than the corresponding outer panels made of the same polymeric materials. These results prompted for further research to better understand the kinetics of degradation and toxicity of FNs on aquatic species. However, our preliminary findings should increase the awareness of the scientific community and the stakeholders involved in the fishing industry to the capacity of FNs to release toxic additives into the aquatic environment during operation and after loss or abandonment. However, as extractions were performed with DMSO, a solvent more aggressive than seawater, further studies closer to environmental conditions are needed to confirm the extent of plastic chemical release and associated risks.

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3.5 Supplementary Materials

3.5.1 Appendix 3.A

Table 3.A.1: : Limits of quantification (LQ, µg/L), recovery rates (%) at 65 ppb and 220 ppb, and blank results for seven trace elements (Ag, Al, As, Cu, Fe, Mn, Zn) analyzed by ICP-MS.
Recovery values assess method accuracy; blanks confirm no contamination (<LQ) in all samples. Values after the trace metal names in are the wavelengths used for each element in nm. .

	Ag 328,068	Al 396,152	As 228,812	Cu 324,754	Fe 238,204	Mn 257,610	Zn 213,857
LQ (µg/L)	**2.49**	**4.03**	**5.43**	**6.74**	**4.90**	**1.45**	**8.66**
Quality Controls (65 ppb)	**Ag 328,068**	**Al 396,152**	**As 228,812**	**Cu 324,754**	**Fe 238,204**	**Mn 257,610**	**Zn 213,857**
Recovery (%)	**97.28**	**127.82**	**96.88**	**97.54**	**96.51**	**98.94**	**98.37**
Quality Controls (220 ppb)	**Ag 328,068**	**Al 396,152**	**As 228,812**	**Cu 324,754**	**Fe 238,204**	**Mn 257,610**	**Zn 213,857**
Recovery (%)	**94.60**	**97.31**	**93.61**	**93.85**	**93.34**	**96.85**	**94.22**
Blanks	**Ag 328,068**	**Al 396,152**	**As 228,812**	**Cu 324,754**	**Fe 238,204**	**Mn 257,610**	**Zn 213,857**
sample 1	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
sample 2	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
sample 3	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ

Table 3.A.2 : Comparisons of trace metal concentrations in DMSO extracts of FNs.
Table A.2.a comparisons between extracts of PA g33 and PBS d35 FNs and Table A2.b comparison between the extracts of PA g60 and PBS d60 FNs. These trammel nets became EOL FN after the same time of operation (624 h) and in the same marine region (English Channel, France). "< LQ" means that metals concentrations were below the limit of quantification. "NA" means that concentrations differences or statistical were not possible to conduct due to values < LQ. Statistical comparisons: Welch's test.

Table A

Metals	Concentration ± SE		New	p-value	Concentration ± SE		EOL	p-value
	PA γ33 new	PBS δ35 new	PBS δ35 - PA γ33		PA γ33 EOL	PBS δ35 EOL	PBS δ35 - PA γ33	
Ag	2.78±1.41	3.7±0.67	0.92	> 0.05	0±0	22.8±3.81	22.8	**< 0.01**
Al	0.37±0.37	0±0	-0.37	> 0.05	0±0	150.93±123.23	150.93	> 0.05
As	7.01±2.51	3±1.38	-4.01	> 0.05	0±0	11.08±1.13	11.08	**< 0.01**
Cu	12.61±10.92	194.55±12.11	181.94	**< 0.05**	0±0	14.35±5.46	14.35	**< 0.001**
Fe	2.72±0.94	0.01±0.01	-2.71	> 0.05	1.56±0.94	132.4±108.82	130.84	> 0.05
Mn	0.68±0.05	4.05±0.58	3.37	**< 0.01**	2.73±1.71	101.03±10.1	98.3	**< 0.0001**
Zn	0±0	278.52±34.75	278.52	**< 0.05**	0±0	1020.71±50.1	1020.71	**< 0.001**

Table B

Metals	Concentration ± SE		New	p-value	Concentration ± SE		EOL	p-value
	PA γ60 new	PBS δ60 new	PBS δ60 - PA γ60		PA γ60 EOL	PBS δ60 EOL	PBS δ60 - PA γ60	
Ag	0±0	1.93±0.82	1.93	**< 0.0001**	0±0	5.24±0.55	5.24	**< 0.0001**
Al	0±0	26.05±26.05	26.05	**< 0.001**	0±0	122.12±89.85	122.12	**< 0.01**
As	0±0	6.75±1.07	6.75	**< 0.0001**	0±0	0±0	0	**< 0.001**
Cu	< LQ	251.59±50.16	NA	NA	0±0	64.83±25.88	64.83	**< 0.0001**
Fe	0±0	15.85±5.66	15.85	**< 0.0001**	0±0	14.6±4.09	14.6	**< 0.0001**
Mn	<LQ	4.09±0.25	NA	NA	0±0	33±8.14	33	**< 0.0001**
Zn	0±0	197.72±7.04	197.72	**< 0.0001**	0±0	409.71±18.37	409.71	**< 0.0001**

Table 3.A.3: Effective concentration of DMSO extracts of FNs on the bioluminescence inhibition of the marine bacteria *A. fischeri*. Values indicate EC20±SE. ">3.33 mg/mL" means that the EC20 were higher than the maximum concentration tested.

Fishing nets	EC_{20} (mg/mL)	
	New FNs	EOL FNs
PE-PP ε4.25	>3.33	>3.33
PA α35	>3.33	1.99±0.21
PA β33	2.55±0.29	>3.33
PA β95	>3.33	>3.33
PA γ33	>3.33	>3.33
PA γ60	>3.33	>3.33
PBS δ35	1.76±0.22	>3.33
PBS δ60	1.38±0.72	>3.33

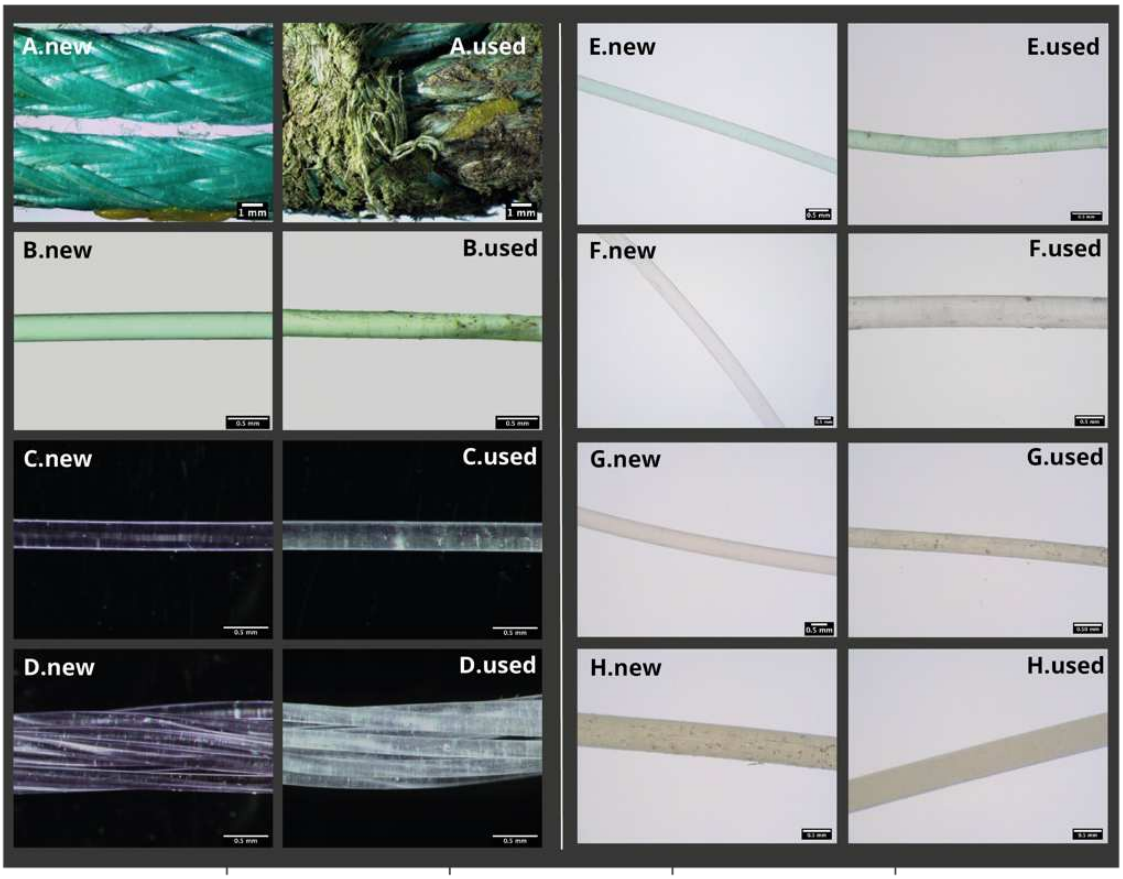


Figure 3.A.1: Brightfield images of the new and EOL filaments of fishing nets (FNs).
A.new and A.EOL are filaments of PE-PP ε4.25 FNs. B.new and B.EOL are filaments of PA α35 FNs. C.new and C.EOL are filaments of PA β33 FNs. D.new and D.EOL are filaments of PA β95 FNs. E.new and E.EOL are filaments of PA γ33 FNs. F.new and F.EOL are filaments of PA γ60 FNs. G.new and G.EOL are filaments pf PBS δ35 FNs. H.new and H.EOL are filaments of the PBS δ60 FNs. "new" indicates images of new fishing nets and "EOL" images of the end-of-life FNs after their operation by fishermen.

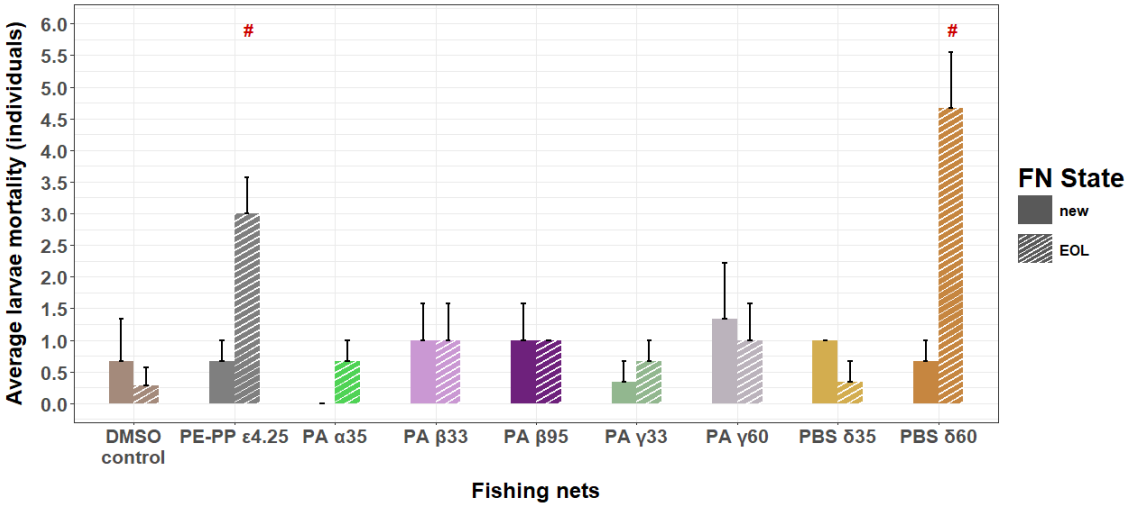


Figure 3.A.2: Mortality of *O. latipes* larvae caused by DMSO extracts of FNs. Top of the bars are the average the mortality.
Full color bars show the new FN extract effects, while the hatched bars represent the EOL FN extract effects. Black lines at the top of the bars represent the standard deviation. Red sharp signs indicate a significant difference of average of extracts of FNs compared to the DMSO control average (Mann–Whitney U-test: # = p-value < 0.05). Sample size by FN state = 3. Number of larvae per replicate = 18 to 20.

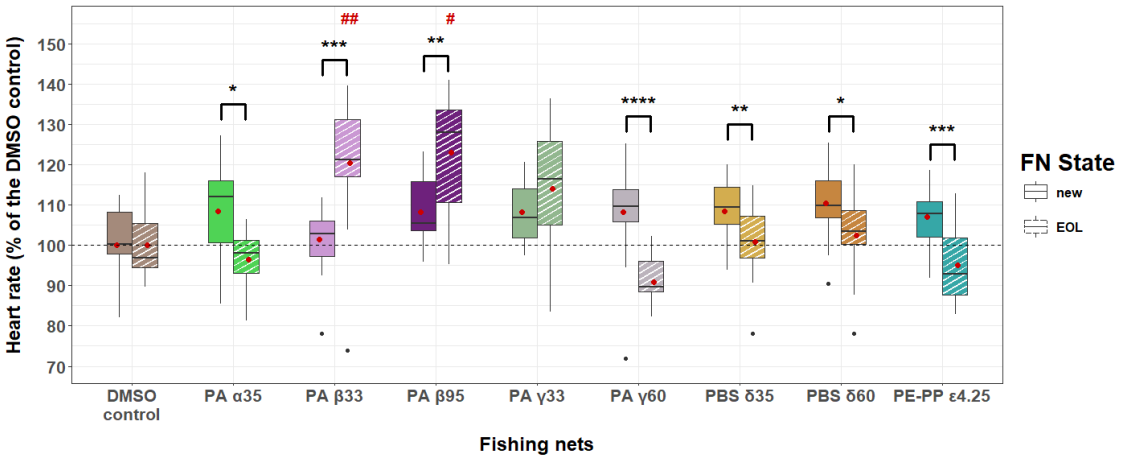


Figure 3.A.3: Heart rate of *O. latipes* larvae measured after 48 h of exposure to DMSO extracts of FNs.
Heart rates of the larvae exposed to the DMSO control were normalized at 100%. Then, the heart rate of the larvae exposed to DMSO extracts of FNs was normalized as a percentage of the DMSO control. The central black line of the boxplot represents the median, and the upper and lower borders are the 25th and 75th percentiles. The whiskers indicate 1.5 times the interquartile range (IQR). The red points represent the means, and the black ones are the outliers. The black dotted line represents the normalized heart rate of the larvae exposed to the DMSO control. Red sharp signs indicate a significant difference of the FN extract average compared to the DMSO control average (Mann–Whitney U-test: # = p-value < 0.05, ## = p-value < 0.01). Black brackets highlight a significant difference between the extracts of new and EOL FNs (Mann–Whitney U-test: * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). Sample size by FN state: n = 16.

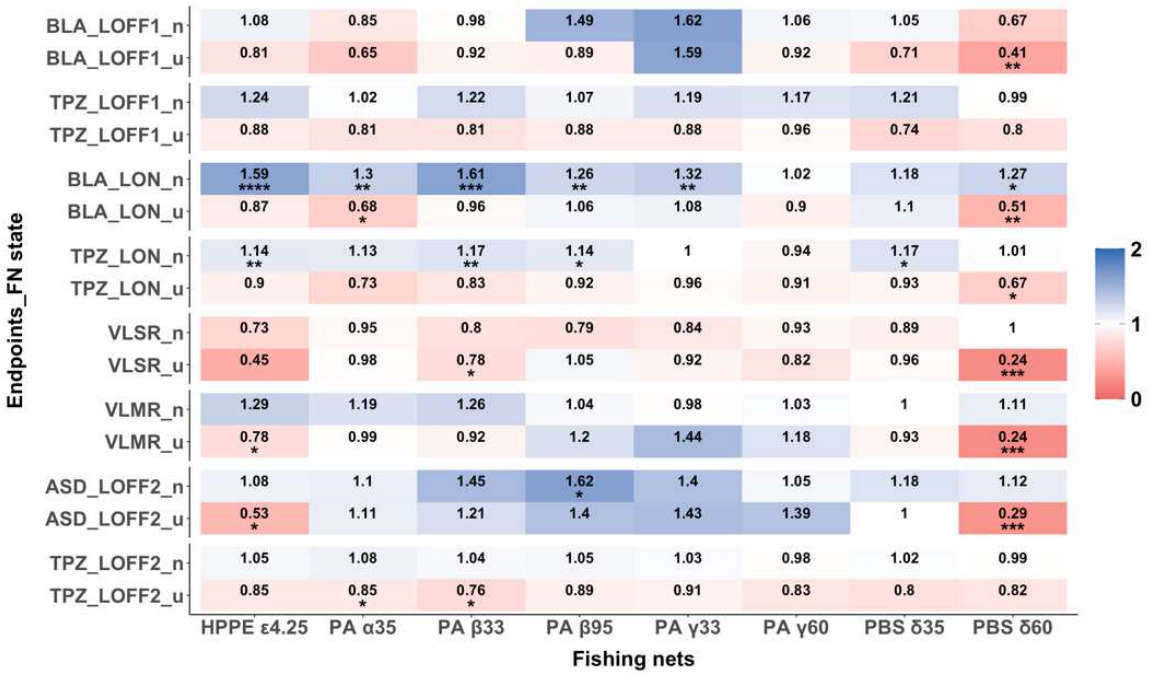


Figure 3.A.4: Effect of DMSO extracts of FNs on the swimming behavior of *Oryzias latipes* larvae.
The abbreviations of the different endpoints are BLA = Basal Locomotor Activity, TPZ = Time in Peripheral Zone, VLSR = Vibrational and Light Startle Response, VLMR = Vibrational and Light Motor Response, ASD = Acclimatization to Sudden Darkness, LOFF = Light Off periods and LON = Light ON period. The letter after each endpoint name ("n", "u") are the FN state (n = new and u = EOL). Values in the cells are the normalized (around 1) deviation from the respective DMSO control value for each endpoint. The scale color on the right represents the deviation from the DMSO control. White cells (value = 1) indicate a low deviation from the DMSO control, red cells illustrate a decrease in the endpoint value according to the DMSO control, and blue cells express an increase in the endpoint values. Asterisks indicate significant differences between larvae exposed to DMSO control and larvae exposed to DMSO extracts of FNs for each FN state and for each endpoint (Independent Dunn's test: * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). Sample size by FN state: n = 48.

3.6 Highlights, main results and conclusions

Highligths of the chapter:

- The operation of the trawl net significantly deteriorated the structural integrity of the cod end net's filaments.
- Partially biobased and biodegradable FNs released more trace metals than oil-based, non-biodegradable ones.
- The bioluminescence of *A. fischeri* was significantly affected by trace metals released by the FNs.
- Most of the extracts from new and EOL FNs significantly affected the RTL-W1 cell line and *O. latipes* behavior.
- The operation time of the FNs led to a change in their toxicity for the three species studied.

Main results of the chapter:

FNs	IMAGING	CHEMICAL COMPOUNDS		BIOASSAYS											
	SEM global aspect	Heavy metals quantif.	POPs quantif.	Marine bacteria (*A.* CE20 (mg/mL)		RTL-W1 cell line Cell viability (ctl. = 100%)		ROS (ctl. = 1 au)		FISH (*O. latipes*) Survival (%)		Heart rate (ctl. = 100%)		Behavior (BSI)	
				new FNs	used FNs	new FNs	used FNs	new FNs	used FNs	new FNs	used FNs	new FNs	used FNs	new FNs	used FNs
PE-PP ε4.25	✓	✓	PLANNED	>3.33	>3.33	**89%**	**91%**	1,04	1,12	96%	82%	105%	93%	**2,5**	**3**
PA6 α35	✓	✓	PLANNED	>3.33	>3.33	**91%**	**75%**	1,01	**1,12**	100%	96%	101%	97%	**1**	**1,5**
PA6 β33	✓	✓	PLANNED	**2.55**	**1.99**	**96%**	**87%**	0,97	0,98	94%	94%	101%	120%	**3,5**	**2**
PA6 β95	✓	✓	PLANNED	>3.33	>3.33	**89%**	**93%**	0,98	0,98	94%	94%	95%	119%	**4,5**	0
PA6 γ33	✓	✓	PLANNED	>3.33	>3.33	**87%**	**90%**	1,06	**1,31**	98%	96%	108%	114%	**1**	0
PA6 γ60	✓	✓	PLANNED	>3.33	>3.33	**91%**	**51%**	1,06	**1,16**	92%	94%	108%	88%	0	0
PBS-PBAT δ35	✓	✓	PLANNED	**1.76**	>3.33	**90%**	**94%**	1,03	1,01	94%	98%	108%	101%	**0,5**	0
PBS-PBAT δ60	✓	✓	PLANNED	**1.38**	>3.33	98%	105%	1,04	1,08	96%	**73%**	110%	99%	**1**	**13,5**

***Values in red and bold represent significant differences between the endpoint and the control.**

Conclusions:

This chapter highlighted that plastic FNs used in the SE BoB possess the potential to release plastic chemicals into the aquatic environment. Furthermore, it showed that partially biobased and biodegradable FNs, which were designed to substitute oil-based, non-biodegradable gillnets, also released trace metals in comparable or greater numbers and quantities. Although the DMSO extracts and exposure conditions constituted a stress test of plastic chemical leaching, the findings underscored that the chemical mixtures derived from FNs can impact multiple aquatic species. The findings also revealed that partially biobased and biodegradable nets exhibited toxicity levels that were comparable to, or exceeded, those of certain oil-based gillnets. In order to comprehensively assess the environmental risks associated with FNs, it is imperative to characterize and quantify the kinetics of their degradation. This includes the subsequent release of degradation products, whether from a chemical, MP, or NP perspective. It would also be interesting to determine whether aging conditions and durations have an impact on the toxicity of FNs' degradation products.

Chapter 4 Distinct toxicity profiles of conventional and biodegradable fishing nets' leachates after artificial aging

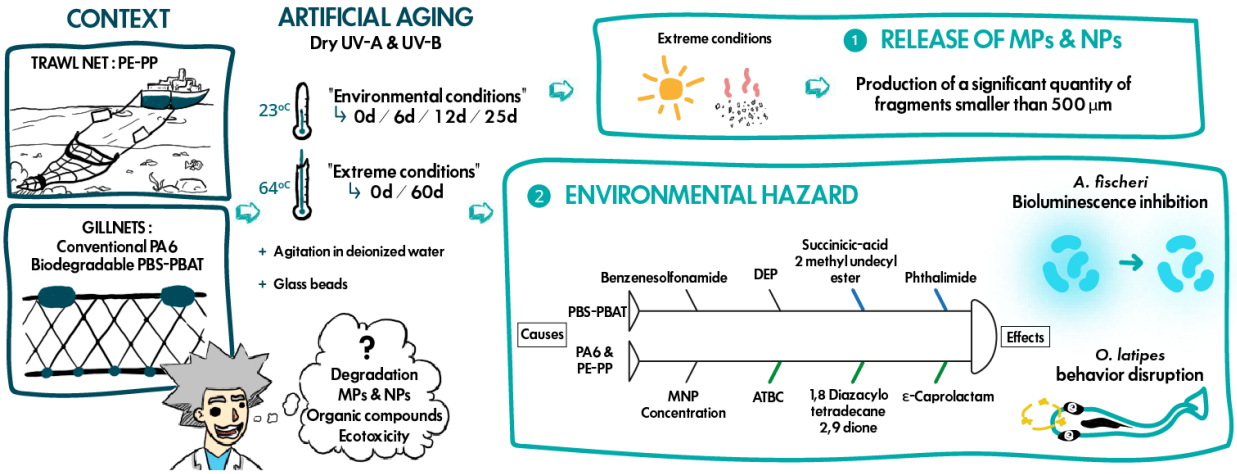
Context:

Given that plastic additives and NIAS contained in FNs are not necessarily covalently bonded to the polymeric matrix, those situated in the superficial layer may be leached during operational activities. However, it remains uncertain whether the aging of FNs contributes to an increase in the quantity of plastic chemicals released. Additionally, it is unclear whether the aging process leads to the formation of MPs and NPs. If so, it is not known whether the duration of aging has an effect on the number of particles produced. A comprehensive investigation into the toxicity of FN degradation products will facilitate a clearer understanding of whether all such products exhibit uniform toxicological effects or if certain degradation products exert a more significant impact. Secondly, using leachates of FNs aged for different durations will help clarify whether the kinetics of degradation products during the aging process results in a linear increase in their toxicity. Thirdly, studying the toxicity of partially biobased and biodegradable FNs will facilitate a preliminary assessment of whether their polymeric and chemical compositions are sufficiently suitable to replace oil-based, non-biodegradable FNs.

Objectives:

To investigate the degradation kinetics of three fishing nets of different polymer composition and different fishing métiers in terms of the release of microplastics, nanoplastics and plastic chemicals and to examine their effects on two aquatic species.

The chapter in one illustration:



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Abstract

Fishing nets (FNs) represent a significant source of plastic waste, but their contribution to pollution by micro- and nanoplastics (MNPs) and associated additives is poorly understood. We studied the degradation of a polyethylene-polypropylene (PE-PP) trawl net and two trammel nets made of polyamide 6 (PA6) or biodegradable polybutylene-succinate-polybutylene-adipate-terephthalate (PBS-PBAT). Accelerated artificial ageing (AA) was performed using UV irradiation under environmental or extreme conditions followed by abrasion in water with glass microbeads. FN degradation and organic compound release were studied as well as the toxicity of leachates on the marine bacteria *Allivibrio fischeri* and larvae of the fish *Oryzias latipes*. AA of FNs under environmental conditions caused slight polymer degradation and did not produce significant MNPs. However, under extreme conditions, PA6 and PBS-PBAT FNs produced 9.1×10^4 MP/mL and 2.0×10^4 MP/mL, respectively. FNs released a total of 27 organic compounds in the leachates from which 7 were quantified at concentrations between 0.35 µg/L (phthalimide) to 200 µg/L (succinic-acid 2-methylallyl-undecyl-ester). Only the PBS-PBAT FN leachates induced significant toxicity on bacteria, bioluminescence inhibition ranging from 26 % to 56 %. Exposure of fish larvae to leachates of AA FNs disrupted their behavior. PBS-PBAT FN leachates caused the highest behavior stress indicator at day 12 (8.5), followed by PA6 at day 25 (8) and PE-PP at day 12 (7). We concluded that the toxicity of FN leachates was related more to the release of organic compounds than to the release of MPs. The toxicity of biobased and biodegradable FNs should be further evaluated before their wider implementation in the fishing sector.

4.1 Introduction

The boom in plastics production starting in the 1950s brought about a profound transformation in all sectors, including the fishing industry (Macfadyen, 2009; Geyer et al., 2017). Cheaper, lighter, more resistant to traction, and offering higher fishing yields, plastic became the primary material used in fishing gear (FG) in the second half of the 20th century (Macfadyen et al., 2009; Dowarah & Devipriya, 2019). It is currently estimated that between 1.5 Mt and 2.09 Mt of plastic FG are used yearly worldwide (Kuczenski et al., 2022). The annual consumption of plastic FG in the European Union is estimated at 53 Kt (Council of the European Union, 2018). Generally, bottom trawl nets are made of polyethylene, polypropylene (PP), or a mixture of PE-PP called polysteel (Napper et al., 2022). Longlines, gillnets, and trammel nets are often made of monofilament polyamide (PA) 6 or 6.6 (Basurko et al., 2023). The lifespan of FG can vary from days to decades, depending on the quality of FG, the fishing fleet, the target fish species and the practices (INdiGO Project, 2021). At their end-of-life, plastic FG are often stored, buried, incinerated or dumped into the environment, contributing to global plastic pollution (Viool et al., 2018; Basurko et al., 2023). Moreover, during the fishing activity FG can be abandoned, lost or otherwise discarded (ALDFG) (Macfadyen et al., 2009). Schwarz et al. (2023) reported that ALDFG represented 6% of annual global ocean macroplastic pollution in 2017, approximately 522 Kt. More conservatively, Kuczenski et al. (2022) estimated a loss of 48.4 to 100 Kt (2.3% to 4.7%) of the global FG used

yearly, while Richardson et al. (2022) estimated a loss of 2.0% annually. More precisely, longline branchlines (3.6%), trawl nets (3.6%) and seines are the three most lost FG. On the European scale, it is reported that the average ALDFG was 20% (10 Kt) of the FG used yearly and 3 to 20% in the number of waste items collected on beaches of the Atlantic and North Sea (Addamo et al., 2017; Council of the European Union, 2018; Viool et al., 2018; Lacroix et al., 2022). Therefore, FG contribute significantly to the pollution of aquatic environments and are a major threat to flora, fauna and marine habitats due to the well-known phenomenon of ghost fishing (Gilman et al., 2016; Lively & Good, 2019; Muñoz-Pérez et al., 2023).

FG and fishing nets (FNs) may degrade and therefore contribute to secondary micro- and nanoplastic (MNP) pollution. Plastic degradation is becoming better understood, and it is recognized that the main driver is photooxidation, followed by thermodegradation, abrasion, and biodegradation (Shamey & Sinha, 2003; Song et al., 2017; Chamas et al., 2020; Hale et al., 2020; Masry et al., 2021; Andrady, 2022). However, limited information is available on the degradation of FNs. Indeed, to our knowledge, only two papers focused on FNs (Brakstad et al., 2022; Le Gué et al., 2023) but they did not study their additive composition, their potential release of MNPs and their potential desorption of chemical compounds. The current trend is rather to solve the problem of ghost fishing and end-of-life FNs by developing biodegradable FNs (Kim et al., 2016; Grimaldo et al., 2018; Grimaldo et al., 2020; Brakstad et al., 2022; Cerbule et al., 2022). A growing number of publications highlighted adverse lethal and sublethal effects of plastic degradation products (Wang et al., 2019; Kögel et al., 2020; Alberghini et al., 2023; Li et al., 2023). Indeed, it has been demonstrated for a wide variety of plastics that toxic effects can be induced by MNPs and/or the release of additives, non-intentionally added substances, and adsorbed pollutants, or a combination of them (Pannetier et al., 2020; Beiras et al., 2021; Cormier et al., 2021a; 2021b; Kang et al., 2021; Katsumiti et al., 2021; Wagner et al., 2024). However, to date, only one work has been published on the ecotoxicological effects of beach-sampled FG (Vilke et al., 2024), but this study did not focus on FNs commonly used by fishermen and did not address the ecotoxicity of their specific degradation products. Concerning the novel biodegradable FNs, no study has been found on their potential effects on aquatic organisms. Thus, this work aimed to investigate the degradation kinetics of artificially aged new FNs through accelerated aging and to study the effects of their degradation products on two aquatic organism models. We hypothesized that the production of MNPs and the composition of additives vary with the FNs. Furthermore, the degradation products of FNs were expected to be toxic, with toxicity increasing with degradation time and the release of MNPs and additives. To test these hypotheses, polysteel, polyamide 6 and polybutylene succinate-polybutylene adipate terephthalate FNs were artificially aged using UV irradiation and then leachates were produced in deionized water using glass microbeads to simulate abrasion. The remaining FN mesoparticles were studied using scanning electron microscopy (SEM), attenuated total-reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, and polarized Raman scattering. The MPs produced were quantified and characterized using a laser direct infrared chemical imaging system (LDIR). The released compounds were analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Finally, the toxicity of the FN leachates was assessed using the bioluminescence

inhibition assay on the marine bacteria *Aliivibrio fischeri* (Microtox®) and the behavioral assay on larvae of the Japanese medaka, *Oryzias latipes*.

4.2 Materials and methods

4.2.1 Fishing nets

Three fishing nets (FNs) of different composition were studied. The first was a conventional green cod end trawl net made of polysteel, referred to as PE-PP in this study (Appendix 4.A, Figure 4.A.1), purchased from the Spanish company Naberan (reseller reference: 3psco0704d, cul de chalut polysteel j-70 4mm double). Polysteel is a copolymer of polyethylene and polypropylene (PE-PP). The diameter of a braided string was 4.25 mm. The second FN studied was a conventional trammel net made of polyamide 6 (PA6) (Appendix 4.A, Figure 4.A.1), purchased from the French company Alprech Filet (reseller reference: Monofilament kit 45x0.33x2496x30.5 mm green - 200x3.5-0.60x416 mm white). In this work, only the two outer panels were studied. Their monofilaments had a diameter of 0.60 mm and were translucent. The third FN tested was a partially biobased 55%, biodegradable and industrially compostable outer panel trammel net made of polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT) (Appendix 4.A, Figure 4.A.1). It also contained polyvinyl acetate at a final concentration of 0.5 to 10%. The monofilament was opaque white with a diameter of 0.60 mm. The Arcachon Bay Marine Natural Park (Office Français de la Biodiversité) provided this FN. The detailed composition of these three plastic FNs remains unknown, although the patent details for PBS-PBAT were available. The two conventional FNs were selected because they were used by fishermen in the southeastern Bay of Biscay (OSPAR Commission, 2020).

4.2.2 Artificial aging

Due to the ubiquitous nature of MNP pollution, strict precautions inspired by Lefebvre et al. (2023) were taken to avoid sample contamination. All handling of FNs and leachates was carried out in a laminar flow hood, regularly cleaned and disinfected. All tools and containers used were made of stainless steel or glass. They were washed three times with filtered (0.22 µm) 70% ethanol and deionized water before each use or sample change. Vinyl gloves and cotton coats were used systematically during the experiments. Blanks were performed in parallel with all steps to estimate cross-contaminations. Any transport of FNs, net pieces, or leachates was conducted only in previously cleaned and sealed containers.

For the AA of the FNs, they were cut into mesopieces of 2 to 2.5 cm, weighed in batches of 0.6 g (environmental conditions) or 0.2 g (extreme conditions), placed in covered quartz pots, and exposed to UV light without water for 6, 12, 25 or 60 days. The UV irradiation process indicated below was partially based on the international guidelines ISO-14892-3:2016(E) for plastic exposure to laboratory light sources (International Organization for Standardization, 2016). Two aging methods were used. First, 60 days of dry UV irradiation (25% UV-$B_{280\text{-}315nm}$ and 75% UV-$A_{315\text{-}400nm}$ at 31 mW/cm^2) at 64°C was used to simulate the worst-case aging scenario

(extreme condition) only for PA6 and PBS-PBAT. Second, an artificial aging protocol with conditions closer to environmental parameters (6, 12 and 25 days of dry UV irradiation with 25% UV-$B_{305\text{-}315nm}$ and 75% UV-$A_{365\text{-}375nm}$, at 40 mW/cm^2 at 23°C) was used to study the degradation kinetics of the three FNs (environmental conditions). The 'environmental' UV A&B chamber was designed and constructed by the Aquatic Ecotoxicology team of the EPOC laboratory (University of Bordeaux, France). This UV chamber was designed to be open source, easy to build, financially accessible, energy efficient, and capable of emitting high irradiation power at low temperatures (23°C) (Dusacre et al., 2024). The correspondence between artificial UV irradiation in the chamber and sunlight was calculated using the method of Gewert et al. (2018). The mean solar irradiance used is the annual average irradiance of the southeastern Bay of and is equal to 68 W/m² (Huld et al., 2012). Therefore, 6, 12, and 25 days of UV irradiation in this chamber are equivalent to one, two, and four years of exposure to sunlight, respectively. After the photo-oxidation period, the leachates were obtained by shaking for 24 h at 190 rpm on an orbital shaker (Ika KS 501 digital, radius 15 mm) with filtered (0.22 µm) deionized water and 62 glass beads (diameter of 1.5 mm) to increase abrasion. Because there was no standardization for the speed of agitation (Da Costa et al., 2018; Mauel et al., 2022; Sun et al., 2022; Wang et al., 2022), we used as shaking speed, the maximum tidal current velocity (0.3 m/s) on the continental margin of the Basque Country and the New-Aquitaine region (Lazure & Desmare, 2012). Conversion from m/s to rpm was performed using the following formula:

$$N_{(rpm)} = \frac{60}{2\pi r} * v_{(m.s^{-1})} \quad \text{(Equation 1)}$$

where N is the number of rotations per minute (rpm), 60 is the number of seconds per minute, r is the radius of the orbital shaker, and v is the tidal current velocity (m.s^{-1}).

Finally, leachates were filtered using a 1 mm stainless steel filter to separate the mesopieces from the leachate. The final volume of the leachates was 60 mL (environmental conditions) and 40 mL (extreme conditions), with initial equivalent mesoplastic concentrations of 1x10^4 mg/L (environmental conditions) and 5x10^3 mg/L (extreme conditions). FN pieces were dried until a constant mass was achieved, weighed, and stored at 4°C. Leachates were divided into different aliquots for the experiments and stored at -20°C. For each condition, three replicates were made and three blanks were also made, following all the steps.

4.2.3 Structural analysis of fishing nets

Images were captured by scanning electron microscopy (SEM) to characterize the effects of AA on the surface structure of FNs. The FN pieces were randomly selected, thinly coated with platinum using a platinum coater (JEOL JFC-1300) and placed in the SEM (JEOL, JSM 6510 LV, IMMM, Le Mans, France). The images were taken at four different magnifications (x50, x100, x250, x500) with an accelerating voltage of 20 kV, a working distance of 20 mm, and a spot size of 30 nm.

Polarized Raman scattering analyses were conducted on FN filaments to calculate the dichroic ratio and to observe the evolution of polymer orientation, particularly the crystalline phase. Analyses were performed using a WITec Alpha 300RS instrument (WITec GmbH, Ulm,

Germany) equipped with an EMCCD high-intensity low-noise camera and a high-precision piezoelectric scanning stage. Raman spectra were recorded from 200 to 3,700 cm^{-1} (600 g per mm grating, spectral resolution of 8 cm^{-1}) using a 532 nm excitation wavelength. The beam was focused on the sample with a Zeiss objective (20×/0.4 NA), and the laser intensity in the sample was approximately 30 mW. A polarizer was used to change the laser orientation, and two spectra were acquired at 0° and 90° with a 10-second acquisition time per spectrum. This process was repeated on five different filaments for each FN. For each FN, two bands sensitive to the crystalline phase were selected to monitor polymer orientation during the AA kinetics. For PE-PP, the band at 1,421 cm^{-1} (assigned to the stretching mode of the crystalline (orthorhombic) C-C bond) and the band at 2,847 cm^{-1} (assigned to the δCH2 of the methylene group) were chosen (Phan et al., 2022). For PA6, the bands at 1,009 cm^{-1} (methylene group) and 1,657 cm^{-1} (assigned to C=O of the amide group) were selected. For PBS-PBAT, the bands were chosen at 1,617 cm^{-1} (assigned to νC=C bond) and 864 cm^{-1} (assigned to δC-C bond). All data were processed using WITec Project FIVE Plus software (WITec GmbH, Ulm, Germany). Preprocessing included cosmic-ray removal and background subtraction using a shape function. Data analysis was focused on the 400-3,600 cm^{-1} range. For each selected band, the area under the curve was measured for the 0°, and 90° spectra and the absolute dichroic ratio (spectra 90°/spectra 0°) was calculated. The dichroic ratio values for each AA time were statistically compared for each FN.

Attenuated-total-reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy (Nicolet, Nexus 870 equipped with MIRacle diamond crystal) was used to monitor the chemical evolution of the surface structure of the FNs. Three spectra (OMNIC software V9.2.98, Thermo Fisher) were acquired for each pristine FN and for each FN at different aging times (6, 12, 25 days) with a spectral range of 400–4,000 cm^{-1}, a spectral resolution of 4 cm^{-1} and an acquisition of 100 scans. Before being superimposed, spectra of the same FN and irradiation time were averaged, and the baselines were manually corrected.

4.2.4 Microplastic quantification and nanoplastic detection in fishing net leachates

After the AA process and after having been dried to a constant mass, remaining FN filaments (> 1 mm) were weighted using a precision balance (Ohaus Discovery DV215CD, Ohaus Corporation, USA) with a readability of 0.1 mg. Their mass after AA was subtracted from the initial mass of FN put under UV irradiation to calculate the mass lost. Regarding the liquid phase, 2 mL of leachate from each condition and time were collected and stored at -20°C in a sterile glass vial. Samples were thawed at room temperature for MP quantification and used directly. The particles in the leachate were quantified using laser direct infrared chemical imaging system (Agilent 8700 LDIR Chemical Imaging System). The company protocol (Agilent Technologies, 2023) was followed with a few adjustments described below. Five hundred microliters of leachate from each sample were filtered using a 0.8 µm polyethylene terephthalate glycol gold-coated filter from Sterlitech (Auburn, USA). To reduce analysis time and filter consumption, the three replicates of each artificially aged FN were combined and

diluted tenfold in 96% filtered (0.22 µm) ethanol to distribute particles homogeneously in the filter and minimize aggregation. The laser direct infrared chemical imaging analysis was performed in two steps. First, particles above 100 µm were quantified in a 180 mm² area (half of the total filter area) using infrared detection mode and scan acquisition (infrared spectrum range: 975-1,800 cm^{-1}). Then, particles between 4 and 100 µm were quantified using the real image mode and scan acquisition for better size resolution (infrared spectrum range: 800-1,800 cm^{-1}). The 180 mm² area was divided into 25 smaller squares of 7.2 mm², and seven squares were randomly chosen. A power statistical analysis determined that this number of squares was representative ($\alpha = 0.05$, $1-\beta = 0.75$, size effect = 0.38, k = 25). The "Microplastic 2.0" library of the "Agilent Clarity" software (version 1.5.58) was used for spectral matching, consolidated with spectra of the new and artificially aged FN samples. Three blanks were prepared for each condition, following all the steps of the AA process. The MP quantification data were corrected using blank values according to the methods proposed by Dawson et al. (2023). The method selected only particles corresponding to the FN polymers with a 'spectral matching score' > 70% and statistical comparisons with blanks.

Detection of nanoplastics (particles < 1 µm) in the leachates using scanning electron microscopy (SEM) was performed in the leachates of FNs artificially aged for 0, 25, or 60 days. After thawing, the leachates were stirred for 10 seconds and 10 µL of each leachate was deposited on a thin copper foil fixed on a support. The support was placed in a closed glass container and incubated at 35°C for 15 hours. The support surface was coated with a thin layer of platinum and placed in the SEM (Hitachi S-4800 SEM, General Service SGiker, UPV/EHU, Leioa, Basque Country, Spain). The images were taken at two magnifications (x5000, x10000) with an accelerating voltage of 5 kV and a working distance of 4.2 mm.

4.2.5 Chemical analysis of organic chemicals in fishing net leachates

A preliminary non-target analysis was performed using artificially aged FN leachates for 25 days. The leachates (200 mL, equivalent mesoplastic concentration: $1x10^4$ mg/L) were frozen on dry ice and sent to LABOCEA (Plouzané, France). First, a purification step was performed using a Florisil® column. Then three extractions were carried out using the following methods: 1) 25 mL of dichloromethane (Pestipur Carlo Erba, Italy); 2) 10 mL of dichloromethane, 15 mL of ethyl acetate (Pestipur Carlo Erba, Italy), 500 µL of acetic acid and 250 µL sulfuric acid; and 3) 5 mL of dichloromethane. The extracts were purified and concentrated for analysis by gas chromatography coupled with a mass spectrometer (GC/MS SCAN) (column HP 5MS 30, 0.25 mm, 0.25 µM Agilent, USA). The obtained mass spectra were compared with the National Institute of Standards and Technology (NIST) (USA) library (version 2.2, June 10, 2014) for compound identification.

In the second phase, using the results of the non-target analysis (Appendix 4.A, Table 4.A.1), ten compounds were selected for quantitative analysis: ε-caprolactam, Tetradecanoic acid, 1,8-diazacyclo-tetradecane-2,9-dione, succinic acid 2-methylallyl undecyl ester, N-butyl benzenesulfonamide, 2-ethyl-1-hexanol, 2,4-di-tert-butylphenol, diethyl phthalate, acetyl-tributyl-citrate, phthalimide (Appendix 4.A, Table 4.A.2). New artificially aged FN leachates (0, 6, 12, 25 days) (80 mL, equivalent mesoplastic concentration $1x10^4$ mg/L) were produced,

frozen in dry ice, and sent to LABOCEA (Plouzané, France) for quantification of the ten selected organic compounds. Unfortunately, the leachate of PA6 FNs artificially aged for 6 days was lost during the analysis process. Standards for target analysis were purchased from Toronto Research Chemicals (Toronto, Canada) or HPC Standards GmbH (Borsdorf, Germany) for the following compounds: ε-caprolactam (CAS: 105-60-2), tetradecanoic acid (CAS: 544-63-8), 1,8-diaza-2,9-diketocyclotetradecane (CAS: 56403-09-9), N-butyl benzenesulfonamide (CAS: 3622-84-2), 2-ethyl-1-hexanol (CAS: 104-76-7), 2,4-di-tert-butylphenol (CAS : 96-76-4), diethyl phthalate (CAS : 84-66-2), tributyl O-acetylcitrate (CAS: 77-90-7), and phthalimide (CAS: 85-41-6). The standard for succinic acid 2-methylallyl undecyl ester was unavailable, so tetradecanoic acid (CAS: 544-63-8) was used to estimate its concentration. Liquid-liquid extraction was used for analysis. Briefly, an aliquot of leachate was diluted in ultrapure water. Then, extraction with a mixture of methyl chloride (Carlo Erba) and ethyl acetate (Carlo Erba) was performed under acidic conditions. The extract was purified in Florisil® and concentrated at 250 µL. The analyses were performed on a GC/MS Agilent (USA) with a VF 5 MS, 30 m column (Agilent). Helium was used as the carrier gas. Each molecule was identified by three ions and the specific retention time was used to ensure reasonable specificity. The quantification limits of the ten organic compounds are described in Appendix 4.A (Table 4.A.3).

4.2.6 Microtox© bioassays

The acute toxicity of leachates to the marine bacteria *Aliivibrio fischeri* was assessed using the Microtox© bioassay and ISO 11348-3 guidelines (International Organization for Standardization, 2007). Marine bacteria cultures were exposed for 30 min to 100% leachates with NaCl at $2x10^4$ mg/L. The pH of the controls and FN leachates was adjusted to 7.3±0.2 (the optimal pH range for *Aliivibrio fischeri* is 6.5-8.5). Data were normalized to the seawater control containing bacteria in seawater and bioluminescence inhibition was calculated for each sample. Finally, data were statistically compared to blanks.

4.2.7 Fish larvae bioassay

4.2.7.1 Exposure conditions, survival, malformations and heart rate

Japanese medaka (*Oryzias latipes*) embryos (Carbio strain) were purchased and received 24 hours after fertilization from the experimental facility PGP (Physiology Genomique Poissons, INRAE, Rennes, France). The reception and development of the embryos were carried out as described by Bedrossiantz et al. (2023). The temperature of the climate chamber (Snidjers Scientific, Tilburg, The Netherlands) was maintained at 24±0.5°C. The embryo development medium was renewed daily (1/2 v/v), and dead embryos were counted and removed. Immediately after hatching (10-day post fertilization), 18 to 20 larvae per replicate were exposed for 48 hours to 100% artificially aged FN leachates. The exposure medium was renewed daily (1/2 v/v). Only FN leachates produced under environmental conditions were used. Triplicates were performed for each condition. A binocular magnifier was used to assess mortality, swimming ability, and abnormal body curvature at 24 and 48 hours after exposure.

The effects on swimming ability and abnormal body curvature were considered according to what was described in the works of Horie et al. (2022) and Yamagishi et al. (2022). Dead fish were counted and eliminated at 24 hours and at 48 hours the cumulated mortality was calculated, while swimming ability and abnormal body curvature data were recorded only at the end of the 48 hour exposure period.

To measure their heart rate, larvae were prepared as described by Bedrossiantz et al. (2023) at a room temperature of 24±0.5°C. Sixteen individuals (12-day post fertilization) per blank or FN leachate sample were analyzed. Only alive and healthy organisms were selected. A near-infrared camera (Basler acA1300-60gm) mounted on a stereomicroscope (Olympus SZX2-TR30), connected to the open-source software Open Broadcaster Software Studio, was used to capture the videos (30 fps, 1280x720 pixels, 60 seconds). The videos were analyzed using DanioScope™ software Version 1.2 (Noldus, Wageningen, The Netherlands). Three 20-second measurements were recorded and averaged for each individual. Heart rates of fish in blanks were normalized to 100, and data of fish exposed to FN leachates were normalized relative to their respective blanks.

4.2.7.2 Fish behavior

The analysis of fish behavior was carried out using the DanioVision System (Noldus, Wageningen, The Netherlands) composed of a DanioVision observation chamber equipped with a near-infrared camera, a panel of LED for light simulation, a vibrational stimulus mechanism (tapping), a Temperature Control Unit to maintain the chamber at 24±0.5°C, and a computer running the analysis software EthoVision XT (version 17.0.1630). The analysis followed methodologies reported by Bedrossiantz et al. (2023).

Larvae (12-day post fertilization) were placed in 48-well plates and subjected to a 51 min routine which enabled the determination of six behavioral endpoints:

Basal Locomotor Activity (BLA): Distance travelled (cm) by the larvae in the dark during the period from 10 to 20 min (LOFF1 period).

Vibrational Startle Response (VSR): Escape reaction to vibrational stimuli (51 stimuli, intensity 8) in the dark, measured as the distance swum (cm) during the second after the first vibrational stimulus.

Habituation: Area under the curve of the distance travelled during the tapping period from minute 20 to 21 in the dark, indicating the non-associative learning process of ignoring irrelevant stimuli (Best et al., 2008; Colwill, 2019).

Visual Motor Response (VMR): Hyperactivity of the larvae in reaction to a sudden reduction of light intensity, estimated by subtracting the distance travelled during the first 2 minutes of the dark period from minute 41 to 51 (LOFF3) and the last 2 minutes of the light period (intensity 100%) from minute 31 to 41 (LON).

Acclimatization to Sudden Darkness (ASD): Ability of the larvae to recover their BLA over an extended period after the light shut off suddenly. Measured by the area under the curve of the swum distance (cm) during the LOFF3 period.

Time in the Peripheral Zone (TPZ): Time spent by larvae in the border area of the well (ring area: ½ of the well area (diameter of 14 mm)), calculated during the LOFF1, tapping, and LOFF3 periods.

Data were normalized to values in their respective blanks, set at 1. To quantify the overall effects of leachates on larvae, a behavioral stress index was determined based on the work of Villa et al. (2018). For each endpoint, a score was assigned based on the deviation from the blank when it was significantly different: deviation ≤ 0.2 = 0.5;]0.2 - 0.4] = 1;]0.4 - 0.6] = 2;]0.6 - 0.8] = 3;]0.8 - 1] = 4; > 1 = 5. The scores were then summed for each FN and AA time. A higher score indicated a greater significant behavioral effect on the Japanese medaka larvae.

4.2.7.3 Data treatment and statistical analysis

Data treatment and statistical analysis were performed using RStudio (R version 4.3.1, RStudio version 2024.04.0+735). Conditions of application of statistical tests were carefully checked for each test (density plots, Shapiro–Wilk tests, and QQ-plots, Bartlett´s test, Levene´s test). When requirements were met, Student´s t test was used for two groups comparisons. If not, non-parametric Wilcoxon-Mann-Whitney´s test was used. A one-way ANOVA was performed to identify significant differences between more than two groups. If necessary, a Tukey´ s HSD post-hoc test was performed. When the validity criteria for the ANOVA were not met, a Kruskal-Wallis test was used, and if necessary, a Dunn's multiple comparison test with Holm´s correction was carried out. For MP quantification after applying the correction method, a Napierian logarithmic transformation was used to meet the conditions for parametric statistical tests. Data were then represented as back-transformed values using the delta method (Onofri et al., 2010). A principal component analyses based on centered and scaled data and a correlation matrix (Spreaman’s method) were conducted to explore the relationships between degradation variables (MP quantification, organic degradation products) and the resulting toxicity. Variables used included MP and organic compound concentrations (both with blank values subtracted), *A. fischeri* bioluminescence inhibition, and the behavioral stress index for *O. latipes*. To reduce redundancy in the principal component analysis, endpoints related to the time spent by the larvae in the peripheral zone of the well (TPZ LOFF1, TPZ LOFF3, TPZ Tapping) were summed.

4.3 Results

4.3.1 Surface structural modifications of artificially aged fishing nets

No visible differences were observed by SEM between the pristine FNs and the artificially aged ones under environmental conditions (6, 12, 25 days) for the three FNs (Figure 4.1 shows only the pristine mesopieces and those aged for 25 days). However, after 60 days of aging under extreme conditions, substantial degradation was observed in PA6 and PBS-PBAT FNs (Figure 4.1). PA6 FNs exhibited fragmentation through ablation and delamination of the plastic surface layers, resulting in small, fine fragments. Longitudinal cracks that extended deeper into the bulk of the plastic were also visible. PBS-PBAT FNs showed surface layer fragmentation, characterized by numerous transverse and smaller longitudinal cracks.

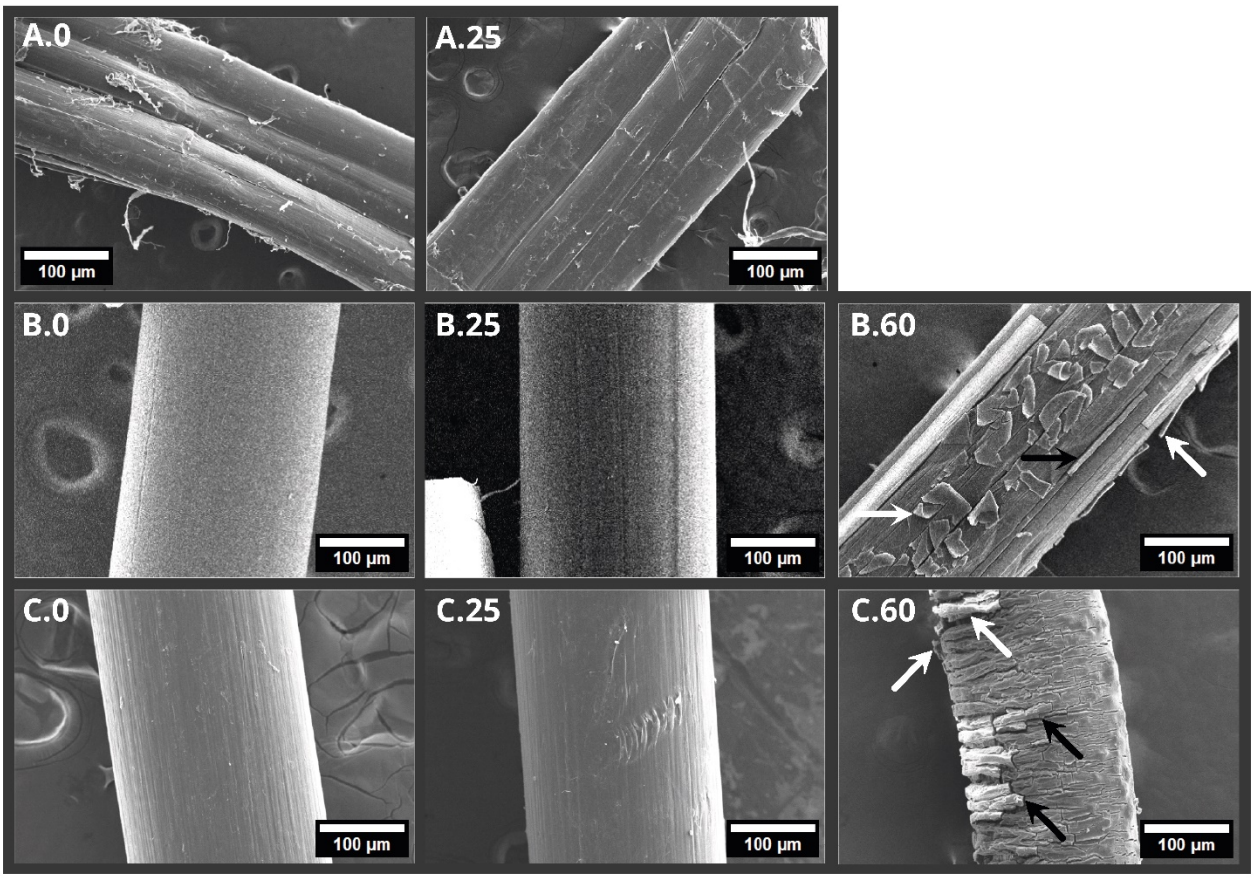

Figure 4.1: SEM images of the pristine and artificially aged (25 and 60 days) mesopieces of fishing nets (FNs).
A.0 and A.25 are the filament of the PE-PP cod end trawl net. A.0 is the filament before artificial aging (AA), and A.25 after 25 days of AA. B.0 and C.0 show the new PA6 and PBS-PBAT FNs, respectively. B.25 and C.25 show the filaments after 25 days of AA. B.60 and C.60 show the filaments artificially aged for 60 days under extreme conditions. In B.60 and C.60, white arrows point to the fragmentation and ablation degradation phenomena. The black arrows highlight longitudinal and transversal cracks. Scale bars are 100 µm.

As to polarized Raman spectroscopy, neither PE-PP nor PA6 FNs showed significant differences in crystallinity after 0, 25, or 60 days of artificial aging (df = 3, p-value > 0.05). On the contrary, for PBS-PBAT FNs artificially aged for 12 or 25 days a significant increase in the crystalline phase of the polymer was observed for both wavelengths studied (864 cm^{-1} and 1,617 cm^{-1}). Significant changes in the crystalline phase occurred between 6 and 12 days of AA with an increase of 0.14 points of the dichroic ratio for the bands at 864 cm^{-1} and 0.13 points of the dichroic ratio for the bands at 1,617 cm^{-1} (Appendix 4.A, Figure 4.A.2). PBS-PBAT FNs irradiated for 60 days were too degraded and emitted too much fluorescence to obtain reliable results using polarized Raman spectroscopy.

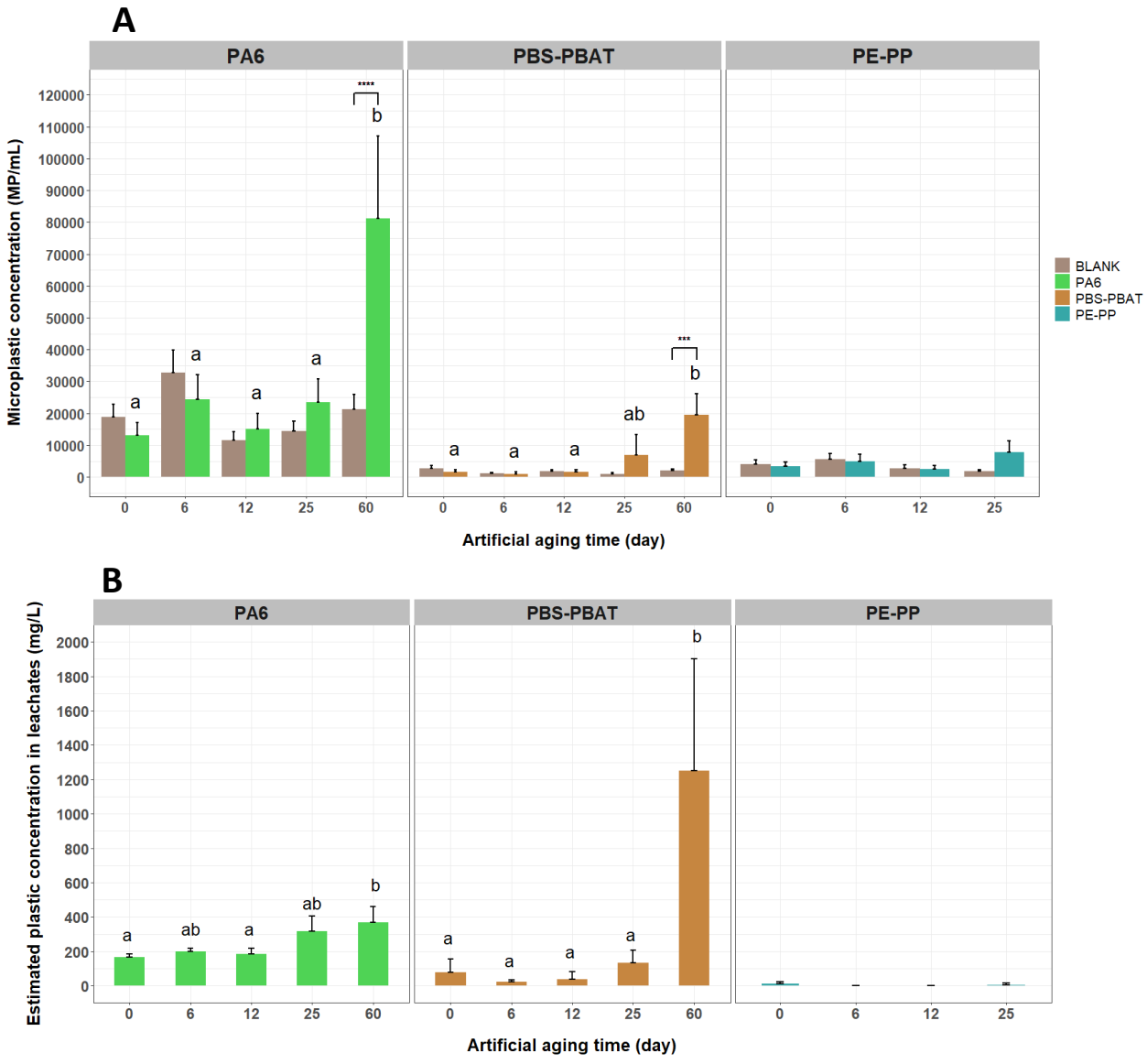

Figure 4.2: A) Microplastic concentration in leachates of artificially aged FNs for 0, 6, 12, 25, or 60 days.
The size range is 4 to 100 µm. The top of the bar represents the back-transformed median and the black line is the back-transformed standard error (SE). Both are calculated with the delta method. Brackets and asterisks indicate significant differences in MP concentration between the FN net and its respective blank for each FN and each time independently (Student's t-test). Sample size by fishing net and AA time: n = 7. **B) PE-PP, PA6, and PBS-PBAT FNs mass loss caused by the AA.** The top of the bars represents the mean percentage, and the black line is the standard deviation (SD). Sample size by FN and AA time: n = 3. In both plots, letters indicate significant differences between AA times for each FN (independent one-way ANOVA, followed by Tukey's HSD post-hoc test).

The surface analysis of the FNs with ATR-FTIR revealed no significant modification of the chemical environment of the PE-PP FN between pristine FNs and FNs artificially aged for 25 days (Appendix 4.A, Figure 4.A.3 and Table 4.A.4). For PBS-PBAT and PA6 FNs, differences between pristine FNs and those artificially aged for 25 days were very slight. However, under extreme AA conditions, there was an overall decrease in PA6 FN absorption intensity, accompanied by a widening of the amine group. While for the PBS-PBAT FN, there was a photooxidation of the methyl and methylene groups, a widening of the carbonyl band, and the disappearance of the bands at 1,270 cm^{-1} and 732 cm^{-1}, indicating a strong impact of AA on this trammel net (Appendix 4.A, Figure 4.A.3 and Table 4.A.4). Therefore, these modifications indicated a change in the chemical environment at the surface of PA6 and PBS-PBAT FNs aged under extreme conditions.

4.3.2 Plastic particle analysis in artificially aged FN leachates

Microplastic quantification by laser direct infrared chemical imaging system revealed a non-negligible amount of MPs in the blanks (Figure 4.2A), likely resulting from contamination during the AA steps or/and potential artifacts detected by the laser direct infrared chemical imaging system. Increasing the system's sensitivity to detect the smallest particles also increased the detection of artifacts, especially when there were very few real particles in the filter. After correcting the data with a "spectral matching score" > 70%, the number of MPs in the leachates (MPs/mL) (Figure 4.2A) and the concentration of plastic particles in the leachates (mg/L) (Figure 4.2B) were significantly correlated for PBS-PBAT FNs (cor.coeff = 0.99, p-value = 0.002), but not for PA6 FNs or PE-PP FNs, primarily due to values being below the mass loss quantification limit for the latter.

AA under environmental conditions did not significantly produce MPs across AA time for PE-PP FN leachates (Figure 4.2A). The mass loss was below the quantification limit (1 mg) and thus under 16.7 mg/L, regardless of the AA time. This corresponds to a mass loss lower than 0.1% (Figure 4.2B). For PA6 FN leachates, the estimated concentration of plastic particles in the leachates ranged from 167±17 mg/L (1.7% mass loss) to 367±95 mg/L (7.3% mass loss) after 0 and 60 days under extreme AA conditions, respectively (Figure 4.2B), with a significant difference only between 0 and 60 days. Similarly, for laser direct infrared chemical imaging MP quantification, only the worst-case scenario caused a significant difference compared to blank leachates (t-value = 10, df = 9, p-value = $6x10^{-6}$) or with the other AA times (F-value = 5, df = 4, p-value = $2.7x10^{-3}$), at a release median of $1.1x10^{5}$ MPs/mL (95% CI: $1.6x10^{4}$ - $1.6x10^{5}$). Blank mean value for PA6 spectral matching was $2.3x10^{4}$ MPs/mL (95% CI: $1.5x10^{4}$ - $4.0x10^{4}$), leading to a corrected value of $9.1x10^{4}$ MPs/mL. Most of the particles were fragments (size ratio L/W = of 1.6). The median particle dimensions were 25 µm in length and 15 µm in width.

Concerning the PBS-PBAT FN leachates, the plastic mass loss and MP quantification did not vary significantly between 0 and 25 days of AA, with a maximum estimated plastic concentration of 133±73 mg/L (1.3% mass loss) and no significant difference in MP concentration between FN and blank. However, under extreme AA conditions, the degradation of PBS-PBAT led to a significant increase in particle concentration in leachates (1,250±651 mg/L, i.e., 25% mass loss) (Figure 4.2B) and a significant production of $2.2x10^{4}$

MP/mL (95% CI: $9.9x10^3$ - $3.8x10^4$) (t-value = 6, df = 10, p-value = $2.2x10^{-4}$) (Figure 4.2). The blank leachate value was $2.4x10^3$ MPs/mL (95% CI: $8.7x10^2$ – $6.4x10^3$), resulting in a corrected value of $2.0x10^4$ MP/mL. Most of the particles were fragments (size ratio L/W = of 1.5). The median particle dimensions were 16 µm in length and 11 µm in width. The size range extended from a minimum of 5 µm for both length and width to maximum dimensions of 43 µm and 33 µm for length and width, respectively (Figure 4.3). For leachates of PA6 and PBS-PBAT FNs, most quantified MPs were between 8 and 12 µm (15% for PA6 FN, 24% for PBS-PBAT FN). The smallest size range (4 to 8 µm) represented only 2% for both FN leachates, mainly because the laser direct infrared chemical imaging system focused on larger particles, preventing the detection of the smallest particles.

SEM analysis of the leachates revealed the presence of nanoplastics below 1 µm (Figure 4.4). The leachates of FNs artificially aged under extreme conditions showed more nanoplastics than those aged under environmental conditions, although a higher abundance of MNPs caused a higher particle aggregation (Figure 4.4).

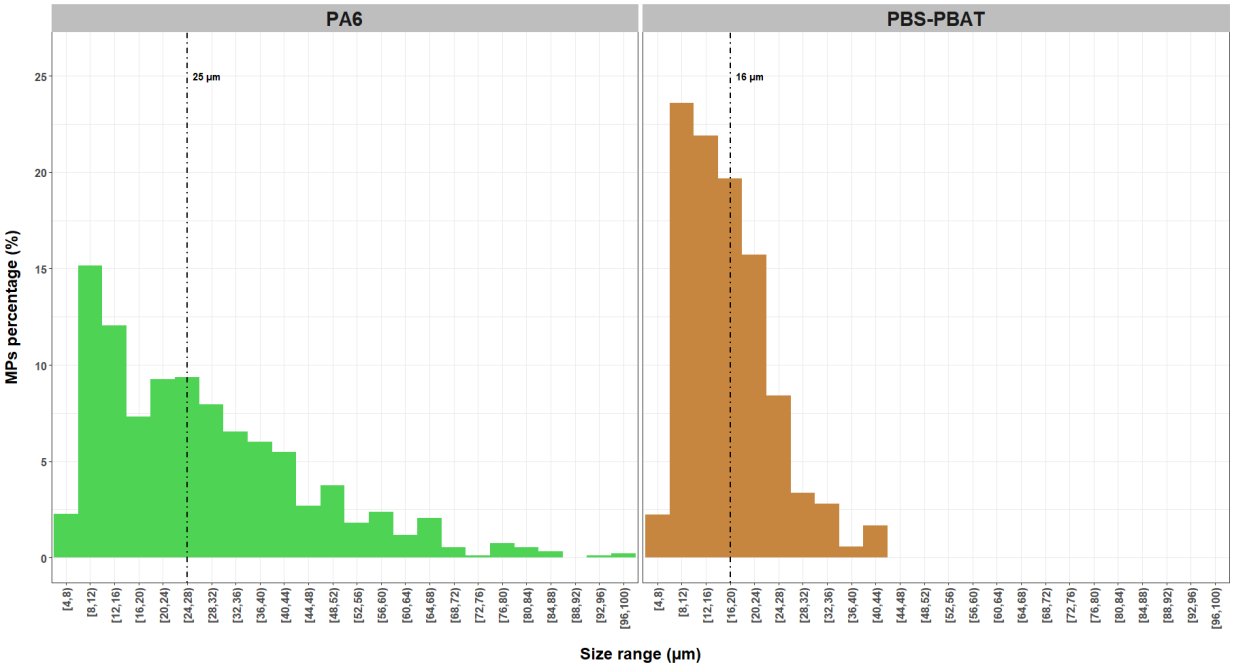

Figure 4.3: MP size distribution quantified in the leachates of PA6 and PBS-PBAT FNs artificially aged for 60 days.
Size range of 4-100 µm with size intervals of 4 µm. The black dotted line is the median length of the quantified MPs.

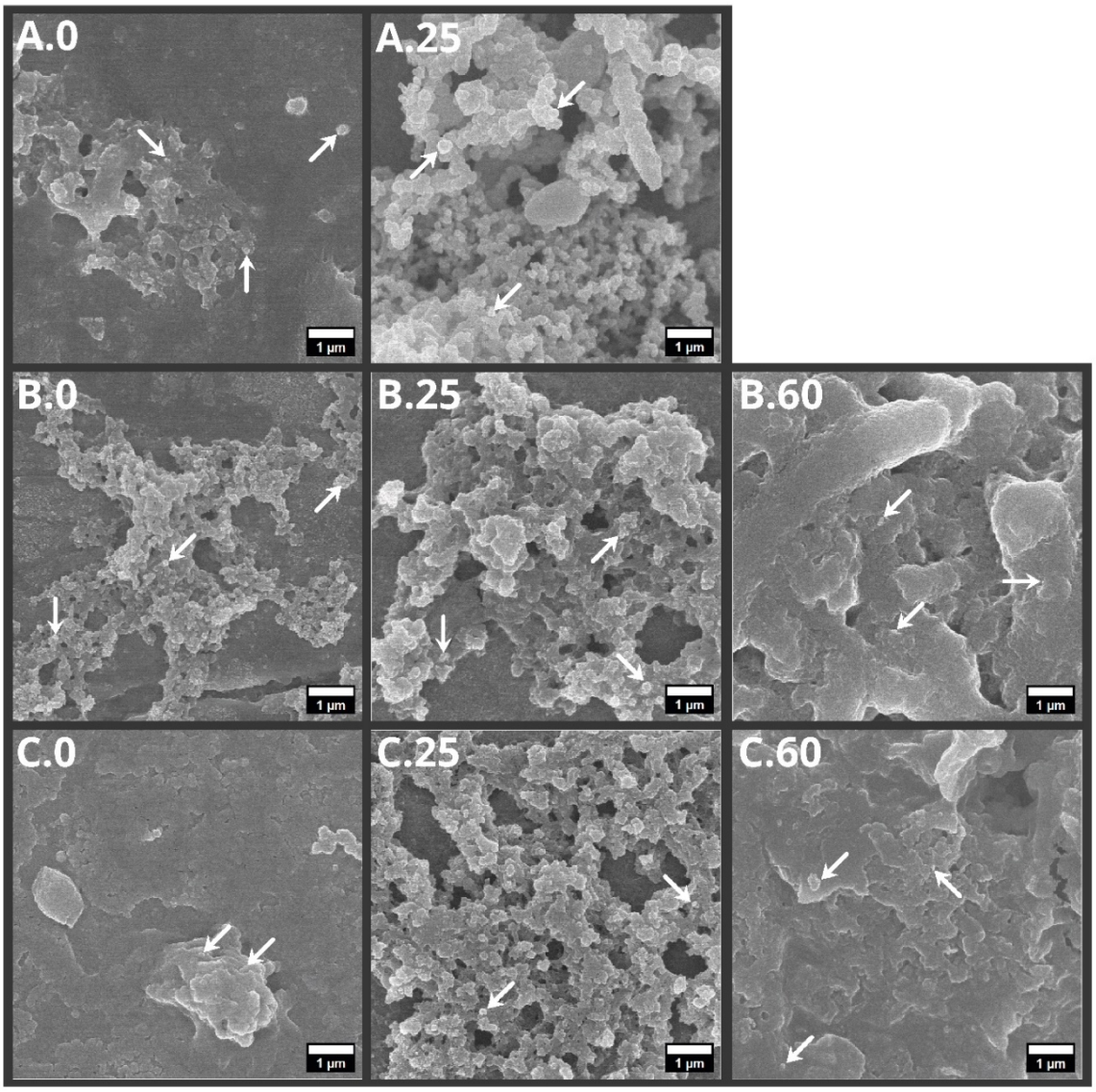

Figure 4.4: SEM images of the leachates of artificially aged FNs for 0, 25 or 60 days (only PA6 and PBS-PBAT).
A.0 and A.25 are leachates of PE-PP FNs artificially aged 0 or 25 days. B.0, B.25, and B.60 are the leachates of PA6 FNs artificially aged 0, 25 or 60 days. C.0, C.25, and C.60 are the leachates of PBS-PBAT FNs artificially aged 0, 25 or 60 days. White arrows indicate nanoparticles inferior to 1 µm. Scale bars are 1 µm.

4.3.3 Organic chemicals in FN leachates

Non-target GC/MS analysis identified 27 compounds in the FN leachates (Appendix 4.A, Table 4.A.1 and Figure 4.A.4). Among these, ten compounds were of particular concern to the aquatic environment, including ε-caprolactam, tetradecanoic acid, 1,8-diazacyclo-tetradecane-2,9-dione, succinic acid 2-methylallyl undecyl ester, N-butyl Benzensulfonamide, 2-ethyl-1-hexanol, 2,4-di-tert-butylphenol, diethyl phthalate, acetyl-tributyl-citrate, phthalamide (Appendix 4.A, Table 4.A.2). The rest of compounds were of lower or unknown hazard. The composition of mineral oil, although unknown, could potentially contain hazardous compounds such as polycyclic aromatic hydrocarbons, alkanes, and isoparaffins.

Quantitative analysis of these ten compounds revealed that seven of them (ε-caprolactam, 1,8-diazacyclo-tetradecane-2,9-dione, succinic acid-2-dimethylallyl undecyl ester,

benzenesulfonamide, diethyl phthalate, acetyl-tributyl-citrate and phthalimide) were present at concentrations exceeding the limit of quantification and blank values (Figure 4.5). In particular, acetyl-tributyl-citrate was the only compound found in PE-PP FN leachates, with its concentration steadily increasing with artificial AA (0 day = 122 µg/L, 6 days = 162 µg/L, 12 days = 182 µg/L, 25 days = 221 µg/L). In PA6 FN leachates, 1,8-diazacyclo-tetradecane-2,9-dione and ε-caprolactam were present at concentrations > 195 µg/L and > 4,200 µg/L, respectively, at each time of AA, while acetyl-tributyl-citrate was quantified above the blank value (69 µg/L) only in leachates of FNs aged 25 days (199 µg/L). The PBS-PBAT FN leachates contained the seven compounds, with 1,8-diazacyclo-tetradecane-2,9-dione slightly exceeding the blank value (28 µg/L), especially in the leachates of FNs aged 12 days (37 µg/L). A similar pattern was observed for acetyl-tributyl-citrate, with concentrations exceeding the blank value (69 µg/L) in the leachates of FNs aged 12 days (85 µg/L) and 25 days (73 µg/L). N-butyl benzenesulfonamide was quantified at all AA times with concentrations > 6.5 µg/L, with a maximum concentration in leachates of FNs aged 12 days (7.1 µg/L). Diethyl phthalate and phthalimide increased until 12 days of AA, with maximum concentrations of 3.3 µg/L and 0.55 µg/L, respectively, before decreasing at 25 days. Finally, the concentration of succinic acid-2-dimethylallyl undecyl ester decreased from 350 µg/L in leachates of FNs that were not irradiated to 200 µg/L in leachates of FNs aged 12 days and increased slightly to 210 µg/L in leachates of the FNs aged 25 days (Figure 4.5).

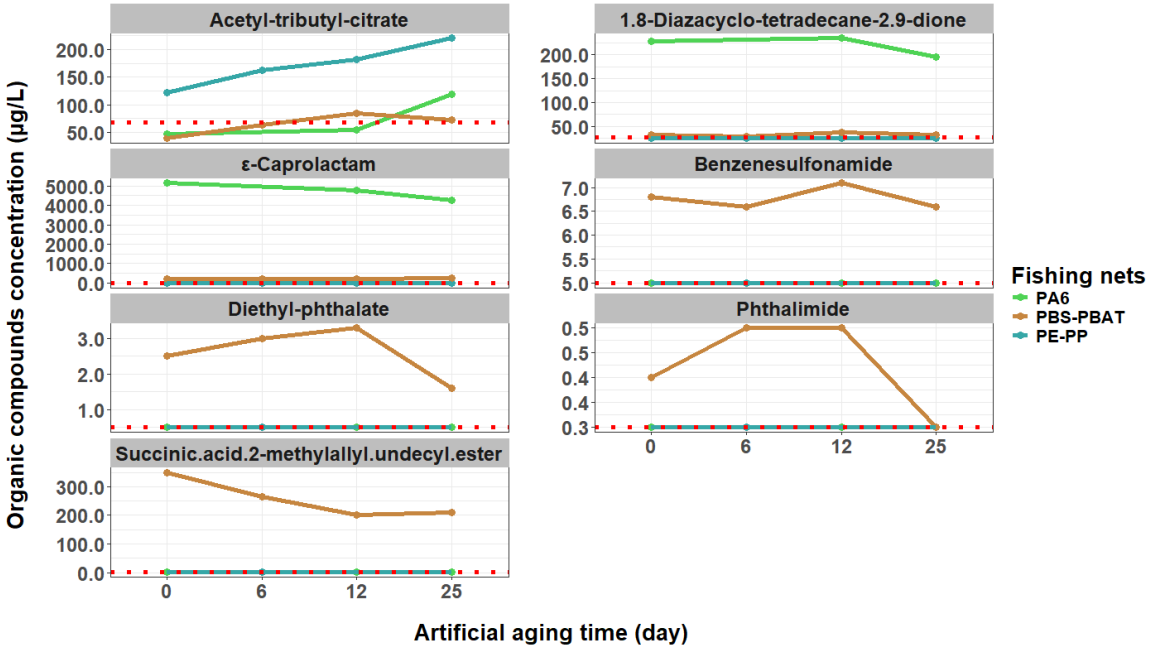

Figure 4.5: Organic compounds leached during the AA of FNs.
The red dotted line represents the higher value between the quantification limit and the concentrations observed in leachates of artificially aged blank samples. The values for succinic acid, 2-methylallyl undecyl-ester, are approximated because the standard of tetradecanoic acid was used for its measurement. Sample size n=1.

4.3.4 Microtox© bioassay

After 30 minutes of exposure to 100% leachates, PE-PP FN leachates did not cause significant bioluminescence inhibition compared to the blank leachates (Figure 4.6). Additionally, no significant difference in bioluminescence inhibition was observed between the different AA times. Leachates of PA6 FNs aged 0, 12, 25 and 60 days caused significant bioluminescence inhibition compared to the blanks. However, bioluminescence inhibition of leachates produced under environmental AA conditions was less than 20%. No significant differences were observed between the different AA times.

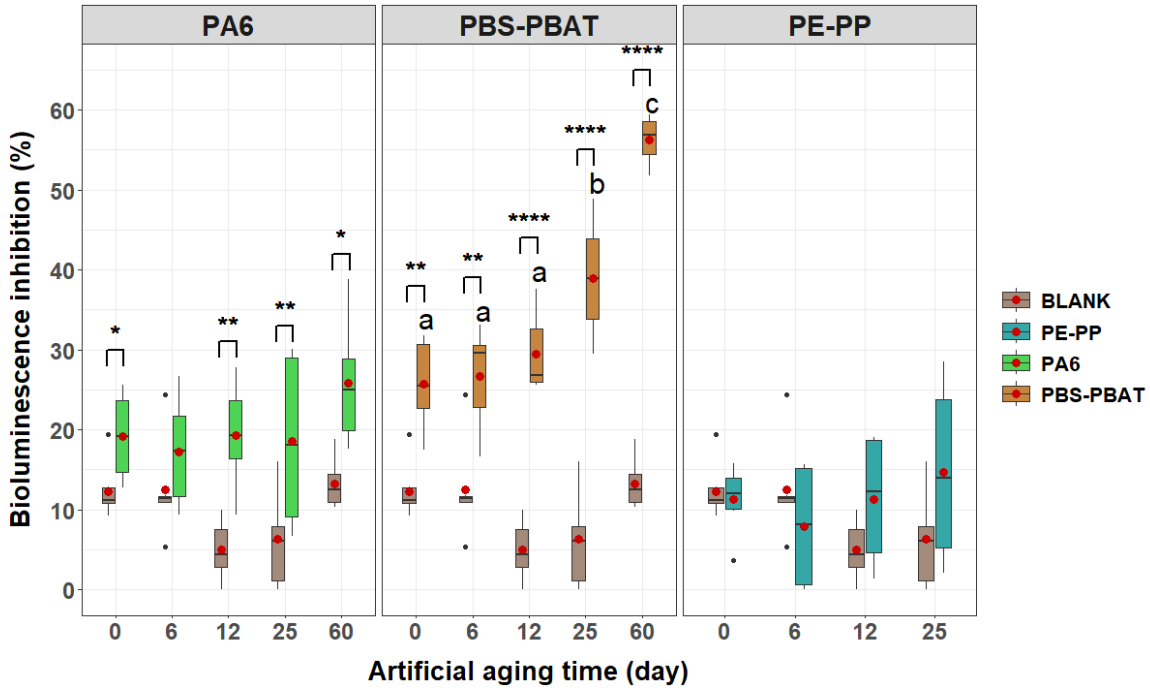

Figure 4.6: Bioluminescence inhibition of the marine bacteria *Aliivibrio fischeri* exposed to FN leachates after various artificial aging times.
Data normalized to seawater controls. The central black line of the boxplot represents the median, and the upper and lower borders are the 25th and 75th percentiles. The whiskers indicate 1.5 times the interquartile range (IQR). The red points represent the means, and the black points are the outliers. Letters indicate significant differences among AA times (One-way ANOVA for each FN, p-value < 0.05, Tukey's HSD post-hoc test with Holm´s correction). Brackets and asterisks indicate significant differences between the blank and FN leachates (Student´s t test for each FN and AA time. * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). Sample size by FN and AA time: n = 6.

On the contrary, for PBS-PBAT FN leachates, the bioluminescence inhibition increased with AA time. The mean inhibition was greater than 20% for all leachates: 25.7±5.7% for leachates of FNs not irradiated, and 26.7±6.5%, 29.5±5.2%, 39±7.3% and 56.3±3% for the leachates of FNs aged 6, 12, 25 and 60 days, respectively. A significant increase in inhibition was observed between the leachates of FNs aged 25 days and all other AA times [One-way ANOVA, F-value = 17, df = 4, p-value < 0.0001], as well as between the leachates of FNs aged 60 days and all the other AA times [One-way ANOVA, F-value = 17, df = 4, p-value < 0.0001] (Figure 4.6). In conclusion, PA6 FN leachates slightly affected *A. fischeri*, while PBS-PBAT FN leachates were the only ones to cause a significant increase in bioluminescence inhibition with aging time and to exceed 20% bioluminescence inhibition for all AA times.

4.3.5 Fish bioassays

4.3.5.1 Survival, malformations and heart rate

FN leachates did not significantly affect *Oryzias latipes* larvae survival, swimming ability, or body curvature after 48 hours of exposure compared to the blank leachates (Appendix 4.A, Figure 4.A.5). FN leachates had little or no effects on heart rate compared to blank leachates (Appendix 4.A, Figure 4.A.6). Only the heart rate of fish exposed to leachates of PE-PP FNs aged 6 days decreased significantly compared to leachates of blanks aged 6 days (Kruskal-Wallis, Chi2 = 12, df = 3, p-value < 0.01; Dunn´s test: p-value < 0.01). The leachate of the PE-PP FN aged 6 days was also significantly different from all other PE-PP FN leachates (Kruskal-Wallis, Chi2 = 21, df = 3, p-value < 0.001; Dunn's test: p-value < 0.01). PA6 and PBS-PBAT leachates did not significantly affect the heart rate ratio of the medaka larvae (Appendix 4.A, Figure 4.A.6).

4.3.5.2 Fish behavior

Sublethal effects on larvae behavior caused by exposure to FN leachates were summarized in a heatmap (Figure 4.7). Various endpoints, including Basal Locomotor Activity (BLA), Vibrational Startle Response (VSR), Tapping habituation, Visual Motor Response (VMR), Acclimatization to Sudden Darkness (ASD), and Time in the Peripheral Zone (TPZ) during LOFF1, tapping, and LOFF3 periods were analyzed. Leachates of PE-PP and PBS-PBAT FNs aged 12 days significantly affected the BLA [H = 13.37, df = 3, p < 0.0001].

For the same period, the TPZ LOFF1 was affected more homogeneously among the different FN leachates. We observed a significant increase in the time spent in the peripheral zone for larvae exposed to leachates of PE-PP, PA6 and PBS-PBAT FNs aged 12 days [H = 30.61, df= 3, p < 0.0001], while for leachates of FNs aged 25 days, only PE-PP and PA6 FNs caused significant behavioral effects [H = 57.99, df = 3, p < 0.0001]). Exposure to FN leachates did not affect the VSR. However, the two other endpoints calculated for the same stimulus period (TPZ tapping, Habituation) showed a significant response. Indeed, the TPZ tapping increased significantly for the leachates of PE-PP and PBS-PBAT FNs aged 12 days [H = 13.12, df = 3, p < 0.001], and for the leachates of the three FNs aged 25 days [H = 62.45, df = 3, p < 0.0001]. The habituation was less affected, with only one significant difference for larvae exposed to leachates of PE-PP FNs aged 25 days [H = 11.88 df= 3, p < 0.001]. Concerning the VMR, a significant decrease occurred for larvae exposed to leachates of the PE-PP and PA6 FNs aged 6 days [H = 32.97, df = 3, p < 0.0001]. The ASD was significantly affected by leachates of PE-PP FNs aged 6 days [H = 19.11, df = 3, p < 0.001]. Furthermore, the leachates of PA6 FNs aged 25 days also impacted the ASD, with a significant and sharp decrease [H = 18.7, d = 3, p < 0.0001]. For PA6 and PBS-PBAT FN leachates, the ASD increased from 0 to 12 days of AA. Finally, for the TPZ during the LOFF3 period, the three FN leachates caused a similar effect. Larvae stayed significantly longer in the peripheral area when exposed to leachates of FNs aged 12 days [H = 159.24, df= 3, p < 0.0001] or 25 days [H = 302.07, df= 3, p < 0.0001]. Interestingly, the effects on the TPZ were

very similar, regardless of the period studied, with a significant impact from 12 days of AA (Figure 4.7).

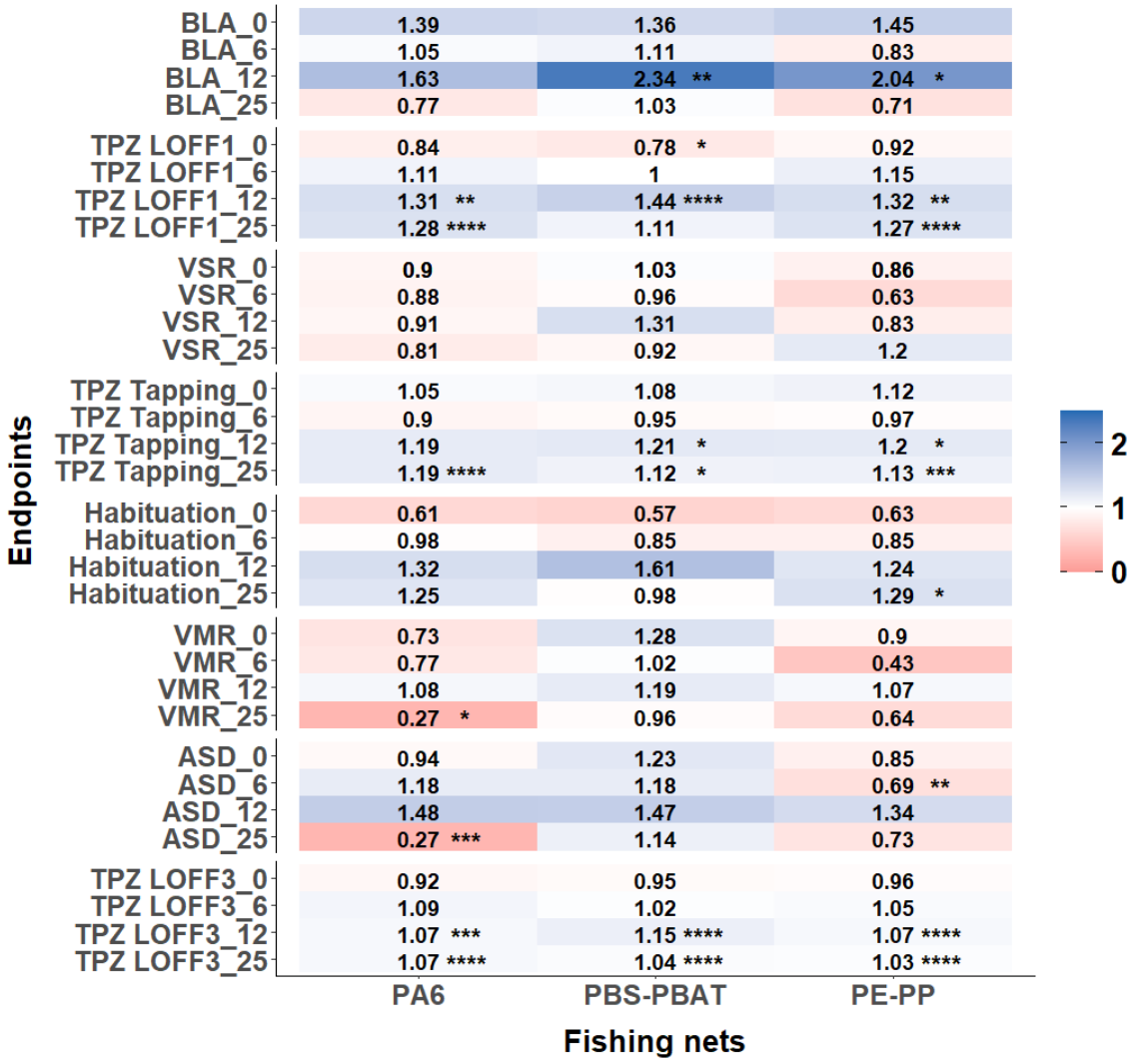

Figure 4.7: Effect of leachates of artificially aged FNs on the swimming behavior of Oryzias latipes larvae.
The abbreviations of the different endpoints are BLA = Basal Locomotor Activity, TPZ = Time in Peripheral Zone, VSR = Vibrational Startle Response, VMR = Vibrational Motor Response, ASD = Acclimatization to Sudden Darkness, and LOFF1 and LOFF3 = Light Off during the first and third periods. The numbers after each endpoint name (0, 6, 12, 25) are the AA times in days. Values in the cells are the normalized (around 1) deviation from their respective blank values for each endpoint. The scale color on the right represents the deviation from the blank. White cells (value = 1) indicate a low deviation from the blank, red cells illustrate a decrease in the endpoint value according to the blank, and blue cells express an increase in the endpoint values. Asterisks indicate significant differences between larvae exposed to blank leachates and larvae exposed to FN leachates at each AA time for each endpoint (Independent Dunn's test: * = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001, **** = p-value < 0.0001). Sample size by FN and AA time: n = 48.

Overall, leachates of FNs irradiated for 12 and 25 days caused more behavioral effects on *Oryzias latipes* larvae than shorter AA (Figure 4.7 and Figure 4.8). However, the behavioral stress index indicated different response patterns among the FNs (Figure 4.8). The highest adverse impacts on fish behavior of PE-PP and PBS-PBAT FN leachates occurred after 12 days of AA. The BLA was the most strongly impacted behavior endpoint since it represented 57% of the PE-PP FN leachates behavioral stress index and 41% of the PBS-PBAT FN leachates behavioral stress index. PBS-PBAT FN leachates had the highest behavioral effect with a

behavioral stress index of 8.5, mainly due to an increased effect on the TPZ during the LOFF1 period. PE-PP FN leachates were the only ones to cause an impact after six days (ASD was significantly affected). PA6 leachates yielded very different outcomes. Indeed, leachates of PA6 FNs began to disrupt larvae behavior after 12 days of AA, as indicated by a behavioral stress index of 1.5. Subsequently, a substantial disruption was observed following exposure to leachates from PA6 FNs aged 25 days (behavioral stress index = 8), with significant impacts on both VMR and ASD, accounting for 86% of the behavioral stress index.

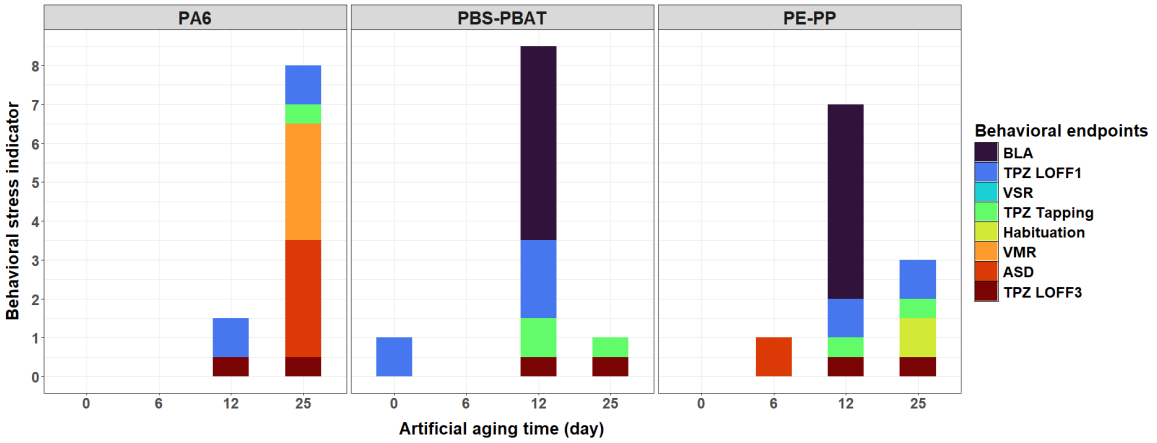

Figure 4.8: *Oryzias latipes*' larvae behavioral stress index.
The stacked bars illustrate the cumulative behavioral stress indexes across different AA times (0, 6, 12, and 25 days) for three types of fishing nets (PE-PP, PA6, and PBS-PBAT). Each colored segment within the bars represents the relative contribution of distinct behavioral endpoints, including Basal Locomotor Activity (BLA), Time in Peripheral Zone (TPZ) during period with light off (LOFF1), Vibrational Startle Response (VSR), TPZ Tapping, Habituation, Vibrational Motor Response (VMR), Acclimatization to Sudden Darkness (ASD), and TPZ LOFF3.

4.3.6 Principal Component Analysis

The principal component analysis aimed to elucidate the relationship between FN degradation products occurring in the leachates and their toxicity. Analysis revealed that three dimensions were necessary to explain most of the variance of data (76.3%) (Figure 4.9). Organic compounds contributed the most to the first two dimensions, followed by the bioluminescence inhibition of *A. fischeri* and three of the six *O. latipes* behavior endpoints (ASD, VMR, and habituation). Four organic compounds (N-butyl benzenesulfonamide, diethyl-phthalate, phthalimide and succinic acid-2-dimethylallyl undecyl ester) were loaded in the same dimension and direction as bioluminescence inhibition, indicating their influence on this endpoint. The correlation matrix (Appendix 4.A, Figure 4.A.7) also showed that the influence of these organic compounds was significantly correlated to the bioluminescence inhibition of *A. fischeri* ($r \geq 0.77$, p-value < 0.01). On the other hand, ε-caprolactam, 1,8-diazacyclo-tetradecane-2,9-dione, and the behavior endpoints VMR and ASD were loaded identically and in the same direction, suggesting a relationship between these variables (Figure 4.9). Additionally, all PA6 FN leachates were grouped in the same area, particularly, leachates of PA6 FNs aged 25 days, indicating their toxicity for Japanese medaka larvae. The principal component analysis also highlighted a relationship between the leachates of PE-PP FNs aged 12 and 25 days, the acetyl-tributyl-citrate and the disruption of the habituation behavior in

response to vibrational stimuli. This suggests that acetyl-tributyl-citrate released by the PE-PP FNs during the AA could had an effect on the behavior of the fish larvae.

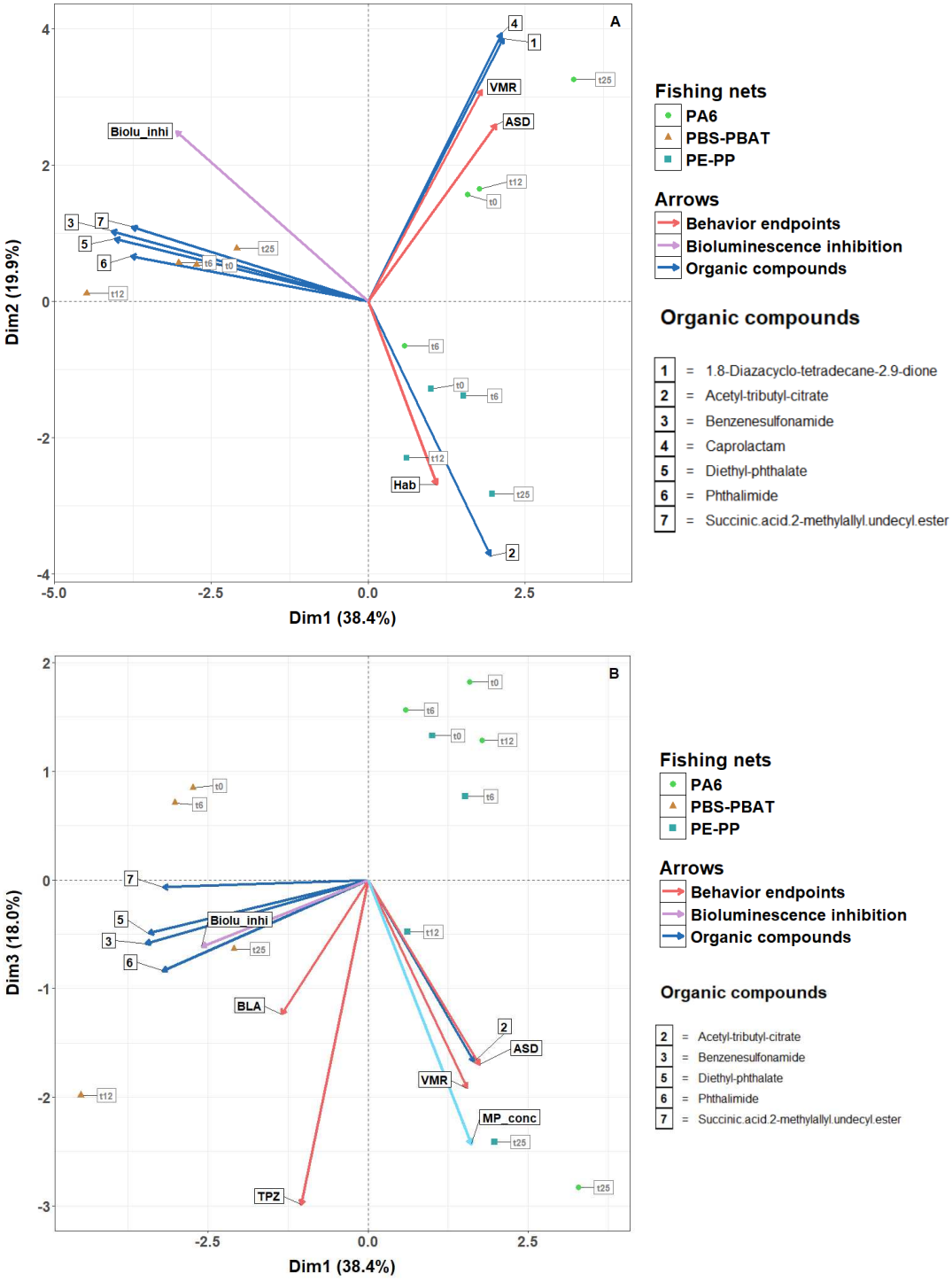

Figure 4.9: Principal component analysis of toxicity endpoints, MP concentrations, and organic compound concentrations. (A) The first and second dimensions explain 58.3% of the variance. (B) The first and third dimensions explain 56.4% of the variance. The pink arrow represents bioluminescence inhibition; the red arrows are the larvae behavior endpoints; the sky blue arrow represents MP concentration, and the dark blue arrows are the seven concentrations of organic compounds. Numbers near the FN points indicate the AA times under 'environmental conditions' (0, 6, 12 or 25 days).

The third dimension provided additional information, particularly on MP concentration and behavior endpoints TPZ and BLA, which had not contributed significantly to the first two dimensions (Figure 4.9). This third dimension strengthened the relationship between the release of phthalimide and the inhibition of bioluminescence in *A. fischeri,* especially for leachates of PBS-PBAT FNs aged 25 days. Furthermore, MP and acetyl-tributyl-citrate concentrations were loaded identically and in the same direction as VMR and ASD, suggesting their role in behavioral effects of leachates. The presence of 25-day PA6 FN leachates and 12-day PE-PP FN leachates in the same sector emphasized their effects on *O. latipes* larvae. On the other hand, the toxicity of PBS-PBAT FN leachates to *O. latipes* larvae was not explained by explanatory variables such MP concentration and quantified organic compounds.

4.4 Discussion

4.4.1 Fishing nets degradation

4.4.1.1 Structural modifications and production of microplastics and nanoplastics

Fishing activity plays a vital role in the global economy and as a primary food source for humans (Macfadyen et al., 2009; Gilman et al., 2016). However, fishing gear, including fishing nets (FNs), contributes significantly to global plastic pollution. Despite the substantial environmental impact of abandoned, lost, or discarded fishing gear (ALDFG), research on its role as a source of micro- and nanoplastics (MNPs) and dissolved chemical compounds remains limited. Understanding the degradation kinetics and potential toxicity of ALDFG is essential for effective mitigation strategies and better management of end-of-life FG.

Under artificial aging conditions closer to the environment (UV irradiation at 23°C for 25 days and shaking at 190 rpm), the structural modifications caused on the three FNs was different, with slight degradation only observed for the crystalline/amorphous phase ratio of the biobased/biodegradable PBS-PBAT trammel net. Because to the best of our knowledge, no studies have been conducted on the controlled degradation of polysteel, we could not compare the results obtained for the PE-PP trawl net. However, a study on polyethylene and polypropylene pellets indicated no degradation after two months of a weak UV irradiation, first cracks appearing after six months, and significant degradation occurring after 12 months for polyethylene pellets (Song et al., 2017). For polypropylene, they observed first cracks after two months and pronounced signs of degradation after six months. On the contrary, in our study, even 25 days of AA (approximately equivalent to 4 years) induced minimal or no structural modifications of the PE-PP FN surfaces, as observed through SEM, polarized Raman, or ATR-FTIR analyses. Furthermore, unlike what is often observed for polyethylene or polypropylene weathering (Song et al., 2017; Andrade et al., 2019; Julienne et al., 2019; Masry et al., 2021), the AA treatment of PE-PP did not provoke photooxidation of the carbonyl band at 1,730 cm^{-1}. This minimal degradation of PE-PP FN was further corroborated by the low mass loss of PE-PP FN after AA and the insignificant release of MPs by PE-PP FN over the UV irradiation period. These differences compared to the literature, can be partially explained by the composition of polyethylene or polypropylene pellets of the raw material that differs from

commercial PE-PP trawl nets, the latter containing more additives, at least partly to withstand physical and chemical degradation processes (Song et al., 2017). Second, the temperature of the experiment made by Song et al. (2017) was approximately twice as high as in our study. It has been reported that elevated temperature, combined with UV irradiation, activates photooxidation and significantly accelerates plastic degradation processes (Gonçalves et al., 2007; Brandon et al., 2016; Masry et al., 2021; Therias et al., 2021). Moreover, the aging and fragmentation of plastics in the environment are highly dependent on several intrinsic parameters, such as the types and contents of their additives, their initial crystallinity, and their shaping and processing, which can explain differences in degradation patterns and kinetic for the same type of plastic.

Concerning the PA6 trammel net, previous studies have highlighted the appearance of holes in PA fibers and filaments when weathered in seawater at room temperature (24-25°C) (Lee et al., 2020; Sørensen et al., 2021). In these works, degradation was hypothesized to be due mainly to hydrolysis, salts, and photooxidation. The low degradation observed in the present work under realistic environmental conditions does not agree with the results of Sørensen et al. (2021). However, the PA used was not the same (PA 6,6 vs. PA 6) and the textile microfibers used in Sørensen et al. (2021) were thinner than the FN filaments studied in this work. Nevertheless, in another experiment, exposure of PA6 to natural weathering for six months caused few visual modifications of the plastic surface (Arrieta et al., 2013). In our case, the low visual degradation was consistent with the non-significant production of microplastics compared to the control. For PBS-PBAT FNs, the results of our study were aligned with the conclusions of Le Gué et al. (2023) who showed few changes in the surface and characteristics of filaments weathered for 18 months in deionized water and seawater at 4 or 15°C. Regarding the chemical structure, there was a slight photooxidation for both trammel nets, which is consistent with several studies (Arrieta et al., 2013; Andrade et al., 2019; Grimaldo et al., 2020; Lee et al., 2020; Sørensen et al., 2021) even though the modifications were less marked in the present study. The significant changes observed by polarized Raman only for PBS-PBAT FNs indicated that this FN was more sensitive to AA than the other oil-based FNs. The modification of the orientation of the polymer chain can be explained by the photooxidation of the amorphous phase (Deroiné et al., 2019; Julienne, 2019; Masry et al., 2021). When the amorphous phase decreased due to oxygen attack, it may have led to chemo-crystallization of the polymer (Masry et al., 2021). Other studies have also reported modifications of polymer properties without visual degradation (Arrieta et al., 2013; Grimaldo et al., 2020; Le Gué et al., 2023). In summary, while remaining conservative and not ignoring the limitations of the laser direct infrared chemical imaging system and the AA protocol, the new PE-PP cod end trawl net and the new 0.60 mm diameter outer panel trammel net of PA6 or PBS-PBAT did not appear to produce any significant quantity of microplastics, after 25 days of AA (equivalent to 4 years in the environment).

However, studying the degradation of the FNs in the worst-case scenario enabled us to understand the evolution of FNs when they become ALDFG and remain in the natural environment exposed to sun light for extended periods. In this context, Grimaldo et al. (2020) and Le Gué et al. (2023) weathered FNs under UV irradiation for 41 days at 38°C and in seawater for 18 months at 40°C and 60°C, respectively. Both studies showed greater

degradation of the filament (longitudinal embrittlement and cracks) under extreme conditions than under environmentally relevant conditions. These observations were consistent with the results of our work, in which extreme conditions led to drastic changes on the surface of the PA6 and PBS-PBAT FNs (ablation, delamination, splitting), as well as to significant modifications of the chemical structure, high mass loss, significant microplastic production, and nanoplastic release (Figure 4.1, Figure 4.2, Figure 4.3, Figure 4.4). The PBS-PBAT FN produced fewer MPs than the PA6 FNs despite experiencing a higher mass loss during the AA process. This could be explained by the biodegradability of the PBS-PBAT FN, which may result in the production of smaller MPs and nanoplastics, as observed through SEM or even in degradation into oligomers, monomers, or other degradation by-products. However, since the complete degradation of PBS-PBAT FNs could not be reached experimentally, it can also be hypothesized that the lower amount of MPs in the PBS-PBAT leachates could come from the impossibility to accurately quantify MPs smaller than 5 µm by laser direct infrared chemical imaging. Indeed, the laser direct infrared chemical imaging system struggled with particles < 10 µm, and robust detection was impossible for particles < 5 µm (Lin et al., 2024). Moreover, the wavelength range of 975 cm^{-1} to 1,800 cm^{-1} of the system was inadequate for identifying polymers with bands above 1,800 cm^{-1}. Nevertheless, the laser direct infrared chemical imaging system and SEM enabled us to study the shape of the released MNPs and revealed that 100% of them were fragments and not fibers, according to the definition of Liu et al. (2019; 2022a). This finding was in line with the results of Napper et al. (2022), who reported that degradation of maritime ropes produced fragments and not fibers. Therefore, our results suggested that the outer panels of both oil-based or biobased trammel nets weathered in the environment for very long periods may produce microplastics and nanoplastics with the shape of fragments.

4.4.1.2 Release of organic chemicals in the leachates

To better understand the potential impact of FNs over time, it was essential to analyze the release of soluble organic compounds in the leachates. Results underlined that even under conditions that caused only slight degradation of the FNs, 27 organic compounds were released in the leachates. Among them, ten were known or suspected to be hazardous to the aquatic environment (ε-caprolactam, tetradecanoic acid, 1,8-diazacyclo-tetradecane-2,9-dione, succinic acid 2-methylallyl undecyl ester, N-butyl benzensulfonamide, 2-ethyl-1-hexanol, 2,4-Di-tert-butylphenol, diethyl phthalate, acetyl-tributyl-citrate, phthalamide) (Chen et al., 2022; Horie et al., 2022; Wagner et al., 2024). Three of these hazardous organic compounds were quantified in all FN leachates (ε-caprolactam, 1,8-diazacyclo-tetradecane-2,9-dione and acetyl-tributyl-citrate), including non-irradiated ones. Two studies also emphasized the release of chemical compounds from fishing gear. Vilke et al. (2024) reported the leaching of 41 organic compounds and 6 trace metals from various ALDFG collected on beaches in southern Portugal. Among the 41 organic compounds, only acetyl-tributyl-citrate and diethyl phthalate, were to the same as those identified and quantified in our work. Chen et al. (2023) quantified 26 additives (mainly antioxidants and plasticizers) in FN leachates commonly used in the Yangtze River and the East China Sea. However, none of the 26 organic compounds they identified were those we detected in this work. Using three different plastic weathering methods, they demonstrated that photooxidation and constant abrasion by waves

contributed to the degradation of plastics and promoted additive leaching. In this present work, we demonstrated that leaching of dissolved organic compounds was not always linearly and positively correlated with the duration of AA. This non-linear relationship between the concentration of organic compounds in leachates and AA time can be explained by the volatility of specific compounds or their photo-oxidation during UV irradiation (Liu et al., 2023; Nzimande et al., 2024). Lastly, it is important to acknowledge that only a fraction of the organic compounds leached by the FNs during AA were identified and quantified. Therefore, further non-targeted analyses methods, such as high-performance liquid chromatography, are required to obtain a more comprehensive list of organic compounds in the FN leachates.

4.4.2 Toxicity of fishing net leachates

4.4.2.1 Effects on the bioluminescence of A. fischeri

After characterizing and quantifying some of the degradation products of artificially aged FNs, we were able to study the toxicity of leachates containing the mixture of MNPs and chemical degradation products. The Microtox© assay revealed varying levels of toxicity depending on the composition of FNs and AA times. For PE-PP FN leachates, the low toxicity for *A. fischeri* can be attributed to the slow degradation rate of this net and the release of compounds below the toxicity threshold of this bacteria (ε-caprolactam and 1,8-diazacyclo-tetradecane-2,9-dione). On the contrary, PA6 and PBS-PBAT FN leachates exhibited significant acute toxicity, but PBS-PBAT FN leachates, which caused bioluminescence inhibition greater than 20% at all AA times, were the most toxic. The organic compounds released (N-butyl benzenesulfonamide, diethyl phthalate, phthtalimide, and succinic acid 2-methylallyl undecyl ester) were strongly linked to this inhibition of bioluminescence and accounted for most of this toxicity. These organic compounds have been reported as endocrine disruptors, cytotoxic agents, and inducers of oxidative stress (Xu et al. 2013; Souza et al., 2020; Zimmermann et al., 2020; Chen, 2022, Wagner et al., 2024). Comparing low density polyethylene, bio-polybutylene succinate, and polybutylene adipate terephthalate pellets, Klein et al. (2021) observed similar results with *A. fischeri*, showing no toxicity after exposure to low density polyethylene leachate but high toxicity with bio-polybutylene succinate and polybutylene adipate terephthalate leachates. They hypothesized that the higher toxicity of biobased and biodegradable plastics was attributed to the release of active chemicals. Lastly, the highly significant toxicity of PA6 and PBS-PBAT FNs artificially aged under extreme conditions raised concerns, as it reproduced the worst-case scenario that ALDFG can suffer.

4.4.2.2 Effects on the behavior of O. latipes larvae

Fish behavior endpoints are increasingly recognized as indicators of physiological disturbances at the individual level (Le Bihanic et al., 2014; Bedrossiantz et al., 2023) and are recommended to be used for risk assessment (Ford et al., 2021). Locomotor disturbances are often considered as a general marker of physiological disorders in response to low concentrations of chemical pollution (Bedrossiantz et al., 2023). Increased basal locomotor activity can lead to excessive energy consumption, potentially disrupting other energy-dependent physiological functions such as growth, reproduction, and foraging (Hirshfield, 1980; Mora et

al., 2016). In the case of FNs, exposure to PE-PP and PBS-PBAT 12 day leachates increased the swimming distance of larvae, potentially causing significant energy loss (Shi et al., 2022). Additionally, the sublethal effects of these two types of FNs affected the time spent in the peripheral zone, which is described as an escape behavior or a sign of anxiety (Chiffre et al., 2016; Cormier et al., 2021b). Anxiety behavior and locomotion perturbations have been observed in the early stages of zebrafish exposed to plastic additives such as 2,4-di-tert-butylphenol and diethyl phthalate. The latter compound, found mainly in PBS-PBAT FN leachates and used in plastic manufacturing, has been described as neurotoxic (Xu et al., 2013; Liu et al., 2022b; Shi et al., 2022), which may partially explain observed behavioral changes. Anxiety could also result from disturbances in steroid biosynthesis induced by endocrine disruptors such as additives potentially originating from plastic degradation, including diethyl phthalate, acetyl-tributyl-citrate, and 2,4-di-tert-butylphenol, even at low concentrations (Xu et al., 2013; Horie et al., 2022; Qiu et al., 2023). In our study, acetyl-tributyl-citrate was strongly related to the toxicity of leachates of PE-PP FNs aged 12 and 25 days and with disruptions in habituation endpoints. The habituation to vibrational stimuli is described as a non-associative learning process in which the organism ignores irrelevant stimuli commonly found in the natural environment. Its disruption may lead to exaggerated reactions to irrelevant stimuli and energy loss (Best et al., 2008; Colwill, 2019). In summary, the leachates of PE-PP and PBS-PBAT FNs predominantly affected behavioral endpoints, which could be associated with a disruption of the energy budget, potentially leading to adverse impacts on the growth and reproduction of fish (Cormier et al., 2021b; Galloway et al., 2016).

On the other hand, leachates of PA6 FNs aged 25 days caused a high level of behavioral disturbance, affecting the visual motor response (VMR) and the acclimatization to sudden darkness (ASD). Shi et al. (2022) described these alterations in the reaction to light stimuli (hyper- or hypoactivity) as impacting the optic nerve, potentially disrupting the development of the visual system and reducing the size of the eyes in early life stages of *Danio rerio*. They suggested using these endpoints as indicators of neurotoxicity that affects the vision of fish. At the ecological level, VMR and ASD are critical responses that enable fish to flee from looming predators (Easter & Nicola, 1996). Consequently, significant decrease in VMR and ASD can, in the natural environment, adversely affect prey-predator interactions, thus provoking ecological disturbances (Weis et al., 2011). In our case, the significant decrease of VMR and ASD were correlated with the presence of ε-caprolactam, 1,8-diazacyclo-tetradecane-2,9-dione, and acetyl-tributyl-citrate in leachates. ε-caprolactam, used extensively in plastic manufacturing, was classified on the red list (chemicals that are hazardous according to well-established criteria and that should be regulated) of the plastic additives of the PlastChem report (Wagner et al., 2024) and described as genotoxic and mutagenic (Kulkarni & Kanekar, 1998). Regarding 1,8-diazacyclo-tetradecane-2,9-dione, the European Chemicals Agency (REACH regulation) suspected it to be hazardous to aquatic species, but our study appeared to be the first to highlight its association with the disruption of the behavior of fish larvae. Additionally, MPs played a role in the toxicity observed for PA6 and PE-PP FN leachates after 25 days of AA, contributing to the disruption of some medaka larvae behavior endpoints (VMR, ASD, and habituation). However, the variability in toxicity among FN leachates appeared primarily attributed to differences in the composition of plastic additives and

chemical degradation products, with MPs playing a less relevant role. As discussed extensively throughout this study and in other research papers, leached plastic additives are now recognized as the main contributors to plastic toxicity (Beiras et al., 2021; Qiu et al., 2022, 2023, Wagner et al., 2024). This article highlighted for the first time that this was also the case for additives and degradation products from both commonly used oil-based and partially biobased/biodegradable FNs.

4.5 Conclusions

Our research reveals that cod end trawl nets made from polysteel and trammel nets composed of PA6 and PBS-PBAT did not release a significant quantity of MPs after artificial aging under environmentally relevant conditions. However, PA6 and PBS-PBAT FNs released a significant amount of MPs under extreme conditions of aging. Further investigations are needed to understand their fragmentation into nanoplastics and to determine whether microplastics from partially biobased and biodegradable nets will fully degrade. This study also confirmed that artificially aged conventional and partially biobased/biodegradable FNs released a substantial amount of soluble organic compounds. These compounds adversely affected the marine bacteria *A. fischeri* and the behavior of *O. latipes* larvae. The leachates of the PE-PP trawl net and PA6 trammel net mainly caused sublethal effects on *O. latipes*, while the partially biobased and biodegradable PBS-PBAT FNs induced the most severe effects on both test organisms. The disruption of fish behaviour could have significant ecological consequences through affecting prey-predator relations, growth and reproduction. Therefore, while biodegradable nets hold promises for mitigating ghost-fishing, further refinement in their composition is necessary. Finally, given these results and those of other studies, the chemical composition of FNs and plastics in general must be monitored and openly accessible, especially for public research. It is fundamental to advocate for mandatory disclosure of plastic composition to ensure a comprehensive understanding and effective management of the environmental impacts of plastics.

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4.6 Supplementary materials

4.6.1 Appendix 4.A

Table 4.A.1: Non-target analysis by GC/MS of organic additives or degradation products in the PE-PP, PA6, and PBS-PBAT FN leachates.

Retention time	Compound	N°CAS
4.23	2-ethyl-1-hexanol	104-76-7
4.33	Benzyl alcohol	100-51-6
7.32	ε-caprolactam	105-60-2
8.23	Non-identified	/
9.94	2,4-di-tert-butylphenol	96-76-4
10.48	Dodecanoic acid	143-07-7
10.91	Dibutyl itaconate	2155-60-4
10.93	Diethyl phthalate (DEP)	84-66-2
11.41	Non-identified diester	/
11.46	Non-identified diester	/
12.42	Non-identified	/
12.70	Tetradecanoic acid	544-63-8
13.50	Benzenesulfonamide, N-Butyl	3622-84-2
14.59	Non-identified diester	/
16.66	1-Propene-1,2,3-tricarboxylic acid,tributyl ester	7568-58-3
16.92	Butyl citrate	77-94-1
17.60	Acetyl tributyl citrate	77-90-7
17.90	1,8-diazacyclotetradecane-2,9-dione	5776-79-4
19.89	Non-identified	/

20.10	2,3,5-Tri-O-acetyl-4,6-di-O-methyl-D mannonitrile	58720-12-0
21.55	Non-identified amide	/
22.37	Dodecanoic acid 3-dodecanoyloxy-propyl ester	26719-54-0
23.40	Non-identified	/
17 to 27	Mineral oil	/
27.51	Non-identified	/
28.13	Succinic acid, 2-methylallyl undecyl ester	/
31.35	Non-identified	/

Table 4.A.2: The ten organic compounds considered hazardous for the aquatic environment and quantified in the PE-PP, PA6, and PBS-PBAT FN leachates.

ID	Compounds	Compounds nature	Ecotoxicity	PlastChem list	References
1	ε-caprolactam	Plasticizer; Catalyst; Monomer; Intermediate; Filler; Solvent; Colorant	Genotoxic Mutagenic	Red list	(Brakstad et al., 2022) (Kulkarni and Kanekar, 1998) (Wagner et al., 2024)
2	Tetradecanoic acid	Lubricant; Intermediate; Heat stabilizer; Monomer; Odor agent	Potential; tumorigenic	Red list	(Lahimer et al., 2017) ("Oleic Lauric", 1987) (Wagner et al., 2024)
3	1,8-diazacyclotetradecane-2,9-dione	NIAS; Degradation product	/	Grey list	(Brakstad et al., 2022) (Dubé, 2016) (Wagner et al., 2024)
4	Succinic acid 2-methylallyl undecyl ester	NIAS; Degradation product	Algae growth inhibitor	/	(Brakstad et al., 2022) (Chen et al., 2022) (Wagner et al., 2024)
5	Benzenesulfonamide, N-butyl	Plasticizer; Filler; Intermediate	/	Red list	(Wagner et al., 2024)
6	2-ethyl-1-hexanol	Plasticizer; Lubricant; Intermediate; Monomer; Colorant; Odor agent	Neurotoxic at high doses	Red list	(Haz-Map, 2023) (Wagner et al., 2024)
7	2,4-di-tert-butylphenol	Light stabilizer; Antioxidant; Intermediate; Lubricant	Hepatoxic; Endocrine disruptor; Locomotion disruptor (fish); Anxiety inducer (fish); Algae growth inhibitor	Red list	(Liu et al., 2022) (Shi et al., 2022) (Wagner et al., 2024)
8	Diethyl phthalate	Plasticizer; Biocide; Colorant; Intermediate; Filler; Solvent; Odor agent; Lubricant	Oxidative stress inducer; Hepatoxic; Potential neurotoxic; Endocrine disruptor	Red list	(Ghorpade et al., 2002) (Tran et al., 2021) (Xu et al., 2013) (Wagner et al., 2024)

Continuation Table 4.A.2

9	Acetyl tributyl citrate	Non-phtalate Plasticizer; Biocide; Lubricant; Intermediate; Colorant; Odor agent	Embryotoxic ; Endocrine disruptor	Red list	(Horie et al., 2022) (Muhammad et al., 2018) (Wagner et al., 2024)
10	Phthalimide	Intermediate in colorant and pigments	Affects the gastro-intestinal tube and the central nervous and respiratory systems	Grey list	(Chen et al., 2022) (Singh et al., 2022) (Wagner et al., 2024)

Table 4.A.3: Quantification limits of the ten organic compounds quantified by analyzed by GC/MS in the PE-PP, PA6, and PBS-PBAT FN leachates.

ID	Compounds	Quantification limit (µg/L)
1	ε-caprolactam	5
2	Tetradecanoic acid	100
3	1,8-diazacyclotetradecane-2,9-dione	1
4	Succinic acid 2-methylallyl undecyl ester	1
5	Benzenesulfonamide, N-butyl	5
6	2-ethyl-1-hexanol	0.5
7	2,4-Di-tert-butylphenol	1
8	Diethyl phthalate	0.5
9	Acetyl tributyl citrate	5
10	Phthalimide	0.3

Table 4.A.4: Evolution of main chemical groups of the FNs due to AA. Measures were made using ATR-FTIR spectroscopy.

Fishing nets	Wavelength (cm^{-1})	Chemical group	Modification
PE-PP	2,961 1,379	Methyl (CH_3)	No
	2,918 2,949 1,464	Methylene (CH_2)	No
	1,745	Carbonyl (C=O)	No
PA6	3,296	Amine N-H stretching	Widening and strong absorption decrease after 60 days of AA
	2,932 2,866	Methylene CH_2 stretching	Absorption decrease from 6 days of AA with a strong decrease after 60 of AA
	1,632	Amide I C=O stretching	Strong absorption decrease after 60 days of AA
	1,539	Amide II C-N stretching & N-H bending	Strong absorption decrease after 60 days of AA
	1,265	Amide III C-N stretching	Strong absorption decrease after 60 days of AA
	681	C-C bending	Strong absorption decrease after 60 days of AA
PBS-PBAT	2,967	Methyl CH_3 stretching	Strong absorption decrease after 60 days of AA
	2,922 2,855	Methylene CH_2 stretching	Strong absorption decrease after 60 days of AA
	1,750	Carbonyl C=O stretching	Widening after 60 days of AA
	1,100	C-O stretching	Disappearance after 60 days of AA
	873	CH_2 in $OC(CH_2)_2CO$ bending	Disappearance after 60 days of AA
	1,270 732	C-O-C bending	Disappearance after 60 days of AA

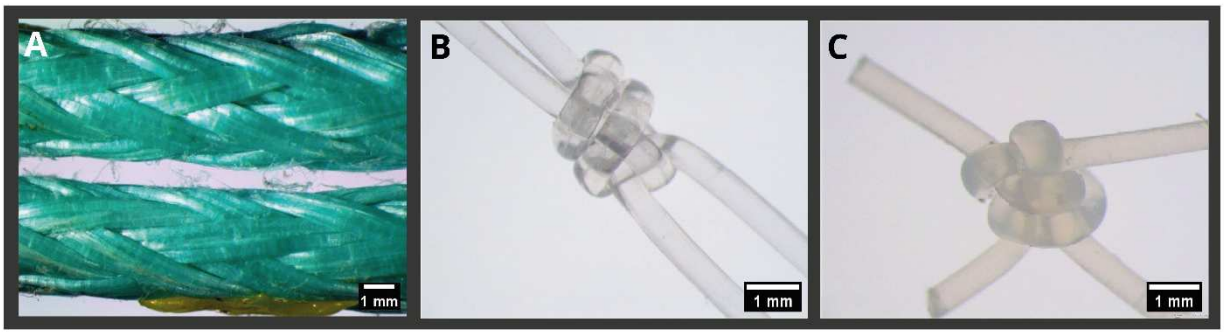

Figure 4.A.1: (A) Fragment of the PE-PP cod end trawl net. (B) Section with a knot from the conventional PA6 trammel net. (C) Section with a knot from a partially biobased and biodegradable trammel net composed of PBS-PBAT.

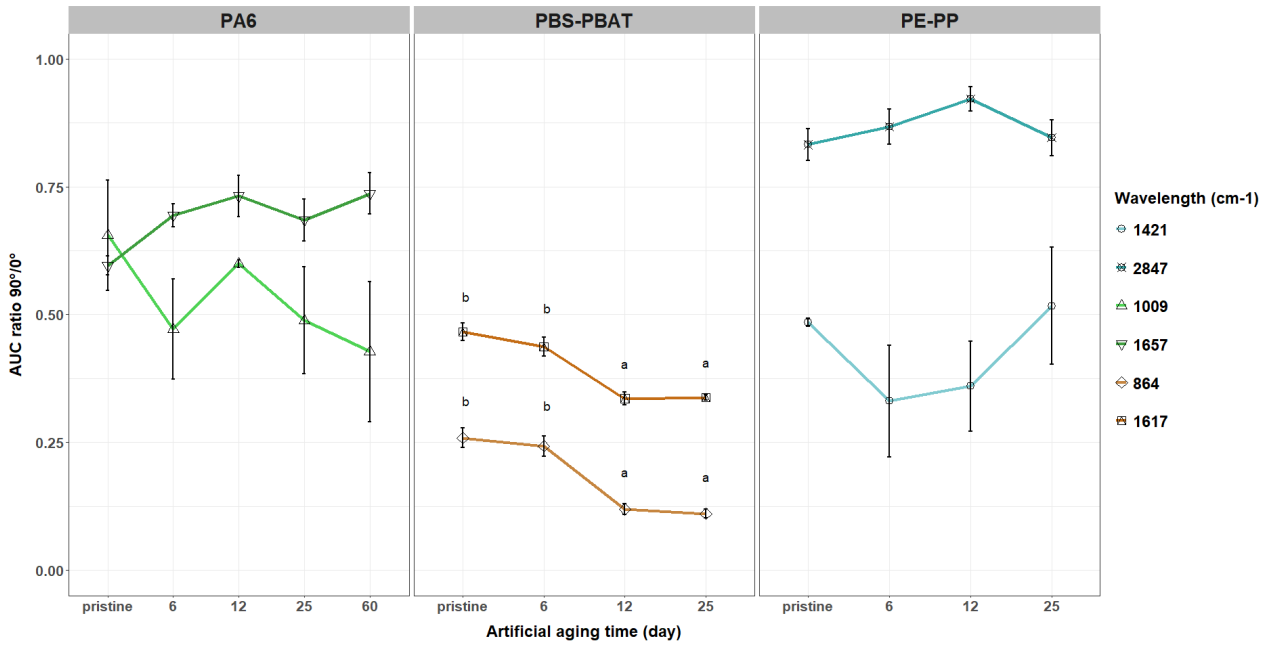

Figure 4.A.2: Evolution of the dichroic ratio for each selected band of each fishing net along the artificial aging time. Letters indicate significant differences among AA times (One-way ANOVA for each FN. If p-value < 0.05, Tukey's HSD post-hoc test with Holm´s correction).

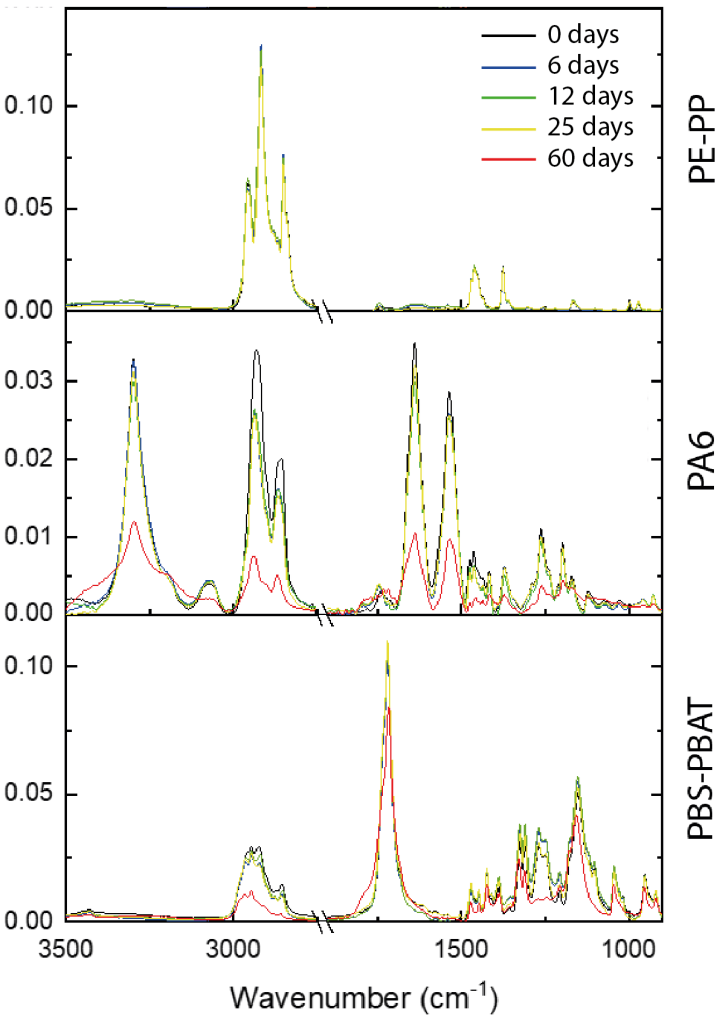

Figure 4.A.3: ATR-FTIR spectra of FN filaments artificially aged for 6, 12, 25, or 60 days. The artificially aged fishing nets are compared with the respective pristine FNs.

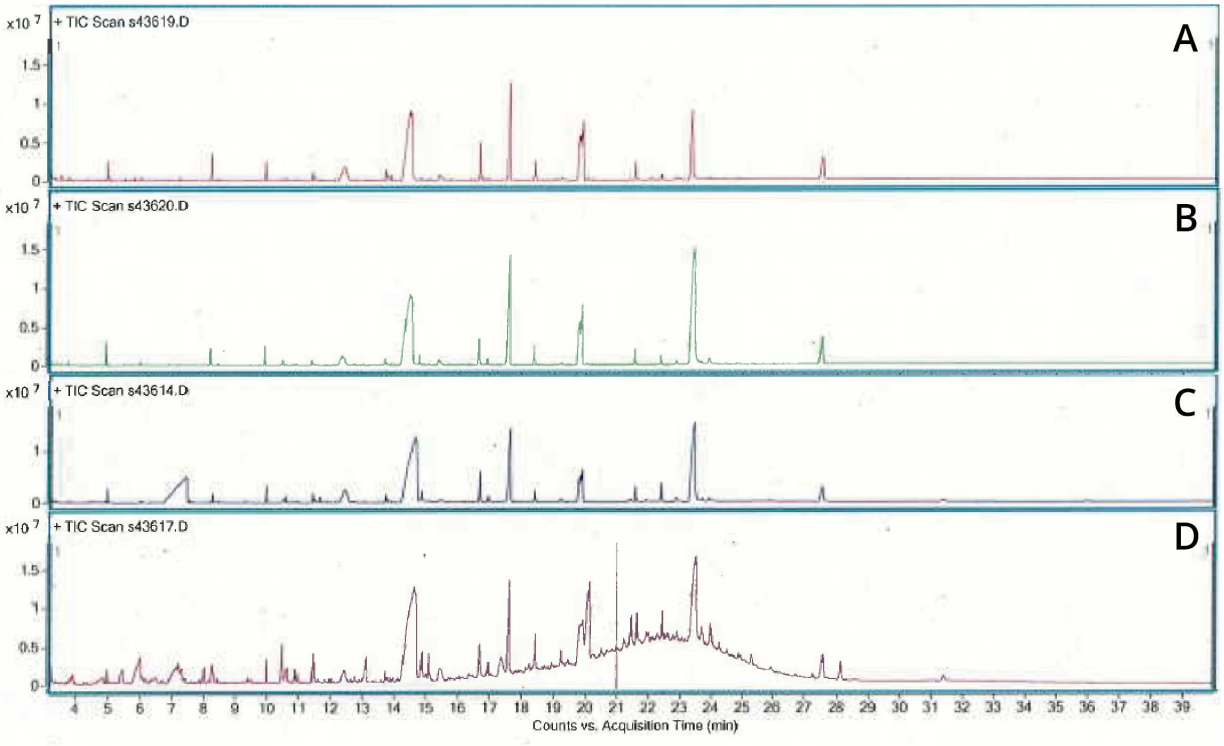

Figure 4.A.4: Chromatograms obtained after GC/MS analysis of leachates from FNs artificially aged for 25 days in scan mode.
(A) Chromatogram of the blank leachate. (B) Chromatogram of the PE-PP leachate. (C) Chromatogram of the PA6 leachate. (D) Chromatogram of the PBS-PBAT leachate.

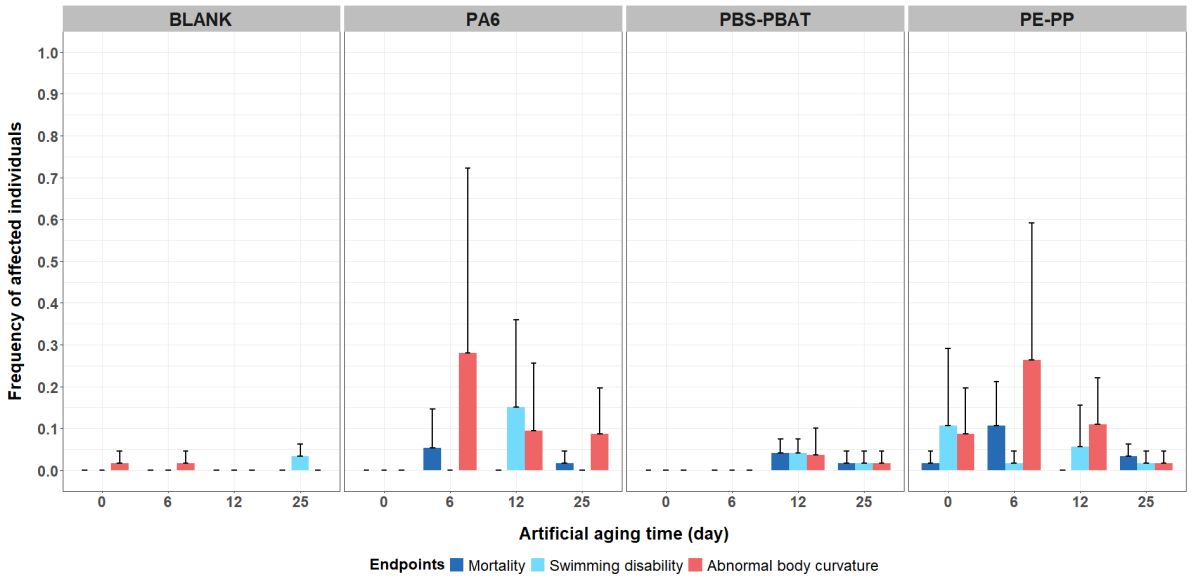

Figure 4.A.5: Frequency of fish mortality, fish showing swimming disability and abnormal body curvature following 48 h of exposure of *O. latipes* larvae to FN leachates.
The top of the bars represents the mean frequency, and the black line is the standard deviation (SD). Statistical analysis: independent Kruskal-Wallis for each FN. Sample size by FN and artificial aging time: n = 54

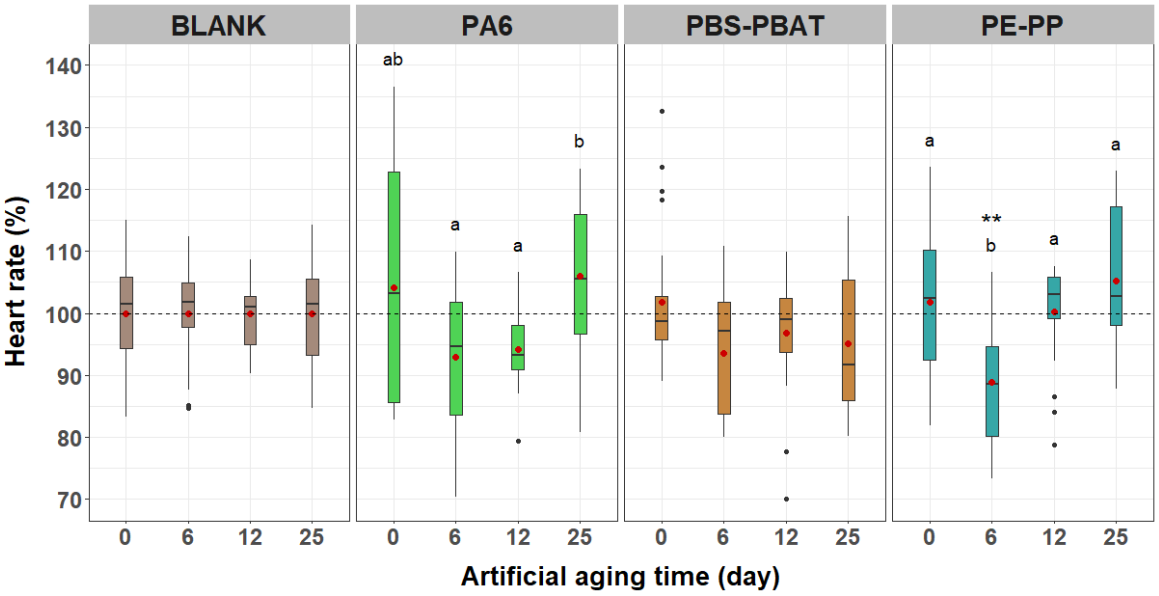

Figure 4.A.6: Effect of artificially aged FN leachates on Japanese medaka larvae heartbeat.
The heart rates of the larvae exposed to the blank leachates were normalized at 100%. Then, the heartbeat of the larvae exposed to FN leachates was normalized as a percentage of their respective blank leachates. The central black line of the boxplot represents the median, and the upper and lower borders are the 25th and 75th percentiles. The whiskers indicate 1.5 times the interquartile range (IQR). The red points represent the means, and the black ones are the outliers. The black dotted line represents the normalized heartbeat of the larvae exposed to the blank leachates. Statistical analysis: independent Kruskal-Wallis for each FN, followed by Dunn test with Holm correction. Letters indicate significant differences among AA times. Asterisks represent significant differences between the blank and FN leachates (Mann–Whitney U test for each FN and AA time. ** = p-value < 0.01). Sample size by fishing net and AA time: n = 16.

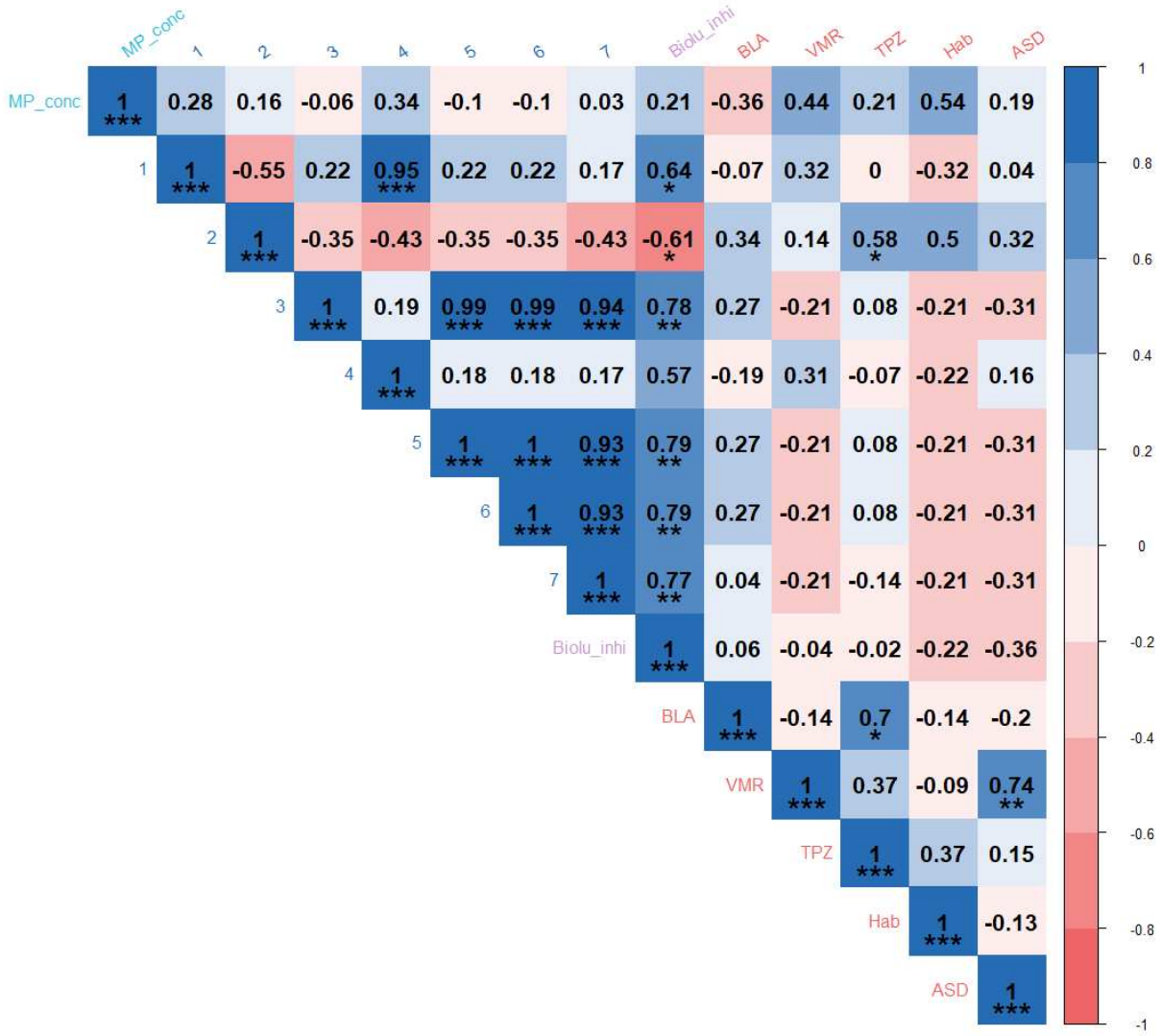

Figure 4.A.7: Correlation matrix of the relationships of the organic compounds and MP concentration in the leachate and their effects on the bioluminescence inhibition (*A. fischeri*) and the fish behaviour (*O. latipes*).
The blue numbers at the top and on the left of the matrix represent the organic compounds: "1" refers to 1.8-diazacyclo-tetradecane-2.9-dione, "2" is acetyl-tributyl-citrate, "3" is N-butyl benzenesulfonamide, "4" is ε-caprolactam, "5" is diethyl-phthalate, "6" is phthalimide, and "7" is succinic acid 2-methylallyl undecyl ester. MP_Conc = microplastic concentration, Biolu_inhi = Bioluminescence inhibition, BLA =Basal Locomotor Activity, TPZ = Time in Peripheral Zone, VMR = Vibrational Motor Response, and ASD = Acclimatization to Sudden Darkness. The matrix uses color-coding to indicate correlation strength: blue for positive and red for negative correlations. The intensity of the color reflects the strength of the correlation, with asterisks denoting statistical significance (*p < 0.05, **p < 0.01, ***p < 0.001). The value inside the cells is the correlation coefficient value.

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4.7 Highlights, main results and conclusions

Highligths of the chapter:

- Fishing nets under extreme aging conditions produced microplastics and nanoplastics.
- Fishing nets released soluble organic compounds that were toxic to aquatic species.
- Leachate toxicity varied among fishing nets and increased with aging time.
- Fishing net leachates significantly disrupted behaviour of fish larvae.
- Toxicity of biobased and biodegradable nets was similar to that of conventional nets.

Main results of the chapter:

FNs	IMAGING & SPECTROSCOPY			DEGRADATION			ORGANIC COMPOUNDS		BIOASSAYS															
	SEM global aspect	ATR-FTIR	Polarized RAMAN	Lost mass	LDIR MPs	SEM NPs	Non-target	Quantif.	Marine bacteria (*A. fischeri*) Bioluminescence inhibition (%)					FISH (*O. latipes*) Survival (%)				FISH (*O. latipes*) Heart rate (ctl. = 100%)				FISH (*O. latipes*) Behavior (BSI)			
									0d	6d	12d	25d	60d	0d	6d	12d	25d	0d	6d	12d	25d	0d	6d	12d	25d
PE-PP ε4.25	✓	✓	✓	✓	✓	✓	✓	✓	11%	8%	19%	19%	15%	98%	89%	100%	97%	102%	**89%**	100%	105%	0	**1**	**7**	**3**
PA6 γ60	✓	✓	✓	✓	✓	✓	✓	✓	**19%**	17%	**19%**	**18%**	**26%**	100%	95%	100%	98%	104%	92%	94%	106%	0	0	**1,5**	**8**
PBS-PBAT δ60	✓	✓	✓	✓	✓	✓	✓	✓	**26%**	**27%**	**30%**	**39%**	**56%**	100%	100%	96%	98%	102%	97%	96%	95%	0	0	**8,5**	**1**

***Values in red and bold represent significant differences between the endpoint and the control.**

Conclusions:

The degradation kinetics of the three FNs analyzed did not exhibit a linear trend in the release of plastic chemicals and MPs. Nonetheless, it was shown that hazardous plastic chemicals such as plastic additives, NIAS, and degradation by-products were released during FNs aging. Regarding MPs, FNs can generate a significant number of microfragments when they are rigorously agitated after extensive aging with UV at high temperature. This degradation rate could be similar to prolonged operational periods or long-term presence (>4 years) in marine environments or on beaches after their loss. Concerning the toxicity of the mixture of degradation products, the findings emphasized an increase with aging, especially when the FNs were highly degraded. Moreover, the toxicity was mainly driven by chemical compounds released by FNs. This suggests that the chemical composition of FNs is a critical factor that requires improvement to mitigate their toxic effects. Initial results on the aging of biobased and biodegradable FNs are inconclusive, in terms of the degradation products and their toxicity, to currently support their replacement of oil-based, non-biodegradable FNs. To formulate more substantiated statements about the toxicity of FNs and their environmental risk, toxicity experiments must be repeated with other FNs, aquatic species, and toxicity endpoints. Furthermore, attempting to identify trace metals that may be potentially released during artificial degradation would be valuable and could help to better understand the cause of the toxicity observed.

Chapter 5 Multi-species toxicity of leachates containing microplastics, nanoplastics and additives from artificially aged oil-based and partially biobased and biodegradable fishing nets

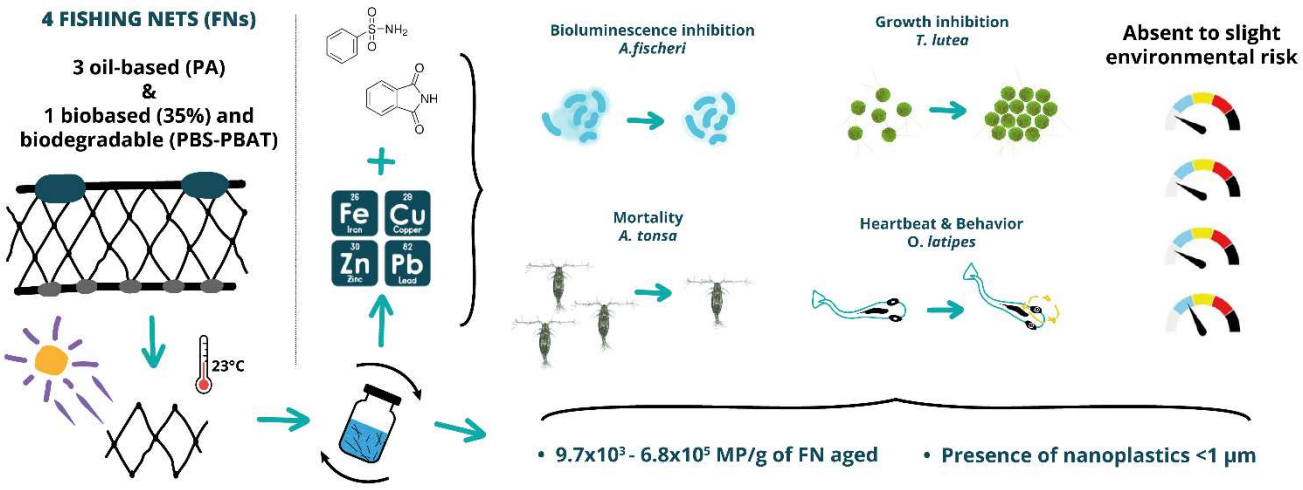
Context:

The preceding chapters highlighted that the FNs used in the SE BoB were mainly composed of gillnets and trawls. The amounts of FG estimated were substantial, with 211 tons used and six tons lost. Additionally, FNs, along with maritime ropes, constituted 9.6% of the plastic litter on the beaches. Findings from the artificial aging process revealed that FNs contribute to MP, NP, and plastic chemical pollution in aquatic environments. Nevertheless, the degradation experiments should be repeated with additional FNs of different characteristics to complement knowledge on their degradation. Previous results indicated that the toxicity levels varied depending on the released plastic chemicals, which could at least be attributed to the diverse polymer composition of the FNs and their aging state. Consequently, it is worthwhile to investigate whether degradation products from FNs of identical polymeric composition but from different manufacturers exhibit comparable or distinct toxicities. An examination of the ecotoxicological effects on various aquatic organisms, from bacteria to fish, would provide a more precise characterization of the toxicity associated with the degradation products of FNs. Ultimately, integrating all these data will enable the estimation of a risk assessment concerning the degradation products of FNs.

Objectives:

To establish a first risk assessment of the degradation products (MPs, NPs and plastic chemicals) of three new fishing nets made of polyamide but with different chemical composition and one biobased and biodegradable fishing net, based on their toxicity to four aquatic species, of which three are present in the Southeastern Bay of Byscay.

The chapter in one illustration:



The content of this chapter reflects a scientific publication that is under preparation to be submitted to the scientific journal "Environment International" as:

Dusacre, E., Cachot, J., Le Picard, C., Hausard, V., Combes, Y., Clérandeau, C., Vilette, Nardi, A., d'Errico, G., S., Lecomte, S. & Miren, P. C., n.d. "Multi-species toxicity of leachates containing microplastics, nanoplastics and additives from artificially aged oil-based and partially biobased and biodegradable gillnets."

Abstract

Plastic gillnets represent one of the most extensively used and lost fishing gear worldwide. However, their contribution to the microplastic (MP), nanoplastic (NP) and chemical pollution of the ocean is still insufficiently understood. To fill this knowledge gap, one partially biobased and biodegradable gillnet made of polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT) and three gillnets made of polyamide were artificially aged (25 days) using UV irradiation and then agitation with abrasion in water. The toxicity of leachates produced was evaluated on four model aquatic organisms. All the studied gillnets produced MPs (median size 23.6 µm) but also NPs and released hazardous trace metals and organic compounds. Based on the quantity of gillnets used daily in 2023 in the SE BoB, PA and PBS-PBAT gillnets would have contributed to MP pollution in the studied area at a level of 2.2 MP/m^3 (95% CI: 0-14.8 MP/m^3) and 1.5 MPs/m^3 (95% CI: 0-3.5 MP/m^3), respectively. The leachates caused significant *A. fischeri*'s bioluminescence inhibition (16% to 23%) and significant *A. tonsa*'s mortality (37.8±13.3% to 41.1±11.3%). They also significantly disrupted the heartbeat (+/- 13% to 18%) and behavior (behavior stress index: 0.5 to 7) of *O. latipes*' larvae. The observed toxicity was predominantly attributed to the chemical compounds released. The partially biobased and biodegradable FN displayed a similar toxicity than the oil-based PA gillnets. Based on the Weight of Evidence approach, the gillnets were estimated to represent no or a slight risk for coastal marine environments.

5.1 Introduction

The substantial diversity in the chemical composition of plastics make them a complex pollutant (Hermabessiere et al. 2017; Hahladakis et al. 2018). First there are nine main solid polymers used worldwide (Carrega & Verney 2017; Geyer 2020). Second, there are numerous different production processes (e.g., radical polymerization under very high pressure, catalyst polymerization at low or medium pressure, polycondensation, cycle opening (anionic or thermal)) (Carrega & Verney 2017). Third, up to 2148 additives are marketed for the plastic production (Wagner et al. 2024). Consequently, the production process and the selection of plastic additives employed in the fabrication of plastics suggest that even if two plastics possess identical polymeric compositions and intended uses, they may not exhibit 100% similarity (Carrega & Verney 2017; Hahladakis et al. 2018; Wagner et al. 2024). In particular, with regard to plastic aging, variation in chemical composition could result in diverse responses to environmental factors, leading to varying degradation processes (Hansen et al. 2013; Gewert et al. 2015; Hahladakis et al. 2018; Masry et al. 2021). Therefore, when plastics degrade, they may not release the same quantity of micro and nanoplastics (MNPs), nor the same type and quantity of chemical compounds (Rani et al. 2015; De Falco et al. 2018; Do et al. 2022; Chen et al. 2023; Wagner et al. 2024). As a consequence, the toxicity of plastic degradation products, already reported on many organisms (Botterell et al. 2019; Cormier et al. 2021; Li et al. 2023; Gambardella et al. 2024), may vary between two items with the same polymeric composition and similar use. For instance, research by Zimmerman et al. (2019, 2021) demonstrated that extracts and leachates from three distinct LDPE food contact packaging or two different PS fruit and vegetable trays exhibited varying levels of toxicity. Consequently, in order to establish the environmental risk of plastic degradation products, it is essential to compare not only analogous items made of distinct polymers, but also items made of identical polymers originating from diverse industrial manufacturers (Chen et al. 2022; Sridharan et al. 2022; Pelegrini et al. 2023).

Regarding plastic fishing nets (FNs), particularly gillnets made of polyamide (PA), there is a substantial variety of manufacturers (Chapter 1), suggesting potential variations in chemical composition. For instance, examining the release of organic compounds from PA gillnets from diverse origins, five different studies identified a multitude of additives, non-intentionally added substances (NIAS), or degradation products (Chaihad et al. 2020; Jang et al. 2021; Brakstad et al. 2022; Vilke et al. 2024; Dusacre et al. 2025). While some compounds were similar across the studied fishing gear (e.g., ε-caprolactam, 1.8-diazacyclotetradecane, diethyl phthalate), the majority were distinct.

Concerning the toxicity of FNs, to the best of our knowledge there is no literature on the comparative toxicity of gillnets of the same polymer composition but from different manufacturers. Nonetheless, a study demonstrated that leachates from a mixture of weathered and beached fishing gear caused significant oxidative damage of the digestive gland of *M. galloprovincialis* (Vilke et al. 2024). The authors also reported genotoxic and neurotoxic effects as well as significant impact on the antioxidant and biotransformation systems of the tissues of the mussels. In a previous work we also highlighted significant effects of leachates from artificially aged FNs of different polymer composition on two aquatic species (Dusacre et al. 2025). It is interesting to note that in both studies, the toxic effects were mainly

linked to the chemical compounds released rather than to the production of MNPs. However, we still know very little about the contribution of FNs to the marine MNPs pollution. Montarsolo et al. (2018) showed that abandoned, lost or otherwise discarded trawl or bottom seine nets made of PA produced short microfilaments after being crushed in a Linitest apparatus (De Falco 2018). Moreover, Grimaldo et al. (2020) showed that ultra-violet (UV) artificial aging of PA gillnets caused long cracks and authors hypothesized that they could have produced MPs. In our previous work, we also showed that a PA trammel net irradiated by UV strong UV irradiation and high temperature (64°C) produced a significant amount of MPs (5 – 455 µm) (Dusacre et al. 2025).

With the aim of reducing the significant threat caused to marine ecosystems by the oil-based gillnets (García-Barón et al. 2019; Roman et al. 2021) and to better manage their end-of-life, biobased and biodegradable gillnets are being developed (Deroiné et al. 2019; Grimaldo et al. 2020; Brakstad et al. 2022; Cerbule et al. 2023; Le Gué et al. 2023; Savina et al. 2024). However, as mentioned in the work of Le Gué (2024), biobased and biodegradable plastic formulation does not guarantee that their degradation products are safe for the marine environment. Indeed, several studies already showed the adverse effects of degradation products of biobased and biodegradable plastics (polyhydroxybutyrate (PHB), polyhydroxyalkanoate (PHA), polylactic acid (PLA), Starch, Cellulose, polybutylene adipate terephthalate (PBAT), polybutylene succinate (PBS), Bio-polyethylene) on various organisms, e.g., marine bacteria, microalgae, zooplankton, bivalves and fishes (González-Pleiter et al. 2019; Zimmermann et al. 2020; de Oliveira et al. 2021; Klein et al. 2021; Chen 2022; Tong et al. 2022; Laranjeiro et al. 2024). However, biobased and biodegradable plastic toxicity need to be further investigated, since studies show different toxicity patterns for identical polymers depending of the studied compartment (sediments, water columns, soils). For example, Campani et al. (2020) showed no effect of elutriates of sediment after composting of bags made of Mater-Bi in *D. tertiolecta*, *P. lividius* and *D. labrax*. While Almeda et al. (2023) reported significant toxicity of leachates compostable carrier bag made of Mater-Bi® in *A. fischeri* and *Paracentrotus lividus*. Regarding FNs, albeit some studies investigated the degradation of biodegradable gillnets (Grimaldo et al. 2020; Brakstad et al. 2022; Wataniyakun et al. 2025), there is little knowledge on the toxicity of their leachates. It is therefore crucial to investigate the degradation of biobased and biodegradable gillnets and the toxicity of their leachates, in comparison to those of oil-based and non-biodegradable ones, before new biobased and biodegradable gillnets are marketed (Gilman 2016; Le Gué 2024; Wataniyakun et al. 2025).

Thus, the objective of this study was to compare the degradation and the toxicity of three oil-based gillnets made of PA and one partially biobased and biodegradable gillnet made of PBS-PBAT. First, their degradation was studied (UV irradiation at 23°C and agitation in water with glass beads) and leachates were produced and characterized (MNPs, trace metals, organic compounds). Then, the toxicity of their leachates was examined on four aquatic species (*A. fischeri*, *T. lutea*, *A. tonsa* and *O. latipes*) using acute lethal (survival, growth rate) and sub-lethal endpoints (bioluminescence, heart rate, behaviour). Finally, we assessed the environmental risk of these gillnets using Weight of Evidence method.

5.2 Materials and methods

5.2.1 Fishing nets

Four gillnets were used through this study. The first one was a green straight monofilament gillnet of 0.35 mm of diameter and made of polyamide 6 (PA). This FN was identified as "PA α35". The three other gillnets were inner panels of trammel nets. The “PA β33” and the “PA γ33” were also made of PA and had a diameter of 0.33 mm. The first was purple and the second translucent white. The last one identified as “PBS δ35” was made of polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT) and had a diameter of 0.35 mm. This last one was 35% biobased and biodegradable. Their origins were previously described in Chapter 3.

5.2.2 Artificial aging and leachate preparation

The method for the FNs artificial aging applied in this study was detailed previously in Chapter 4. Briefly, 0.6 g of FN pieces (2-2.5 cm) were irradiated dry during 25 days using ultraviolet (UV) irradiation. The ultraviolet (UV) chamber (UV-B, UV-A) was configured to operate at an intensity of 40 mW/cm², with a stable temperature maintained at 23°C (Dusacre et al. 2024). In this UV chamber, 25 days of irradiation was equivalent to four years of exposure under the environmental conditions of the SE BoB (average day/night irradiation 68.86 W/m^2 with 10% being UV) (Gewert et al. 2015; European Commission 2021; Beani 2022). Subsequent to irradiation, the FN pieces were subjected to orbital agitation in deionized water (filtered through 0.22 µm filter) for a duration of 24 hours at 190 rpm (Ika KS 501 digital shaker, 15 mm radius), in the presence of 62 glass beads (diameter of 1.5 mm). FN mesoplastic concentration was 10 g/L. Following this step, the mixture of aged FN and water was filtered at 1 mm with a stainless filter. The remaining pieces of FNs (> 1 mm) were dried until constant weight and their mass was measured (precision of 0.1 mg). The leachates containing the dissolved degradation products and the MNPs were stored at -20°C. FN pieces that were not subjected to irradiation were also processed through the same leaching procedure and designated as “non-aged” FNs. FN UV irradiated for 25 days and agitated in water will be named “aged” FNs. Blanks, which underwent identical procedural steps without FNs, were used in parallel alongside non-exposed experimental controls for all experiments. These samples named blanks were used throughout all the experiments as controls compared to the leachates from FNs.

5.2.3 Analyses of dissolved degradation products and MNPs

5.2.3.1 Organic compounds and trace metals

The quantification of dissolved organic compounds released by the FNs during artificial aging was conducted by the analytical laboratory LABOCEA (Plouzané, France) using the same methodologies outlined in Dusacre et al. (2025). Metal quantification was performed using a 720-ES ICP-OES (Varian, Agilent Technologies Inc, USA) system. The instrument was calibrated

using multi-element standard solutions (SCP S210323034), and quality control samples at 65 ppb and 220 ppb were analyzed to ensure accuracy. Recovery rates for quality controls ranged from 85.01% to 100.83% for all measured elements including Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V and Zn. The limit of quantification was set at the first point of the calibration range, due to the quality of the signal observed at this concentration. This point lies in the linear zone of the curve and corresponds to expected sample concentrations, thus ensuring reliable quantification without extrapolation. Limits of detection, limits of quantification and wavelengths used for each element are available in supplementary material (Appendix 5.A, Table 5.A.1). Samples were directly acidified with 10% purified HNO_3 just after sampling. Blank samples were analyzed to assess potential contamination. All the trace metals were below the LQ in the blank leachates.

5.2.3.2 Microplastics and nanoplastics

MPs were quantified using laser direct infrared chemical imaging system (Agilent 8700 LDIR Chemical Imaging System). The method was previously described in Dusacre et al. (2025). The limit of quantification was 5 µm. Only the particles with a "spectral matching score" above 70 % were considered. Subsequently, to estimate the concentration of MPs in the leachates, the values obtained from the FN leachates were corrected using the measurements from the blanks, as proposed by Dawson et al. (2023). Ultimately, the power law relationship provided by the R package poweRlaw was employed to evaluate the relation between the size and abundance of MPs (Gillespie 2015; Erni-Cassola et al. 2017).
NPs (< 1 µm) present in the leachates were identified using scanning electron microscopy (Hitachi S-4800 SEM, General Service SGiker, EHU, Leioa, Basque Country, Spain). Preparation of the samples was described in Dusacre et al. (2025). Images were obtained at two distinct magnifications (x5000, x10000), utilizing an accelerating voltage of 5 kV and maintaining a working distance of 4.2 mm.

5.2.4 Bioluminescence inhibition of *Aliivibrio fischeri*

The acute toxicity of leachates to the marine bacteria *A. fischeri* was evaluated utilizing the Microtox© bioassay in accordance with ISO 11348–3 guideline (International Organization for Standardization 2007). Marine bacterial cultures were exposed for a duration of 30 min to 100% leachates containing NaCl at a concentration of 20 g/L. The pH of both controls, blanks and FN leachates was adjusted to 7.3±0.2. The exposure temperature was 15°C. Data were normalized relative to the seawater control comprising non-exposed bacteria, and bioluminescence inhibition was calculated for each sample. Subsequently, the data were statistically analyzed in comparison to the blanks.

5.2.5 Growth inhibition of *Tisochrysis lutea*

Microalgae *T. lutea were* obtained from the Animal Physiology laboratory at the University of the Basque Country (UPV/EHU, Basque Country, Spain) and were exposed to FN leachates in accordance with the OECD/OCDE guideline N°201 (OECD/OCDE 2011). Culture method of the microalgae is available in supplementary materials (Appendix 5.A, section 5.6.1.1). The

microalgae were exposed to FN leachates with a mesoplastic equivalent concentration of 8 g/L within 10 mL glass conical flasks, which were covered with glass petri dishes. Filtered (0.2 µm) artificial seawater (ASW) (Aquamedic Reef Salt) was used to reach a salinity of 34±1 psu. "Guillard's (F/2) Marine Water Enrichment Solution" was added to enrich the medium with required nutrients. At the beginning of the bioassay, the concentration of microalgae was established at 9×10^4 cells/mL to facilitate exponential growth over the exposure period of 72 h. The conical flasks were incubated at a temperature of 22 ± 1°C under fluorescent lighting, with an intensity of 96±3 µmol/s/m² and a gentle orbital agitation (30 rpm). Cell density measurements were conducted every 24 hours for each conical flask using the Beckman Coulter Z2 Cell and Particle Counter (Beckman Coulter, Inc, USA). A 0.5 mL sample from each flask was analyzed thrice daily for cell sizes ranging from 2.5 µm to 6.5 µm. Three replicates were prepared for each FN leachate and blank, and five replicates were prepared for both filtered seawater and artificial seawater. The experiment was conducted in duplicate. To eliminate interference from MPs that could fall within the size range of the microalgae (2.5 and 6.5 µm), each FN leachate sample free of microalgae was pre-analyzed using the Coulter Z2 Cell and Particle Counter, and these values were subsequently subtracted from the microalgae cell counting. Growth rate and growth inhibition were calculated as described in the OECD/OCDE guideline.

5.2.6 Survival of *Acartia tonsa*

The acute survival assay followed the ISO 14669 standard (International Organization for Standardization 1999). The method for the culture of the copepods *A. tonsa* obtained from Algova® (Germany) is detailed in the supplementary materials (Appendix 5.A, section 5.6.1.2). FN leachates at 100% (10 g/L of mesoplastic equivalent concentration) were prepared in 50 mL glass beakers using artificial sea salt (Aquamedic Reef Salt), to reach a salinity of 31±1 PSU. The pH was buffered to 8.0±0.1 and dissolved O_2 concentration was considered enough if above 80%. Finally, 160 µL/L of antibiotic mixture (penicillin and streptomycin) was added in each beaker to avoid any bacterial contamination. Using stereomicroscopes ten adult copepods (without male and female distinction) were randomly allocated to the beakers. Beakers were then covered with glass Petri dishes. During the assay, copepods were not fed. The vials were maintained in an incubator at 22±1°C, under an illumination of 1,000–1,500 lux and a 14:10 light:dark cycle (Gorbi et al. 2012; Hafez 2021). Copepod mortalities were recorded at 24, 48, and 72 h using the same stereomicroscope. Copepods were considered dead if no movement was observed for 30 seconds. Three replicates per FN leachate and blank were prepared, alongside control groups (five replicates with ASW and five with filtered SW). The experiment was performed twice and the test was considered valid if the mortality in the controls did not exceed 20% over 72 hours.

5.2.7 Survival, malformation, heart rate and behaviour of *Oryzias latipes* larvae

Embryos of the Japanese medaka *O. latipes* (Carbio strain) were obtained 24 hours post-fertilization from the PGP experimental facility (Physiology Genomique Poissons, INRAE, Rennes, France). Embryo culture and experimental procedures for larval exposure to FN leachates were conducted in the same conditions to those detailed previously (Dusacre et al. 2025). In brief, *O. latipes* larvae at 10 days post-fertilization were exposed for 48 h (daily half medium renewal) to non-aged and aged FN leachates, as well as to blanks. Three replicates were conducted for each condition. Survival was examined after 24 h and 48 h of exposure. Larvae body development and swimming ability were recorded after 48 h of exposure on 18-20 larvae per replicate. Heartbeats were measured on 16 larvae per replicate after 48 h of exposure. A near-infrared camera (Basler acA1300–60gm) with a stereomicroscope (Olympus SZX2-TR30) was used to record 60-second videos in a thermoregulated room at 24±0.5°C. Three 20 second measurements were averaged for each individual. Videos were then analyzed by the DanioScope™ software (Version 1.2). Heartbeat of larvae in blanks were normalized to 100, and data of larvae exposed to FN leachates were normalized relative to their respective blanks.

Behavior analysis was conducted on 16 larvae per replicate using the DanioVision System (Noldus, Wageningen, The Netherlands) featuring an observation chamber with a near-infrared camera, LED light panel, tapping mechanism, temperature control unit set to 24±0.5°C, and a computer with EthoVision XT software (version 17.0.1630). Larvae were placed in 48-well plates and subjected to a 51 min routine which enabled the determination of six behavioral endpoints defined in Bedrossiantz et al. (2023), i.e., the Basal Locomotor Activity (BLA); the Vibrational Startle Response (VSR); the Habituation to continuous vibrational stimuli; the Visual Motor Response (VMR); Acclimatization to Sudden Darkness (ASD); and the Time in the Peripheral Zone (TPZ). Data of each endpoint and for each FN leachate were normalized to values in their respective blanks, set at 1.

In order to integrate the impacts of FN leachates on larvae behavior, a behavioral stress index was calculated as explained in Dusacre et al. (2025) Briefly, for each endpoint, a score was assigned based on the deviation from the control when it showed statistical significance: deviation ≤ 0.2 = 0.5;]0.2–0.4] = 1;]0.4–0.6] = 2;]0.6–0.8] = 3;]0.8–1] = 4; > 1 = 5. Subsequently, the scores were aggregated for each FN leachate. Elevated scores indicated enhanced behavioral effects on the Japanese medaka larvae.

5.2.8 Fishing net risk assessment

The first step of the risk assessment of FNs was to calculate the contribution to the MP pollution of the gillnets used (GO_{MP}) and lost (GL_{MP}) in the SE BoB in 2023 making the proxy that the studied area representing 71,422 km^3 of water (Chapter 2) was closed and without exchange. This value was obtained using the following equations:

$$GO_{MP} = \sum_{i=1}^{6} \frac{MP_{FN0,i} \cdot FN_{O,i} \cdot (\frac{t_o}{24})}{V}$$

$$GL_{MP} = \sum_{i=1}^{6} \frac{MP_{FN25,i} \cdot FN_{L,i}}{V}$$

$MP_{FN0,i}$ is the quantity of microplastics produced per kilogram of non-aged gillnet for each size range i, and $MP_{FN25,i}$ is the quantity of MPs produced per kilogram of aged gillnet for each size range i. The size ranges considered were 1–20 µm, 20–50 µm, 50–100 µm, 100–300 µm, 300–500 µm, and 500–1,000 µm. FN_o is the weight in kilograms of gillnet used and FN_L is the weight in kilograms of gillnet lost; t_o is the mean time of operation in hours of the gillnetters equipped with AIS (960 h); and V is the volume of water in the studied area. FN_o, FN_L, and t_o were estimated in Chapter 2. The calculation was performed separately for each size range, and the total quantity of MPs for each FN obtained by summing the values computed for all size intervals.

Based on these data of MPs produced by the FNs used and lost in 2023, we created four scenarios of FN contamination of the SE BoB. The first scenario (S1) was designed to assess the environmental risk of gillnets during their daily operation in 2023 and was based on the data of MPs produced and the toxicity of the FNs non-aged. The second scenario (S2) was designed to estimate the cumulative environmental risk for the yearly operation of gillnets since 1970. The year 1970 was selected since around this time, the majority of FNs used were made from plastic, whereas earlier some FNs could be still made from natural fibres. The third scenario (S3) was made to assess the environmental risk of aged gillnets four years after their loss and was based on the data of MPs production and the toxicity of the FN aged. The fourth scenario (S4) was similar to the S3 but was make to look at the cumulated environmental risk of FNs four years after their loss since 1970.

The risk assessment of FNs was performed using the Sediqualsoft Weight of Evidence (WOE) model (Regoli et al. 2019) adapted to MPs. Two lines of evidence (LOEs) were used: “Microplastic characterization in abiotic matrices” (LOE1) and “ecotoxicological bioassays” (LOE4). For LOE1, the characterization and quantification hazard of MPs in the water column was estimated based on the four scenarios described above and the MPs hazard quotient (HQ) was calculated as:

$$HQ_{MP} = N \cdot (W_{shape}) \cdot (W_{size}) \cdot (W_{polymer})$$

where N was the number of MP and W_{shape}, W_{size}, $W_{polymer}$ were weights assigned to MP characteristics based on their environmental risk potential (e.g., fragment = 1.2, fiber = 1.0; size < 100 µm = 1.3, 100–500 µm = 1.0; polymer hazard weights) (Avio et al. 2015; Pittura et al. 2018; Regoli et al. 2019; Gonçalves et al. 2023). HQ_{MP} values were ranked in five exposition levels: absent (0 MP/m^3), slight (0 > MP/m^3 ≤ 10), moderate (10 > MP/m^3 ≤ 50), major (50 > MP/m^3 ≤ 100), or severe (> 100 MP/m^3) (Paul-Pont et al. 2018; Lefebvre et al. 2023; Reineccius & Waniek 2024). For LOE4, the ecotoxicological hazard was assessed using a battery of bioassays, including all endpoints measured in the four species in this study including heart rate and swimming behavior of fish that were not included in the latest version of the model

(Gambardella et al. 2024). The behavioral response was scaled according to the BSI, dividing the maximum BSI index (40) into 4 ranges (absent: BSI = 0; slight: 0 > BSI ≤ 12; moderate: 12 < BSI ≤ 24; major: 24 < BSI ≤ 36; severe: BSI > 36). As significant heart rate disruption was correlated with later mortality, it is considered as a good proxy for subsequent mortality for fish (Schweizer et al. 2017, 2022; Krzykwa et al. 2018; Tschirren et al. 2018; Massei et al. 2021; Agathokleous 2022; Qiu et al. 2022). Consequently, a hazard level of 1.5 was given to the heart rate response of *O. latipes*. The $HQ_{Battery}$ was computed as:

$$HQ_{battery} = \sum_{k=1}^{T} (Effect_w(k) \cdot w_2$$

$Effect_w$ is the normalized effect for each endpoint and w_2 an exposure correction factor.

Integration of LOE1 and LOE4 was achieved by normalizing HQ_{MP} and $HQ_{Battery}$ to a common 0–10 scale and combining them into a single WOE index using the formula:

$$WOE = (HQ_{MP} \cdot 1.0) + \left(HQ_{battery} \cdot 1.2\right)$$

The final WOE was used to assign the overall risk to one of five classes (absent, slight, moderate, major, or severe).

5.2.9 Statistical analysis

The data treatment and statistical analysis were conducted utilizing RStudio (R version 4.3.1, RStudio version 2024.04.0 +735). Each statistical test's applicability conditions were rigorously validated through methods such as density plots, Shapiro–Wilk tests, QQ-plots, and Bartlett's test. When conditions were satisfied, a Student's t-test was applied for comparisons between two groups. Otherwise, the Welch's test or the non-parametric Wilcoxon-Mann-Whitney test were applied. To determine significant differences between the means of more than two groups, a one-way ANOVA was performed, followed by Tukey's HSD post-hoc test if needed. In cases where ANOVA's assumptions were not met, the Kruskal-Wallis test was used, with Dunn's multiple comparison test and Holm's correction applied when required.
A principal component analysis using centered and scaled data was carried out to investigate the potential associations among degradation products and the resulting toxicity. The degradation products analyzed included MPs, organic compounds, and trace metals. The toxicity variables were the bioluminescence inhibition of *A. fischeri*, the 24-hour growth inhibition of *T. lutea*, the mortality of *A. tonsa*, and the behavioral stress index for *O. latipes*. Only behavioral endpoints with significant differences were considered. To minimize redundancy in the principal component analysis, the three endpoints concerning the time spent by the larvae in the peripheral zone were aggregated.

5.3 Results

5.3.1 Degradation of the fishing nets

5.3.1.1 Production of microplastics and nanoplastics

The artificial aging of FNs caused the production of MPs for most of the FNs (Table 5.1). For non-aged gillnets, similar quantities of MP were observed in the leachates of the different PA gillnets, i.e., 192±1,043 MP/mL, 251±1,595 MP/mL, and 201±1,101 MP/mL for PA α35, PA β33, and PA γ33, respectively. The PBS δ35 produced significantly more MP than the PA α35 and PA γ33 (252±274 MP/mL) (Kruskal-Wallis' test: chi^2 = 11.33, df = 3, p-value = 0.01; Dunn's test with holm correction p-value < 0.03) (Table 5.1). For the gillnets aged, the MP concentrations in the leachates of the PA α35 (4,344±3,013 MP/mL) and PA γ33 (6,834±1,010 MP/mL) were significantly higher than those of the leachates of non-aged FN (Welch's tests: PA α35: W = -3.12, df = 13, p-value = 0.016; PA γ33: W = -11.1, df = 13, p-value < 0.0001) (Table 5.1). For the PA β33 the concentration of MPs was also higher (1,111±1,112 MP/mL) but without to be significant (Welch's test: W = -0.6, df = 13, p-value = 0.538) (Table 5.1). The leachate of the PA γ33 contained a significant higher mean concentration compared to the PA β33 and PBS δ35 (Kruskal-Wallis' test: chi2 = 16.48, df = 3, p-value = 0.0009; Dunn's test with holm correction p-value < 0.03) (Table 5.1). On average, the concentration of MPs in the leachate of the PBS δ35 gillnet aged was lower (98±169 MP/L) compared to that of the non-aged net, although this difference was not statistically significant (Welch's test: W = 2.43, df = 9, p-value = 0.05) (Table 5.1). When compared to the blank values as recommended in Dawson et al. (2023), only the aged gillnets made of PA produced a significant amount of MPs (Table 5.1).

Regarding the number of MPs released by gram of non-aged FN, the PA gillnets produced in average $4.05x10^4$±$8.76x10^4$ (SD) MP/g of FN (Table 5.1). The PBS δ35 gillnet produced in average $2.72x10^4$±$2.46x10^4$ (SD) MP/g of FN (Table 5.1). For those aged, the PA gillnets released more MPs ($3.08x10^5$±$3.17x10^5$ (SD) MP/g of FN) than the PBS δ35 one ($9.75x10^3$±$1.69x10^4$ (SD) MP/g of FN) (Table 5.1).

Considering the shape of the MPs, 100% were fragments (Liu et al. 2019, 2022). The median size of the MPs produced were similar between FNs with a median size of 23.6±6.3 (SD) µm when considering all the FNs. However, the PA gillnets tended to produced bigger particles than the PBS δ35 FN (Table 5.1). The calculated scaling factor for MP size relative to their abundance was 1.22 for a MP minimum size of 10 µm and 2.17 for a minimum size of 20 µm. Specific ratios between size intervals (1-20 µm, 20-50 µm, 100-300 µm and 300-500 µm) are available in supplementary material (Appendix 5.A, Table 5.A.2). NPs were identified in all leachates of artificially aged FNs (Figure 5.1). In the SEM images obtained, MPs and NPs were agglomerated, but in general they were irregular fragments.

Table 5.1: MPs produced after artificial degradation of FNs. Values for the blanks of each polymer is also given to compare it with the values of the FNs.
In the column "statistical differences", the first sub-column represents the individual statistical comparison of each FN and its corresponding control (student's t-test: ns = p-value > 0.05, * = p-value < 0.05, ** = p-value < 0.01, **** = p-value < 0.0001). "ns" means no significant difference. Letters in the "Non-aged" column represent the significant differences between groups of non-aged FN and the column "Aged" the groups of aged FN. The statistical test ran for these two columns were Kruskal-Wallis' tests (df = 3, p-value < 0.05), followed by Dunn's tests with holm's corrections (df = 3, p-value < 0.05). "Ratio L:W" represents the ratio of length and width of particles.

Fishing nets	AA time (day)	MPs concentration (MPs/mL)			Statistical differences				Particle sizes (µm)			MP concentration (MP/g of FN)		
		Average	CI 0.05	CI 0.95	FNs vs. Blanks	Non-aged vs. aged	Non-aged	Aged	Median	Min	Max	Average	CI 0.05	CI 0.95
BLANK PA	0	1462	602	2877					22	5	67			
	25	1069	477	1775					18	5	50			
PA α35	0	1654	605	3377	ns	*	a		21	5	77	$4.6x10^4$	0	$1.9x10^5$
	25	5414	2149	10056	**			AB	26	5	430	$4.3x10^5$	$1.1x10^5$	$9.0x10^5$
PA β33	0	1713	643	4620	ns	ns	ab		18	5	72	$6.7x10^4$	0	$3.2x10^5$
	25	2180	994	3877	*			AC	21	5	211	$1.1x10^5$	0	$2.8x10^5$
PA γ33	0	1662	620	3538	ns	****	a		23	5	275	$4.9x10^4$	0	$2.1x10^5$
	25	7903	6936	9348	****			B	26	5	335	$6.8x10^5$	$5.9x10^5$	$8.3x10^5$
BLANK PBS-PBAT	0	249	63	459					19	5	27			
	25	58	58	58					12	5	15			
PBS δ35	0	501	152	933	ns	ns	b		21.5	5	78	$2.7x10^4$	1.5x103	$6.8x10^4$
	25	156	76	513	ns			C	38	16	38	$9.7x10^3$	$2.2x10^{-4}$	$2.8x10^4$

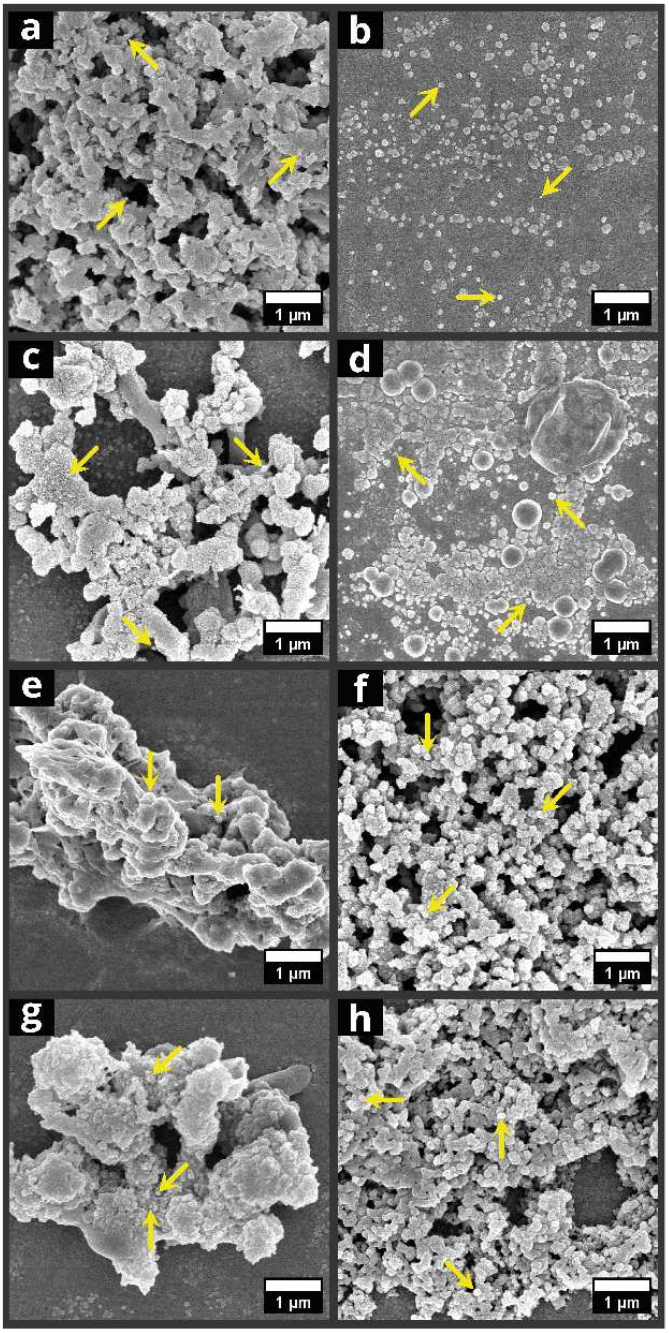


Figure 5.1: SEM images of the MNPs from leachates of non-aged and aged FNs.
"a" and "b" represent the leachates of non-aged and aged PA α35 FNs respectively. "c" and "d" were the leachates of non-aged and aged PA β33 FNs. "e" and "f" were the leachates of non-aged and aged PA γ33. "g" and "h" were the leachates of non-aged and aged PBS δ35. Yellow arrows indicate nanoparticles inferior to 1 µm. Scale bars are 1 µm.

5.3.1.2 Release of trace metals and organic chemical compounds

The analysis of trace metals leached from the artificially aged FNs revealed distinct patterns. Overall, the PBS δ35 released the highest number of trace metals (four) and most of the time in a significant higher amount than the PA gillnets (Figure 5.2). Copper (Cu) and zinc (Zn) concentrations demonstrated a significant increase (Student's t-tests: $W < -3$, $df = 2$, $p < 0.03$) in aged PBS δ35 nets, reaching 17.5±3.0 µg/L and 45.2±2.2 µg/L, respectively, compared to the concentration in the leachate of the non-aged FN (Figure 5.2). With regard to lead (Pb), it was released by the aged PA α35, as well as by both PBS δ35 (non-aged: 44.7±7.2 µg/L and aged: 22.9±12.1 µg/L). The leachate of the aged PA γ33 contained significantly more Iron (Fe) (32±9 µg/L) than the leachates of the PA β33 (8.2±3.7 µg/L) and PBS δ35 (4.17±3.2 µg/L) (one-way ANOVA: $F = 15.6$, $df = 3$, p-value = 0.001; Tukey HSD post-hoc: $df = 8$, p-value < 0.001) (Figure 5.2). While the aged PA α35 had an intermediary concentration (19.45±4.6 µg/L) without significant difference with the other leachates of the aged FNs. Zinc (Zn) was also leached by all FNs. Significant higher concentrations were quantified in the leachates of all aged FNs compared to non-aged FNs (Student's t-tests: $df = 5$, $p < 0.05$). Once again, the PBS δ35 released significantly more Zn than the other FNs, whether non-aged or aged (two one-way ANOVA: $df = 3$, p-value = < 0.00001; Tukey HSD post-hoc: $df = 8$, p-value < 0.0001) (Figure 5.2).

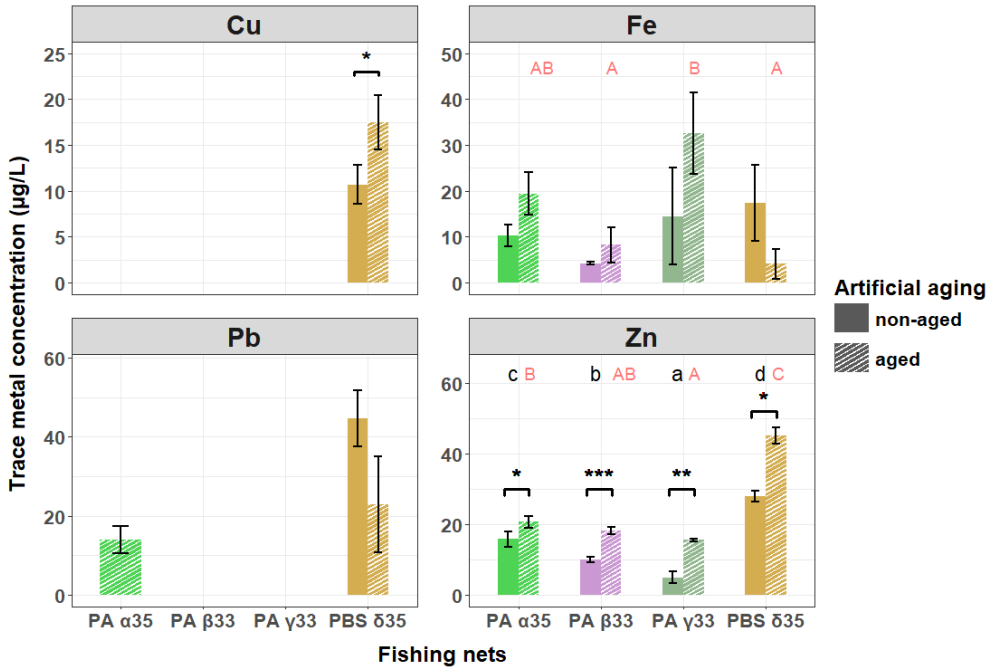


Figure 5.2: Trace metals leached during the accelerated aging of FNs.
Top of the colored bar are the average concentrations (µg/L). Full color bars show the FN leachates from non-aged FNs, while the hatched bars represent the leachates of aged FNs. Black lines at the top of the bars represent the standard deviations. Different lowercase letters above the bars indicate statistically significant differences between the leachates of non-aged FN, while the capital letters highlight differences between leachates of aged FNs. Based on two one-way ANOVAs followed by post-hoc tests (sidak) ($df = 3$, p-value < 0.05). Groups sharing the same letter are not significantly different. Sample size n = 3.

The release of organic compounds from artificially aged FNs varied significantly among the different PA FNs as well as the PBS δ35 FN (Figure 5.3). The PA β33 net was the predominant source of 1,8-diazacyclo-tetradecane-2,9-dione, a degradation by-product, with concentrations at 1,259 µg/L (non-aged) and 1,303 µg/L (aged). The PA α35, PA β33 and PBS δ35 contained lower levels with concentrations from 64 µg/L to 467 µg/L (Figure 5.3). For acetyl-tributyl-citrate, a plasticizer additive, the PA β33 and PA γ33 showed similar patterns with the highest concentrations in the leachates of non-aged FN (141 µg/L and 170 µg/L respectively) and a decrease in the leachates of aged FNs (69 µg/L and 107 µg/L respectively). N-butyl benzenesulfonamide, a non-intentionally added substance (NIAS), was released at high concentrations by the PA α35 whether non-aged (549 µg/L) or aged (516 µg/L). The ε-caprolactam, a residual PA monomer, was quantified in substantial concentrations in the PA β33 and PA γ33, with concentrations exceeding 3,000 µg/L. The PBS δ35 distinguished by its substantial release of 2-ethyl-1-hexanol, a plasticizer, which increased after aging to above 0.57 µg/L. Furthermore, the PBS δ35 released succinic acid-2-methylallyl-undecyl ester, which is supposed to be a degradation by-product, with a decrease following the aging process.

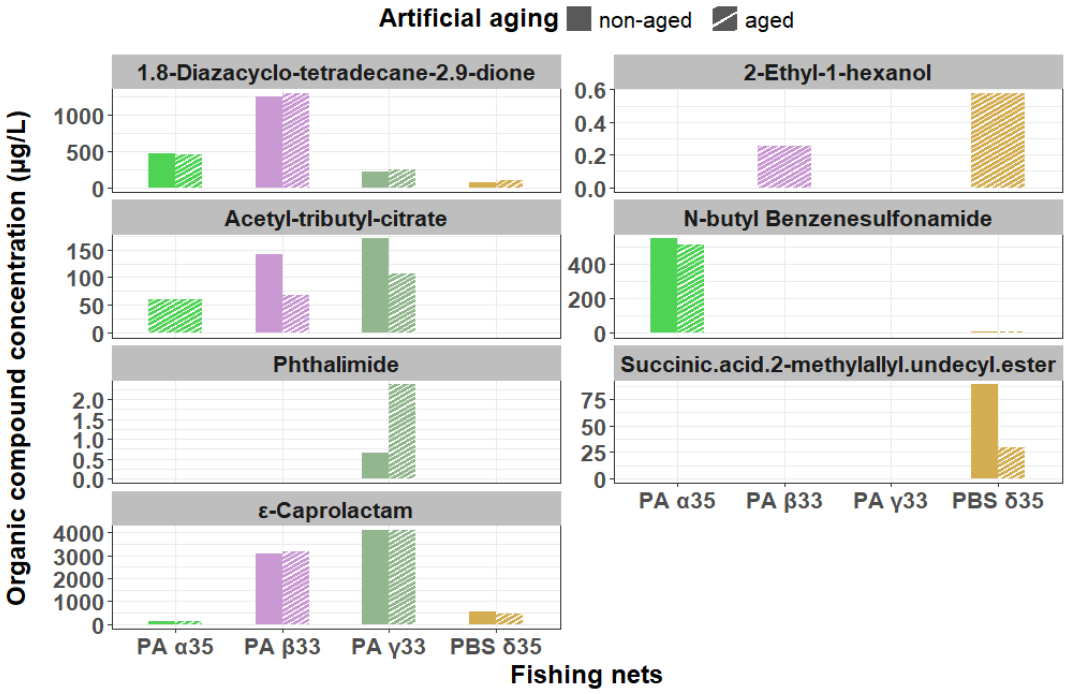


Figure 5.3: Organic compounds leached during the AA of FNs.
Top of the colored bar are the average concentrations (µg/L). Full color bars show the FN leachates from non-aged FNs, while the hatched bars represent the leachates of aged FNs. Data corrected with the concentration quantified in the blanks. Three replicates per condition were pooled but only one sample was analyzed.

5.3.2 Toxicity of the fishing nets

5.3.2.1 Effects of the FN leachates on Aliivibrio fischeri

The toxicity assessment of artificially aged FNs using the marine bacteria *A. fischeri* bioluminescence inhibition assay revealed varying degrees of toxicity between the different FNs and the different artificial aging times. The leachates of non-aged PA β33, PA γ33 and PBS δ35 had a significant toxicity compared to the extraction blanks (Welch's test: W = 3 to 4, df = 9 to 17, p-values < 0.01) (Figure 5.4). The leachate of the PBS δ35 exhibited the highest toxicity (20±5%). Concerning the leachates of aged FNs, only the leachate of the PBS δ35 caused a significant bioluminescence inhibition (23±6%) compared to the blanks (Welch's test: W = 4.99, df = 8.12, p-value = 0.001). For the other FN leachates, a slight bioluminescence inhibition was observed without to be significant (Welch's test: W = -1 to 0.4, df = 5 to 10, p-values > 0.05) (Figure 5.4). Two independent one-way ANOVA analyses revealed no significant differences in the bioluminescence inhibition between each leachate of non-aged FNs (df = 3, p-values > 0.05) and then between each leachate aged (df = 3, p-values > 0.05) (Figure 5.4).

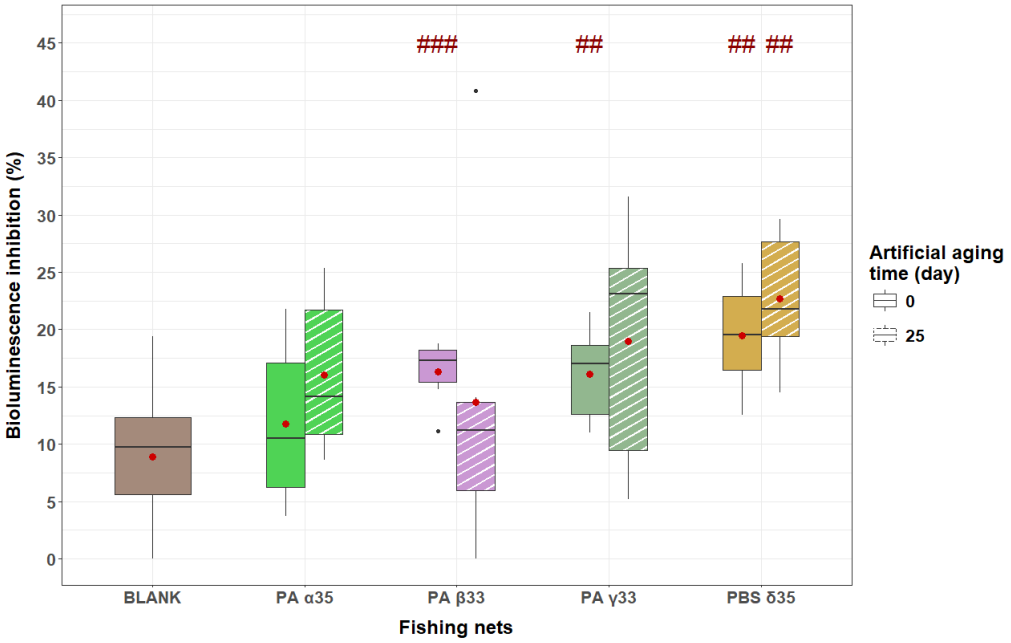


Figure 5.4: Bioluminescence inhibition of the marine bacteria *A. fischeri* exposed to FN leachates after artificial aging. Data normalized to seawater controls. The central black line of the boxplot represents the median, and the upper and lower borders are the 25th and 75th percentiles. The whiskers indicate 1.5 times the interquartile range. The red points represent the means, and the black points are the outliers. Red sharp signs indicate a significant difference of the FN leachate compared to the blank average (Welch's test: df = 6 to 17, ## = p-value < 0.01, ### = p-value < 0.001). No significant differences were observed between non-aged and aged FNs for each FN (Welch's test: df = 6 to 10, p-value > 0.05). No significant differences were found whether between each non-aged FNs or between aged FNs (two one-way ANOVAs: df = 3, p-value > 0.05). Sample size n = 6.

5.3.2.2 *Effects of the FN leachates on T. lutea cell growth*

In accordance with the calculation method outlined by the OECD/OCDE guideline n°201 (2011), exposure to leachates from artificially aged FN for a period of 72 h did not result in any inhibition of the growth rate of the microalgae *T. lutea*. However, the daily growth curves of the microalgae cultures (Figure 5.5) indicated an initial lag phase in cell growth within the first 24 hours of exposure to the leachates from the FNs PA α35, PA γ33 and PBS δ35. Nevertheless, in comparison to the blanks, only the leachate of the aged PA α35 FN resulted in a statistically significant reduction in the average cell concentration at 24 h (Student's t-test: t = -2.46, df = 15, p-value = 0.027) (Appendix 5.A, Figure 5.A.1).

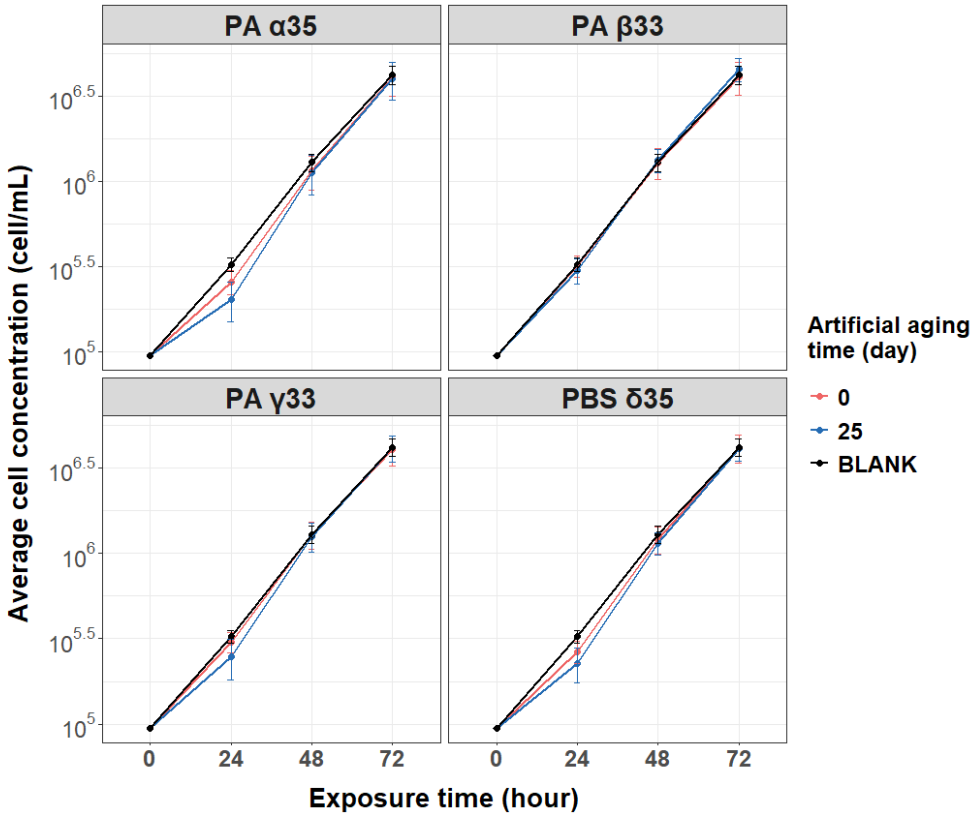


Figure 5.5: Daily growth of *T. lutea* cultures during the 72 h-exposure to leachates FNs.
Points represent the daily average cell concentrations, with error bars indicating standard errors. The black line corresponds to the growth curve of microalgae exposed to blank leachates. Blue and red lines represent growth curves for microalgae exposed to leachates from non-aged FNs (blue) and aged FNs (red). Sample size = 6.

5.3.2.3 Effects of the FN leachates on Acartia Tonsa survival

Leachates of aged FN caused a higher mortality in adult copepods than the leachates of non-aged FNs (Figure 5.6). However, no significant increase was found between the two artificial aging times (MWU test: U = -8 to 17, n = 6, p-values > 0.05). In comparison to the blank, only the non-aged FN PBS δ35 resulted in a statistically significant mortality of the adult copepods (66.7±13.3 (SE) %) (Figure 5.6). Upon exposure to the leachates of FNs artificially aged, PA α35, as well as PBS δ35, exhibited a significant lethal effect on *A. tonsa*, with mortality of 66.7±13.3 (SE) % and 70±11.3 (SE) %, respectively (MWU test: U = 45 and 49, n = 6, p-values = 0.03 and 0.007) (Figure 5.6).

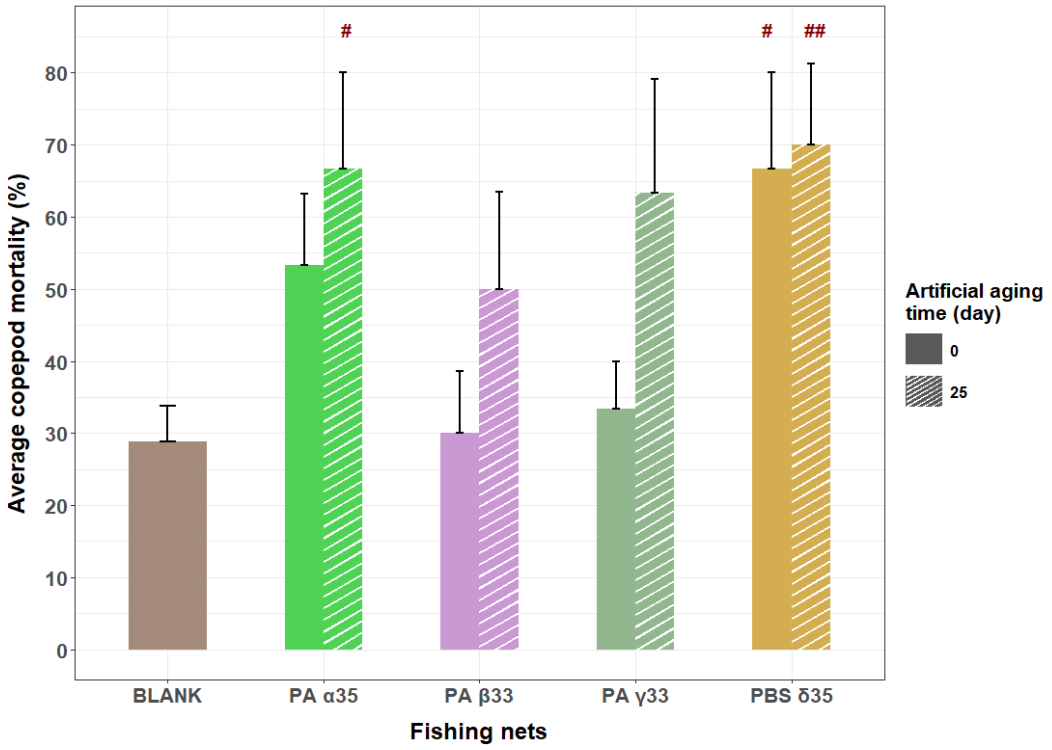


Figure 5.6: Effects of the leachates of FNs artificially aged on the survival of adult copepods (*A. tonsa*) exposed for 72 hours. Top of the bars are the average mortality percentage. Full color bars show the leachates of the non-aged FNs, while the hatched bars represent the leachates of aged FNs. Black lines at the top of the bars represent the standard errors. Red sharp signs indicate a significant difference of the FN leachate compared to the controls (MWU test: # = p-value < 0.05, ## = p-value < 0.01). No significant differences were observed between non-aged FNs and aged ones for each FN (Welch's test: df = 6 to 10, p-value > 0.05). No significant differences were found whether between non-aged FNs or between aged FNs (two one-way ANOVAs: df = 3, p-value > 0.05). Sample size n = 6.

5.3.2.4 Effects of the FN leachates on O. latipes survival, heart rate and behavior

5.3.2.4.1 Survival, development and heart rate

After being exposed for 48 hours, FN leachates did not affect the survival, swimming ability, or body curvature of *O. latipes* larvae in comparison to the blank (Appendix 5.A, Figure 5.A.2). The heart rate of fish larvae exposed to leachates of PA α35 and PA β33 FNs at both artificially aged times, showed significant alterations in comparison to the blank (MWU's test: W = 108 to 308, p-values < 0.02) (Figure 5.7). Regarding PA γ33, the leachate from the aged FN caused a statistically significant tachycardia in comparison to the blank (MWU's test: W = 346, p-values = 0.005). The leachates of the PBS δ35 caused no effects on the heart rate of the larvae (Figure 5.7). Statistical analyses of non-aged FNs indicated that PA α35 and PA β33 exerted a significant opposing effect (Dunn's test: p-value < 0.0001), with PA α35 causing bradycardia and PA β33 causing tachycardia. Furthermore, PA α35 exhibited a significantly greater impact compared to PBS δ35 (Dunn's test: p-value = 0.00) (Figure 5.7). Statistical analyses of the leachates from aged FNs revealed a contrasting effect between PA α35 (bradycardia) and PA γ33 (tachycardia) (Dunn's test: p-value = 0.0001). Finally, the leachates of PA β33 and PA γ33 highlighted a significantly greater toxicity than those of PBS δ35 (Dunn's test, p-value < 0.02) (Figure 5.7).

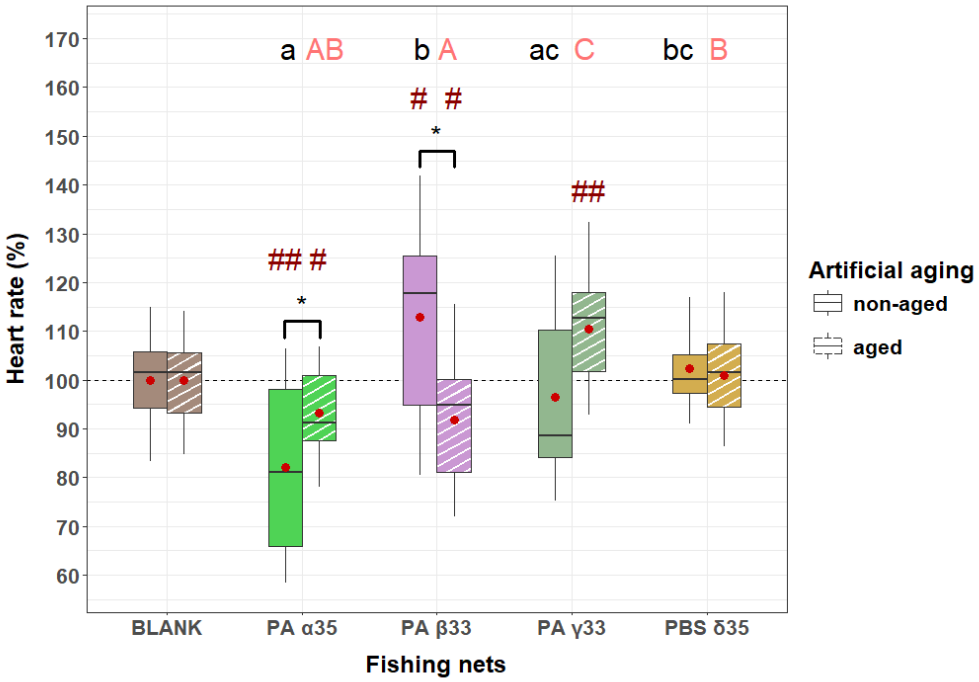


Figure 5.7: Impact of leachates of artificially aged FNs on the heart rate of Japanese medaka larvae.
The heart rates of the larvae exposed to the blank leachates were normalized at 100%. Then, the heart rates of the larvae exposed to FN leachates was normalized as a percentage of their respective blank leachates. The central black line of the boxplot represents the median, and the upper and lower borders are the 25th and 75th percentiles. The whiskers indicate 1.5 times the interquartile range. The red points represent the means, and the black ones are the outliers. The black dotted line represents the normalized heart rate of the larvae exposed to the blank leachates. Red sharp signs indicate a significant difference of the FN leachates compared to the blank average (MWU test: # = p-value < 0.05, ## = p-value < 0.01). Black brackets with stars mean significant differences between artificial aging times (MWU test: * = p-value < 0.05). Different lowercase letters above the bars indicate statistically significant differences between the leachates of non-aged FNs (Kruskal-Wallis' test: df = 3, p-value < 0.0001 and Dunn's test). Capital letters highlight differences between leachates of aged FNs (one-way ANOVA: F = 15.83, df = 3, p-value < 0.0001 and Tukey HSD). Groups sharing the same letter are not significantly different. Sample size by fishing net and AA time: n = 16.

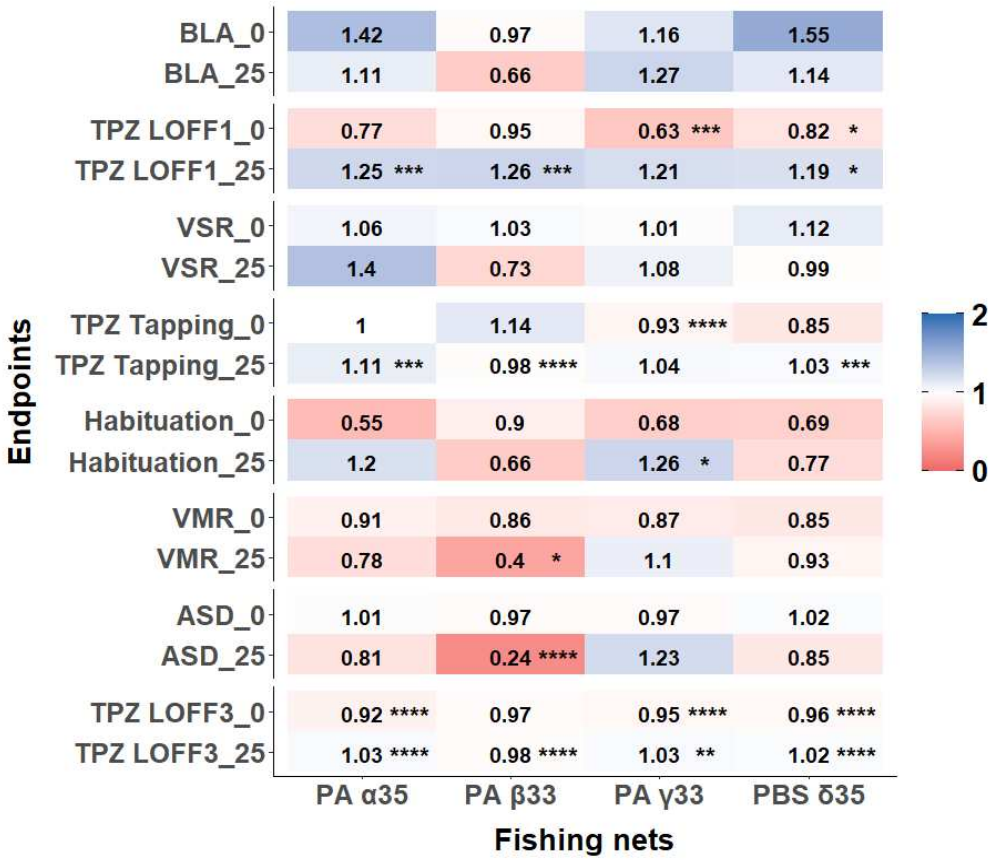


Figure 5.8: Swimming behavior of *O. latipes* larvae exposed to leachates from artificially aged FNs.
Behavioral endpoints are abbreviated as follows: BLA = Basal Locomotor Activity, TPZ = Time in Peripheral Zone, VSR = Vibrational Startle Response, VMR = Vibrational Motor Response and LOFF1/LOFF3 = Light Off during the first and third periods of test. Numbers following endpoint names (0, 25) represent the non-aged (0) and aged FNs (25). Values in the cells correspond to normalized deviations (centered around 1) from blank values for each endpoint and each FN. The color scale indicates deviations according to the blank: white cells (value = 1) show minimal deviation, red cells indicate decreased endpoint values relative to the blank, and blue cells represent increased endpoint values. Asterisks show significant differences between larvae exposed to blank leachates and FN leachates at each AA time for each endpoint (Independent Dunn's test: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$). Sample size per FN and AA time: n = 48.

5.3.2.4.2 Swimming behavior

Overall, the behavior of the larvae exposed 48 h to leachates of non-aged FNs was slightly affected compared to the blank (Figure 5.8 and Figure 5.9). The behavioral endpoint mostly affected was the time in peripheral zone (TPZ) and especially the second period of light off at the end of the assay. The larvae exposed to the leachate of the non-aged PA γ33 moved less, which highlighted hypoactivity (Figure 5.8). The larvae's response to repeated vibrational stimuli (habituation) was impacted by the leachates of non-aged FN, as evidenced by a reduced distance travelled (Figure 5.8). The habituation to vibrational stimuli was particularly affected by the leachate of the non-aged PA α35. Concerning the leachates of aged FNs, the TPZ endpoint was affected by all the FNs (Figure 5.8). Moreover, considering all the behavioral endpoints the leachates of the PA β33 and PBS δ35 caused an hypoactivity of the larvae. However, it was more significant for the larvae exposed to the leachate of the PA β33. This latter FN leachate significantly reduced the behavioral activity of the fish after stimuli related to the light (visual motor response and acclimatization to sudden darkness) (Figure 5.8).

Although not significant, it substantially reduced the larvae's response to vibratory stress (vibratory startle response and habituation) (Figure 5.8). Because of these effects, the leachate of the aged PA β33 was the one with the highest behavioral stress index, i.e., a BSI of 7 Figure 5.9). On the contrary, the leachate of the FN aged PA γ33 led to a general hyperactivity of the larvae with higher basal locomotor activity and higher TPZ. Moreover, its habituation to repeated vibrational stimuli was significantly affected with a higher distance travelled (Figure 5.8).

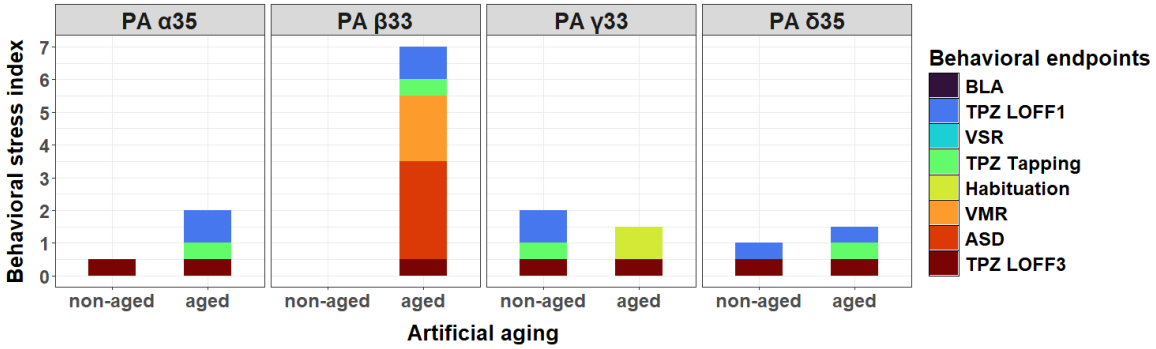


Figure 5.9: Behavioral stress index of *O. latipes* larvae exposed to leachates from artificially aged FNs.
Stacked bars represent the cumulative behavioral stress index across different artificial aging (AA) times (0 and 25 days). Each panel represents a FN. Each colored segment within the bars indicates the relative contribution of specific behavioral endpoints: Basal Locomotor Activity (BLA), Time in Peripheral Zone (TPZ) during the LOFF1, Vibrational Startle Response (VSR), TPZ Tapping, Habituation, Vibrational Motor Response (VMR), Acclimatization to Sudden Darkness (ASD), and TPZ during LOFF3.

5.3.3 Relation between degradation products and toxicity

The principal component analysis (PCA) revealed distinct associations between FN degradation products and their ecotoxicological effects. The first 5 dimensions supported 91.8% of the variances. Overall, the PCA clearly differentiated polymer compositions, with PA FNs contrasting with PBS-PBAT FNs (Figure 5.10). Dimensions 1 (29.2% of the variance supported), 2 (24.8%) and 5 (9.4%) highlighted clear positive association of zinc, copper and succinic.acid.2-methylallyl-undecyl.ester with bioluminescence inhibition of *A. fischeri* (Figure 5.10 and Appendix 5.A, Figure 5.A.4). The presence of leachates of the PBS δ35 non-aged and aged in the same area of the plot underlined their higher toxicity than the other FNs to *A. fischeri*. Furthermore, lead and the plastic additive phthalimide were associated with the disruption of the habituation behavioral endpoint, especially for the leachate of the aged PA γ33 (Figure 5.10). Dimension 1 and dimension 3 (17.4%) revealed that MPs released in higher quantity by the FNs PA α35 and aged PA γ33 were positive related to the microalgae growth lag phase and the VMR alteration (Figure 5.10). Concurrently, the N-butyl benzenesulfonamide showed positive association with the VMR alteration. Moreover, the dimensions 1, 2 and 3 highlighted that the organic compounds 1.8-diazacyclo-tetradecane-2.9-dione and acetyl-tributyl-citrate released mainly by the PA β33 (non-aged and aged) and PA γ33 (non-aged), were negatively linked to microalgae growth inhibition and copepods survival. This observation suggested that these two compounds may enhance *T. lutea* growth and *A. tonsa* survival. The dimension 1, 2, 4 (10.9%) and 5 demonstrated that zinc and copper

were positively linked with the increase of *A. tonsa* mortality, while the 2-ethyl-1-hexanol exhibited a negative relationship with heartbeat alterations (Figure 5.10; Appendix 5.A, Figure 5.A.3 and Figure 5.A.4). Dimensions 1, 4 and 5 revealed that the heart rate disruption of the larvae was positively linked to the release of iron and 1.8-diazacyclo-tetradecane-2.9-dione and negatively related to 2-ethyl-1-hexanol. Finally, the negative relationship between bioluminescence inhibition and the release of iron and 1.8-diazacyclo-tetradecane-2.9-dione indicated that these two chemical compounds may activate the metabolism of the marine bacteria or facilitate their growth (Appendix 5.A, Figure 5.A.4).

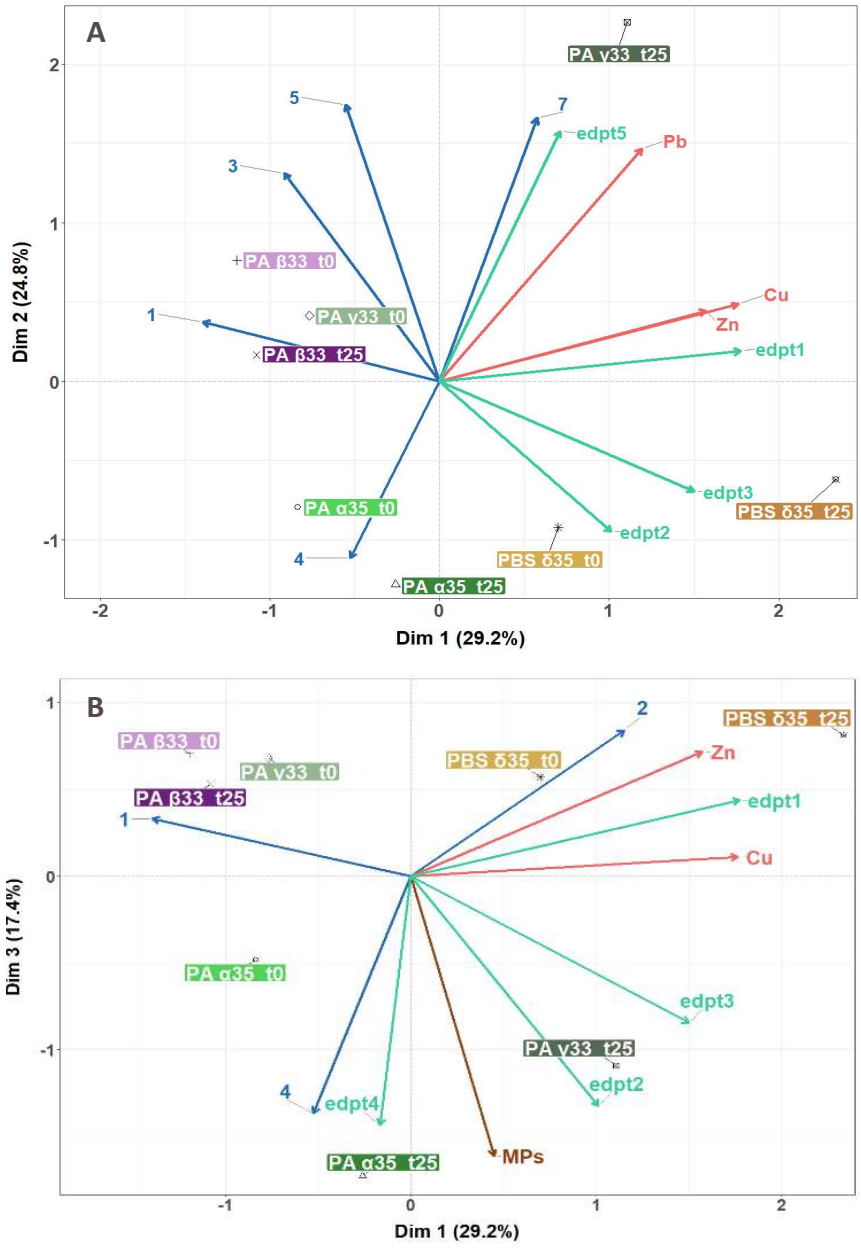


Figure 5.10: Principal component analysis of trace metals, organic compounds, MPs and toxicity endpoints.
(A) The first and second dimensions explain 54 % of the variance. (B) The first and third dimensions explain 46.6 % of the variance. The red arrows are the trace metal concentrations. The brown arrow represents MP concentration, and the dark blue arrows are the seven organic compounds detected: 1 = 1.8-diazacyclo-tetradecane-2.9-dione; 2 = 2-ethyl-1-hexanol; 3 = acetyl-tributyl-citrate; 4 = N-butyl benzenesulfonamide; 5 = ε-caprolactam; 6 = succinic.acid.2-methylallyl-undecyl.ester; 7 = phthalimide. The green arrows represent toxicity endpoints. Edpt 1 = *A. fischeri* bioluminescence inhibition; edpt2 = *T. lutea* growth rate inhibition; edpt 3 = *A. tonsa* mortality; edpt4 = *O. latipes* VMR; edpt5 = *O. latipes* habituation; edpt4 = *O. latipes* TPZ; edpt4 = *O. latipes* heartbeat. Numbers near the FN names indicate the AA times., t0 for non-aged FNs and t25 for aged FNs.

5.3.4 Risk assessment of the gillnets used in the Southeastern Bay of Biscay

Based on the quantity of PA gillnets that was used daily in 2023 in the SE BoB (Dusacre et al., 2025), it has been estimated that their average contribution to MP pollution was $1.57x10^{14}$ MPs (95% CI: 0 – $1.06x10^{15}$ MPs). This corresponds to approximately 2.2 MP/m^3 (95% CI: 0-14.8 MP/m^3) of water, taking into account the variability of degradation of the three different PA gillnets (Table 5.2). If all these oil-based gillnets had been replaced by partially biobased and biodegradable FN, their contribution to MP pollution would have been $1.05x10^{14}$ MPs (95% CI: $5.96x10^{12}$ – $2.66x10^{15}$ MPs), i.e., 1.48 MPs/m^3 (95% CI: 0-3.5 MP/m^3) (Table 5.2). Moreover, based on the data of aged FNs (equivalent to 4 years in the environment), the average contribution of the lost gillnets made of PA would be $8.46x10^{11}$ MPs (95% CI: 0 - $2.38x10^{12}$) in four years, i.e., 0.01 MPs/m^3 (95% CI: 0-0.03 MP/m^3). In general, the use of partially biobased and biodegradable FNs (PBS δ35) would likely result in the generation of fewer MPs compared to oil-based and non-biodegradable PA FNs (Table 5.2). Data for each FN were presented in Table 5.2.

Table 5.2: Estimation of MPs produced by the FNs used and lost in the Southeastern Bay of Biscay (SE BoB) in 2023.
Empty cells of a variable means that data of this variable is not related to the specific row. "nb. of part." means number of particles. "AA" means artificial aging and "FN" fishing net.

Fishing nets	AA time (day)	FN used 2023 (kg)	FN lost 2023 (kg)	MP produced (nb. of part.) by the FNs used and lost			MP/m^3 produced by the FNs in the SE BoB in 2023		
				Average	CI 0.05	CI 0.95	Average	CI 0.05	CI 0.95
PA α35	0	92,700		$1.78x10^{14}$	0	$7.43x10^{14}$	2.49	0	10.40
	25		2,750	$1.19x10^{12}$	$2.97x10^{11}$	$2.47x10^{12}$	0.02	$4.16x10^{-3}$	0.03
PA β33	0	92,700		$2.59x10^{14}$	0	$1.23x10^{15}$	3.63	0	17.16
	25		2,750	$3.11x10^{11}$	0	$7.72x10^{11}$	$4.4x10^{-3}$	0	0.01
PA γ33	0	92,700		$1.91x10^{14}$	0	$8.05x10^{14}$	2.68	0	11.28
	25		2,750	$1.88x10^{12}$	$1.61x10^{12}$	$2.28x10^{12}$	0.03	0.02	0.04
PBS δ35	0	92,700		$1.05x10^{14}$	$5.69x10^{12}$	$2.65x10^{14}$	1.48	0.08	3.72
	25		2,750	$2.68x10^{10}$	$5.95x10^{2}$	$7.64x10^{10}$	$3.75x10^{-4}$	$8.33x10^{-12}$	$1.06x10^{-3}$

The Weight of Evidence (WOE) approach enable us to estimate that after one year of operational use, gillnets are anticipated to result in a slight level of MP pollution in the marine environment (LOE1). Nevertheless, within an accumulation scenario, MPs generated by gillnets since the 1970s may have led to a moderate level of MP pollution in the SE BoB (LOE1) (Table 5.3). Regarding the lost gillnets in 2023, their predicted production of MP after four years of degradation is expected to lead to a slight level of pollution in the SE BoB (LOE1). In the scenario involving the accumulation of MPs generated by gillnets lost since 1970, three out of the four gillnets studied were predicted to have resulted in a slight level of pollution, whereas one specific gillnet (PA γ33) is predicted to have induced a moderate level of MP pollution (LOE1) (Table 5.3). With respect to the level of ecotoxicological hazard (LOE4), the degradation products of the FNs were estimated to have no or slight risk hazard (Table 5.3). The leachates from the three non-aged used gillnets made of PA were assessed to have no ecotoxicological hazard (LOE4), whereas the leachate from the non-aged PBS δ35 was estimated to have a slight ecotoxicological hazard (LOE4). Regarding the leachates from aged

gillnets, the PA β33 and PA γ33 were found to have no ecotoxicological hazard (LOE4), while PA α35 and PBS δ35 were associated with a slight ecotoxicological hazard (LOE4) (Table 5.3). Integration of the two LOEs indicated an absence of environmental risk for PA gillnets used in 2023, while a slight level of risk was identified for PBS δ35. According to the scenario of accumulation of MPs from the gillnets used since 1970 (based on leachates of non-aged FNs), a slight level of risk was predicted regardless the gillnet. Concerning the gillnets that were lost (based on leachates of aged FNs), whether in 2023 or since 1970, they were associated with an environmental risk level ranging from absent to slight (Table 5.3).

Table 5.3: Risk assessment of the degradation products of the different gillnets studied, under four exposure scenarios (S1–S4) in the SE BoB in 2023.
Hazard Quotients (HQ) were calculated at two effect levels: LOE1 "Microplastic characterization in abiotic matrices" and LOE4 "ecotoxicological bioassays". Details of the specific HQ for each toxicity response studied are available in supplementary materials (Appendix 5.A, Table 5.A.3).

Scenarios	FNs	LOE1 (HQ)	LOE4 (HQ)	Environmental risk	
S1	PA α35	SLIGHT (2.15)	ABSENT (0.87)	ABSENT	
	PA β33	SLIGHT (2.2)	ABSENT (0.22)	ABSENT	
	PA γ33	SLIGHT (2.18)	ABSENT (0.29)	ABSENT	
	PBS δ35	SLIGHT (2.09)	SLIGHT (1.19)	SLIGHT	
S2	PA α35	MODERATE (4.67)	ABSENT (0.87)	SLIGHT	
	PA β33	MODERATE (4.95)	ABSENT (0.22)	SLIGHT	
	PA γ33	MODERATE (4.84)	ABSENT (0.29)	SLIGHT	
	PBS δ35	MODERATE (4.28)	SLIGHT (1.19)	SLIGHT	
S3	PA α35	SLIGHT (2)	SLIGHT (1.27)	SLIGHT	
	PA β33	SLIGHT (2)	ABSENT (0.46)	ABSENT	
	PA γ33	SLIGHT (2)	ABSENT (0.67)	ABSENT	
	PBS δ35	SLIGHT (2)	SLIGHT (1.43)	SLIGHT	
S4	PA α35	SLIGHT (2.45)	SLIGHT (1.27)	SLIGHT	
	PA β33	SLIGHT (2.13)	ABSENT (0.46)	ABSENT	
	PA γ33	SLIGHT (2.68)	ABSENT (0.67)	ABSENT	
	PBS δ35	SLIGHT (2)	SLIGHT (1.43)	SLIGHT	

5.4 Discussion

5.4.1 Degradation of FNs into microplastics and nanoplastics and leaching of plastic chemicals

In 2018, Montarsolo et al., first empirically demonstrated that two braided FNs made of PA6 could produce MPs if they are strongly abrased. Then in 2022, another study highlighted that ropes used to haul fishing gear could produce between 14±3 and 767±75 MPs/m of rope hauled, according to their age (Napper et al. 2022). Moreover, in our previous work, we highlighted that under strong UV-irradiation and high temperature (64°C), outer panels (0.60 mm of diameter) of gillnets made of PA and PBS-PBAT produced a significant amount of micro-fragments of plastic (Dusacre et al. 2025). In this present study, we highlighted that inner panels of trammel nets or straight gillnets (0.33 or 0.35 mm in diameter) made of PA produced a significant amount of MPs (between $1.1x10^5$ and $6.8x10^5$ MP/g of FN) when aged. As observed for the degradation of ropes used in the fishing sector (Napper et al. 2022), the aging of gillnets produced microfragments but no microfibres (Liu et al. 2022). Furthermore, the abundance of MPs produced by the gillnets followed a power law relationship with a scaling factor of 1.22 and 2.17 according to the MP size range (respectively, 10 – 430 µm and 20 – 430 µm). The difference was mainly due to the limits of the laser direct infrared chemical imaging system used to quantify MPs, because the resolution started to decrease below 20 µm and was critically reduced below 10 µm (Lin et al. 2024; Dusacre et al. 2025). The power-law relationship between the abundance of MPs and their size was previously highlighted by various studies for MPs present in the environment, exhibiting scaling factors ranging from 1.96 to 4.42 (Enders et al. 2015; Erni-Cassola et al. 2017).

Even if the gillnet made of PBS-PBAT produced significantly less MPs than some FNs made of PA, it still produced some despite its biodegradable property. This partial mineralization could be due to the lack of microorganisms in the aging method (Kim et al. 2023a; Kothawale et al. 2023) or due to additives in the gillnet composition that could interfere with the biodegradability of the polymer (Paul-Pont et al. 2023). The production of MNPs by biodegradable FNs was not unexpected, as previous researches focused on the degradation of PBSAT and PBS-PBAT FNs revealed surface degradation, suspecting the formation of MNPs (Grimaldo et al. 2020; Brakstad et al. 2022; Le Gué et al. 2023). Moreover, other studies have already highlighted the production of MNPs during the aging of other types of biobased and biodegradable plastics (PLA, Mater-Bi®, PBAT, polycaprolactone, PHA, PHB, poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) (Weinstein et al. 2020; Qin et al. 2021; Wei et al. 2021). Although some other studies have examined their long-term degradation, they reported that knowledge is still insufficient to determine the environmental persistence of MNPs from biobased and biodegradable plastics (Accinelli et al. 2020; Wang et al. 2021; Colwell et al. 2023). Therefore, even if partially biobased and biodegradable gillnets could be a solution to limit ghost fishing (Cerbule et al. 2022, 2023; Wataniyakun et al. 2025), their composition need to be improved to avoid their contribution to MNP pollution.

The concentrations of MPs (from $9.8x10^4$±$1.68x10^5$ to $6.8x10^6$±$1x10^6$ MP/mL; median size 23.6 µm) in the leachates used to expose the four organisms studied in this work reproduced the most severe scenario of contamination with high concentrations compared to environmental

MP pollution (Botterell et al. 2019; Kögel et al. 2020; Ebbesen et al. 2024). Indeed, most of the marine MP concentrations reported ranged from 0.001 to 1,770 MP/L (Dubaish & Liebezeit 2013; Barrows et al. 2018; Rist et al. 2020; Campillo et al. 2023; Reineccius & Waniek 2024).
In addition to the production of MNPs, the studied gillnets released trace metals, plastic additives, NIAS and degradation by-products. Overall, the partially biobased and biodegradable gillnet released more trace metals and organic compounds than gillnets made of oil-based PA. The frequent detection of elevated quantities of additives in biobased and biodegradable plastics (Zimmermann et al. 2020; Brakstad et al. 2022; Savva et al. 2023) constitutes a significant limitation to their application as a safer alternative to oil-based and non-biodegradable plastics. Non-sustainable additives are utilized in the production of biobased and biodegradable plastics to improve certain deficiencies in their mechanical properties, such as strength and thermal stability, as well as their sanitary properties, including gas and moisture exchange (Savva et al. 2023; Siddiqui et al. 2023). Among the 16,325 plastic chemicals potentially used or found in plastics (Wagner et al. 2024), Savva et al. (2023) found an average of 121 of them in 5 different PLA and 123 in 3 PHB plastic items. Zimmerman et al. (2020) identified more than 1,000 chemical compounds in more than 80% of the 43 biobased and biodegradable plastic items they analyzed. Among the 27 organic compounds identified in the leachates of artificially aged FNs (Dusacre et al. 2025), the partially biobased and biodegradable gillnet released a higher number of organic chemicals (23) compared to gillnets made of PA (20 chemicals in average).

5.4.2 Toxicity of the degradation products of gillnets

Leachates derived from both artificially aged oil-based and partially biobased and biodegradable gillnets, exhibited significant toxicity in the four exposed species. The impact of these gillnet leachates was particularly pronounced on sublethal endpoints such as delayed growth in *T. lutea*, heart rate and behavioral disruption in *O. latipes* compared to lethal endpoints (Cormier et al. 2019; Kim et al. 2023b; Gambardella et al. 2024). Based on the ecotoxicological hazard of FNs (LOE4), FNs mostly affected the survival of the copepod *A. tonsa* and the behavior of the fish *O. latipes.* Given the ecological importance of copepods in the food chain (Mauchline 1998) and in the carbon pump (Turner 2015), these results are concerning. However, Ebbesen et al. (2024), recently showed that non-aged and aged MPs (<120 µm) from household plastics (PP, PS, PVC, PA) did not cause significant ecotoxicological effects in a mesocosm with bacteria, protists, phytoplankton and zooplankton, including copepods. Therefore, further research is needed, especially on the chronic toxicity of degradation products of FNs.
Overall, the hazard quotient (LOE4) of the partially biobased and biodegradable gillnet was higher than the gillnets made of PA, whether it was the degraded products of aged or non-aged FNs. These results were aligned with previous findings showing similar or higher multi-species toxicity of other types of biobased and biodegradable plastics (e.g., food packaging, pellets, shampoo bottle, cutlery) (Zimmermann et al. 2019, 2020; Savva et al. 2023). Interestingly, the three different gillnets made of PA did not have the same ecotoxicological hazard quotient as PA α35 > PA γ33 > PA β33, whether they were aged or non-aged. Unfortunately, knowledge of the toxicity of plastics made from PA was very limited and did

not allow comparisons with other types of products. As presented in the introduction, Zimmerman et al. (2021) have shown that extracts or leachates of different plastics made from the same polymer had different toxicities, notably due to the different plastic chemicals released. Compared to the ecotoxicological hazards results (moderate to major) of leachates of ALDFG in *Mytilus galloprovincialis*, the degradation products of the gillnets exhibited a lower hazard (Vilke et al. 2024). The higher hazard of the ALDFG could be explained mainly by two factors. First, the environmental pollutants adsorbed on ALDFG could have enhanced the toxicity (Pittura et al. 2018; Le Bihanic et al. 2020; Costigan et al. 2022). Second, the fact that these ALDFG were degraded due to the time spent in the environment could have increased the release of plastic chemicals (Liu et al. 2020; Al Harraq et al. 2022; Chen et al. 2023). With regard to the findings of Gambardella et al. (2024), the aquaculture ALDFG of the Bay of Biscay had an absence of ecotoxicological hazard, similar to the gillnets examined in our study. Conversely, results reporting the toxicity of leachates of aquaculture ALDFG of the Adriatic Sea indicated a moderate hazard closer to the results of Vilke et al. (2024).

Upon examining the ecotoxicological impacts caused by the leachate of gillnets, PCA explicitly highlighted the chemical degradation products as the main contributors to their toxicity. Concerning the trace metals released, the elevated levels of lead, copper, and zinc detected in leachates were linked to the bioluminescence inhibition of *A. fischeri*, the mortality of adults of *A. tonsa* and the behavioral disruption of *O. latipes* larvae. The findings of these relationships were not unexpected, given that concentrations of the trace metals below those quantified in this study, have been documented to cause significant toxic effects in various organisms, including *Isochrysis galbana*, *Dunaliella tertiolecta*, *Acartia tonsa*, and *Mytilus galloprovincialis* (Yap et al. 2004; Bielmyer et al. 2006; Schiavo et al. 2021; Vilke et al. 2024). Moreover, four of the quantified organic compounds (acetyl-tributyl-citrate, 2-ethyl-1-hexanol, N-butyl benzenesulfonamide, ε-caprolactam) were classified as hazardous for the environment and human health, as well as in critical need of regulation (Wagner et al. 2024). Acetyl-tributyl-citrate exposure in zebrafish embryos was identified to suppress the expression of the thyroid-related genes *tshβ*, *dio1*, *dio2*, and *trα, dio2*, *trα*, *trβ* in zebrafish and Japanese medaka, respectively (Horie et al. 2022). Furthermore, this plastic additive disrupted thyroid signaling via competitive binding to thyroid hormone receptors, leading to fish swim bladder malformations (Horie et al. 2022). Muhammad et al. (2018) also showed that six-month acetyl-tributyl-citrate exposure reduced gonado-somatic index of zebrafish by 43% in females and 17% in males. The authors also reported histopathological ovarian alterations, and impaired fecundity (-69% of fertilization rate). Concerning N-butyl benzenesulfonamide, chronic exposure of rabbits (for 12 months at 10, 50 or 100 µg per inoculation) caused progressive motor dysfunction, identified as spastic myelopathy, along with neuroaxonal and dendritic degeneration (Strong et al. 1991). In our study, acetyl-tributyl-citrate, 2-ethyl-1-hexanol, N-butyl benzenesulfonamide and ε-caprolactam could be related to disorders in light sensors development. Indeed, Shi et al. (2022) showed similar behavioral disruption in *D. rerio* after exposure to plastic additives (butylated hydroxytoluene, 2,4-di-tert-butylphenol, 4-tert-octylphenol). Faucher et al. (2006) also showed that a disruption of fish behavior (*Dicentrarchus labrax*) to vibrational stimuli could be related to metabolic dysfunction in the lateral line (Faucher et al. 2006; Bleckmann & Zelick 2009). The disruption of the heart rate of fish (*Oryzias melastigma* and *D. rerio*) caused by the leachates of non-aged and aged FNs could

also be due to some plastic chemicals, particularly phthalates (Hamid et al. 2020; Qiu et al. 2022). The three others organic chemicals quantified in FN leachates, i.e., phthalimide, 1.8-diazacyclotetradecane-2,9-dione, succinic acid, 2-methylallyl undecyl ester, had not enough data to be ranked as hazardous for the environment or human health (Wagner et al. 2024). Despite the quantification of seven organic compounds, other observed toxic effects such as increased anxiety (Chiffre et al. 2016; Bedrossiantz et al. 2023), represented by more time spent by the fish in peripheral areas, could not be linked to plastic chemicals. Further chemical analyzes should be performed using other methods such as HPLC (Savva et al. 2023). Even though chemical compounds in FN leachates were mainly responsible for toxicity, MP concentrations were also linked to possible eye malformations in fish larvae (Shi et al. 2022) and growth delays in microalgae (Zhang et al. 2017; Prata et al. 2019; Parsai et al. 2022). Concerning microalgae, Meng et al., (2024), reported similar results with an initial growth rate inhibition of *Isochrysis galbana* when exposed to 1 µm aged PS MPs at 10 mg/L. Moreover, they highlighted molecular effects such as high total protein and malondialdehyde content, reactive oxygen species over-production and over-expression of ATPases activity in *I. galbana* exposed to pristine and aged PS MPs, even for the treatment that only caused an initial growth delay (Meng et al. 2024).

5.4.3 Risk assessment of fishing nets' degradation products

Fishing gear were estimated to represent 6% of the emissions of macroplastic debris in the ocean annually (Schwarz et al. 2023). With this work, we reported new estimates of the contribution of gillnets to MNPs and chemical pollution of the ocean. Moreover, we went further than the ecotoxicological hazard assessment, as we estimated the environmental risk of FNs' degradation products. Their artificial aging in control laboratory conditions predicted that the 92.7 tons of PA gillnets used in 2023 in the SE BoB (Chapter 2) released about $1.57x10^{14}$ MPs (95% CI: 0-$1.06x10^{15}$ MPs) (median size 23.6 µm) which corresponds to an average of 2.2 MP/m^3 (95% CI: 0-14.8 MP/m^3) for the whole SE BoB. Although these estimates were derived from laboratory artificial aging and with only one method of quantification (LDIR), methods which had limits (Tosin et al. 2012; Therias et al. 2021), the value tended to be environmentally realistic. Indeed, through a literature review, Mendoza et al. (2020) reported a MP concentration in the Bay of Biscay between 2 and 2.25 items/m^3 for particles above 250 µm. Moreover, recently Lefebvre et al. (2023) quantified 0.16±0.08 MP/m^3 (2.3% of PA) and 561.7±68.5 MP/m^3 (5.9% of PA), respectively at the surface and in the water column of the Arcachon Bay (Bay of Biscay, France). In this latter study, the MP size threshold was 300 µm at the surface and 50 µm in the water column. In the same area, Lefebvre et al. (2024) showed that marine animals ingested between 0.10±0.37 MP/ind. (seabass) and 1.09±3.06 MP/ind. (spider crab) (MP sizes 40 – 1,960 µm). The lower abundance of MP ($1.05x10^{14}$ MPs, i.e., 1.48 MPs/m^3 (95% CI: 0-3.5 MP/m^3)) produced by partially biobased and biodegradable PBS-PBAT gillnets did not appear to be sufficient to reduce the level of exposure from slight to absent. In fact, the levels of exposure in the LOE1 of the scenario 1 were estimated to be slight levels of exposure of MP pollution for all the FNs studied and used in 2023 in the SE BoB. Even if the aged FNs produced more MPs per gram of FN, the fact that only 2.7 tons of gillnets were lost in 2023 in the SE BoB (Chapter 2) implies that they will have only a negligible

contribution in the emission of secondary MPs in this area (0.01 MPs/m^3 (95% CI: 0-0.03 MP/m^3)).

To conclude based on the results of this study and the WOE approach, it is estimated that the MPs released annually by the operation and loss of oil-based and non-biodegradable (PA) gillnets do not represent a significant risk for marine ecosystems of the SE BoB. If all the gillnets in PA used in the SE-BoB had been replaced with partially biobased and biodegradable (PBS-PBAT) nets, this risk would have been classified as slight. However, in a context of the accumulation of MPs derived from used gillnets, the level of risk is estimated to slight for all the FNs. Patra and Baitharu (2024), using the species sensitivity distribution approach combined with the predicted no-effect concentration and the risk quotient, estimated that MPs from the Hirakud Reservoir (Odisha, India) had a negligible ecological risk for fresh aquatic systems. The concentrations of MP quantified by the authors were 3,954 MPs/m^3 with a minimum size of 53 µm and a majority of fibres (44.86%). However, few publications have reported data on the environmental risk of secondary MPs derived from plastics used in daily life, complicating comparisons.

This first environmental risk assessment of degradation products of gillnets should be treated with caution, as it is based on several proxy, which could underestimate or overestimate the level of environmental risk. (i) The degradation of the gillnets was simulated in laboratory, which does not exactly represent environmental conditions (Therias et al. 2021). (ii) The quantity of secondary MPs produced by FNs was predicted for a pollution-free closed area excluding the toxicity of the other MPs and other pollutions. (iii) The accumulation of MPs derived from the gillnets since the year 1970 excluded the sedimentation, degradation in NPs of the MPs or the exit of some MPs from the area. (iv) The released plastic chemicals were omitted from the WOE due to insufficient environmental or regulatory information (thresholds) concerning the concentrations of plastic chemical in the environment. (v) The study identified NPs within the leachates, yet they were not measured in this research and consequently omitted from the WOE analysis. (vi) The estimation of used and lost gillnets excluded the small-scale fishing fleet not equipped with automatic identification system.

5.5 Conclusions

Plastic gillnets are among the most widely used fishing gear worldwide, accounting for approximately 3.65×10^5 km^2 and 6.01×10^4 tons. Through the artificial aging of three different oil-based (PA) and one partially biobased and biodegradable (PBS-PBAT) gillnets, we showed that they contributed to the micro, nanoplastic, and chemical pollution in oceans. In 2023, the degradation of gillnets made of PA was estimated to have polluted the SE BoB at a concentration of 2.2 MP/m^3 (95% CI: 0-14.8 MP/m^3). This annual emission of MP was estimated to be slight. However, considering the potential accumulation of the MPs produced by the gillnets since 1970 their level of pollution was estimated as moderate. Furthermore, their degradation products were shown to have toxic effects on the four aquatic species studied. They notably impacted the metabolism of the marine bacterium *A. fischeri*, the survival of adult copepods *A. tonsa*, and the heart rate and behavior of Japanese medaka larvae. Partially biobased and biodegradable PBS-PBAT gillnets did not seem to be a current suitable alternative to replace PA ones, as they also produced MNPs, release plastic chemicals

and displayed higher toxic effects. Therefore, their composition must be improved to be an eco-friendly and sustainable solution. The risk assessment of MPs produced by gillnets used and lost in 2023 in the SE BoB allows us to conclude that they represented a low or no risk to marine ecosystems. However, their potential accumulation in the marine environment over time could increase this risk.

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5.6 Supplementary materials

5.6.1 Appendix 5.A

5.6.1.1 Culture of the marine microalgae T. lutea

The initial inoculum of *Tisochrysis lutea* was procured from the Animal Physiology laboratory at the University of the Basque Country (UPV/EHU, Basque Country, Spain). Subsequently, the inoculum was scaled up, and the culture was sustained at a temperature of 22 ± 1°C, under fluorescent lighting at an intensity of 96±3 µmol/s/m^2. To maintain the culture, filtered seawater (0.2 µm) at 34±1 psu with a pH range of 7.3 to 7.8 was utilized. The culture was regularly diluted to ensure continuous exponential growth, and following each dilution, "Guillard's (F/2) Marine Water Enrichment Solution" was added to supplement the necessary nutrients.

5.6.1.2 Culture of the marine copepod A. tonsa

Cold-stored eggs of *Acartia tonsa* preserved at 4°C and distributed by Algova® (Germany) were hatched following the instructions provided by the producer. Eggs were washed from the buffer solution with 41 µm mesh sieve and 0.2 µm filtered seawater (salinity = 31±1 psu; pH = 8.0±0.1). Then they were incubated for 72 h at 22 ± 1°C in a 2 liters glass decantation funnel at the density of 200,000 eggs/L with air bubbling to prevent sedimentation. The newly hatched nauplii of A. tonsa at NI stage were transferred to a 10 L aquarium and maintained in a climate-controlled room at 23±1°C under a 14:10 h light:dark cycle. Cultures were fed with a mixture of two microalgae species in order to maintain a concentration of 3,000 cells/mL. During the naupliar stage, only Isochrysis galbana was provided, whereas for copepodites and adults, a 1:3 ratio of *I. galbana* to *Tetraselmis chuii* was used (Hafez, 2021). Aeration was maintained at a light bubbling level using a glass tube and a 0.22 µm air filter. To avoid any bacterial and cyanobacterial development, an antibiotic treatment (160 µL/L, providing 5000 units/mL of penicillin and 5,000 µg/mL of streptomycin) was added to the culture medium at every water change (Agostini et al., 2016). Every 1.5 weeks cultures were transferred in clean aquarium, with a renewal of 80% of the seawater. In order to obtain F1 or Fx generations of uniform age, nauplii were collected from the aquarium using 100 µm mesh nets and transferred to a separate aquarium 3±1 days following their initial observation.

Table 5.A.1 : Limits of detections, limits of quantifications and wavelengths used to quantify each element. Analyses were made in two different experiments.
Trace metals in the leachates of the FNs PA β33, PA γ33, and PBS δ35 were quantified during the first experiment. The leachates of the FNs PA α35 and the blanks were analyzed during the second experiment.

Trace metals Wavelength (nm)		Ag 328.1	Al 396.2	As 189.0	Cd 214,4	Co 238,3	Cr 267,7	Cu 224,7	Fe 238,2	Mn 257,6	Mo 277,5	Ni 216,6	Pb 220,4	V 268,8	Zn 213,9
First analysis	Detection limit (µg/L)	3.27	2.69	12.10	0.88	3.21	0.95	9.76	1.06	0.22	6.84	3.27	8.34	3.76	0.14
	1st range point (µg/L)	8.83	62.17	68.62	5.41	13.28	7.66	18.92	10.49	5.54	15.06	7.76	31.92	11.12	8.23
Second analysis	Detection limit (µg/L)	3.32	3.78	19.20	0.75	16.96	2.14	20.23	3.61	0.66	5.47	1.99	9.99	10.72	0.54
	1st range point (µg/L)	7.31	60.08	63.49	5.32	30.89	8.83	58.89	18.07	5.37	19.15	8.54	55.02	18.48	12.57

Table 5.A.2: Comparison of the number of microplastics between different size ranges, presented as a ratio.
Microplastic quantified using laser direct infrared chemical imaging system (Agilent 8700 LDIR Chemical Imaging System).

Size range comparison	Ratio
1-20 µm vs. 20-50 µm	27±102
1-20 µm vs. 50-100 µm	79±183
1-20 µm vs. 100-300 µm	1,301±1,959
1-20 µm vs. 300-500 µm	1,209±1,838
20-50 µm vs. 50-100 µm	20±40
20-50 µm vs. 100-300 µm	795±1,165
20-50 µm vs. 300-500 µm	712±961
50-100 µm vs. 100-300 µm	132±157
50-100 µm vs. 300-500 µm	127±131
100-300 µm vs. 300-500 µm	0±1

Table 5.A.3: Ecotoxicological hazard of the degradation products of the different gillnets studied, under four exposure scenarios (S1–S4) in the Southeastern Bay of Biscay in 2023. Hazard Quotients (HQ) – Battery were calculated based on the Specific HQ of each ecotoxicological assay.

Scenario	FNs	Species	Endpoints	Specific HQ	HQ-Battery	Level of hazard for bioassay
Scenario 1 Scenario 2	PA α35	*A. tonsa*	Survival	3.68	0.87	ABSENT
		A. fischeri	Bioluminescence	0.05		
		O. latipes	Behavior	1.03		
		O. latipes	Development	0		
		O. latipes	Heart rate	1.3		
		O. latipes	Survival	0.03		
	PA β33	*A. tonsa*	Survival	0.2	0.22	ABSENT
		A. fischeri	Bioluminescence	0.52		
		O. latipes	Behavior	0.8		
		O. latipes	Development	0.03		
		O. latipes	Heart rate	0		
		O. latipes	Survival	0		
	PA γ33	*A. tonsa*	Survival	0.37	0.29	ABSENT
		A. fischeri	Bioluminescence	0.51		
		O. latipes	Behavior	1.17		
		O. latipes	Development	0		
		O. latipes	Heart rate	0.06		
		O. latipes	Survival	0		
	PBS δ35	*A. tonsa*	Survival	5.49	1.19	SLIGHT
		A. fischeri	Bioluminescence	0.76		
		O. latipes	Behavior	1.07		
		O. latipes	Development	0		
		O. latipes	Heart rate	0		
		O. latipes	Survival	0		
Scenario 3 Scenario 4	PA α35	*A. tonsa*	Survival	5.49	1.27	SLIGHT
		A. fischeri	Bioluminescence	0.74		
		O. latipes	Behavior	1.17		
		O. latipes	Development	0		
		O. latipes	Heart rate	0.45		
		O. latipes	Survival	0		
	PA β33	*A. tonsa*	Survival	1.03	0.46	ABSENT
		A. fischeri	Bioluminescence	0.16		
		O. latipes	Behavior	1.63		
		O. latipes	Development	0		
		O. latipes	Heart rate	0.54		
		O. latipes	Survival	0		
	PA γ33	*A. tonsa*	Survival	2.03	0.67	ABSENT
		A. fischeri	Bioluminescence	0.95		
		O. latipes	Behavior	1.23		
		O. latipes	Development	0.08		
		O. latipes	Heart rate	0		
		O. latipes	Survival	0		
	PBS δ35	*A. tonsa*	Survival	5.94	1.43	SLIGHT
		A. fischeri	Bioluminescence	1.32		
		O. latipes	Behavior	1.13		
		O. latipes	Development	0		
		O. latipes	Heart rate	0		
		O. latipes	Survival	0		

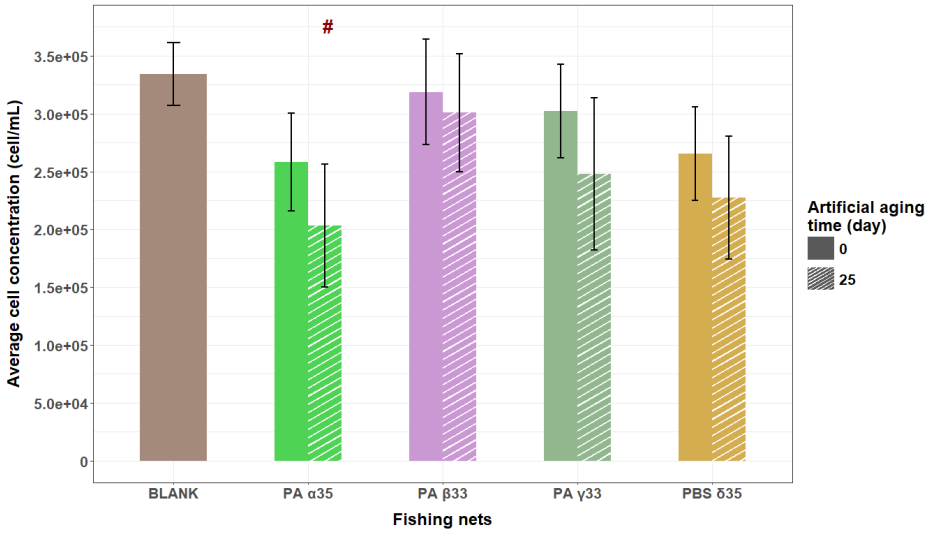

Figure 5.A.1: Average cell concentration of *T. lutea* cultures after 24 h of exposure to FN leachates artificially aged for 0 and 25 days.

Top of the colored bar are the average concentration (µg/L). Full color bars show the FN leachates from not irradiated FNs, while the hatched bars represent the leachates of FN irradiated for 25 days. Red sharp sign indicates a significant difference of the FN leachate compared to the blank average (Student's t-test: # = p-value < 0.05). There was no statistically significant difference in cell concentrations among the various FN leachates aged for 0 days, nor among those aged for 25 days (One-way ANOVAs: p-value > 0.05). For each FN there was no significant difference between the leachates of FN aged 0 day and 25 days (Welch's test: p-value > 0.05). Sample size n = 6.

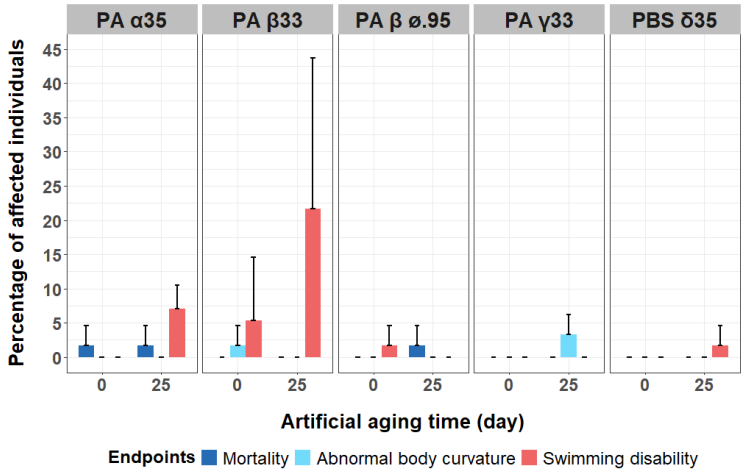

Figure 5.A.2: Mortality of *O. latipes* larvae caused by FN's DMSO extracts.

Top of the bars are the average the mortality. Full color bars show the new FN extract effects, while the hatched bars represent the used FN extract effects. Black lines at the top of the bars represent the standard deviation. Red sharp signs indicate a significant difference of the FN extract average compared to the DMSO control average (Mann–Whitney U-test: # = p-value < 0.05). Sample size by FN state = 3. Number of larvae per replicate = 18 to 20.

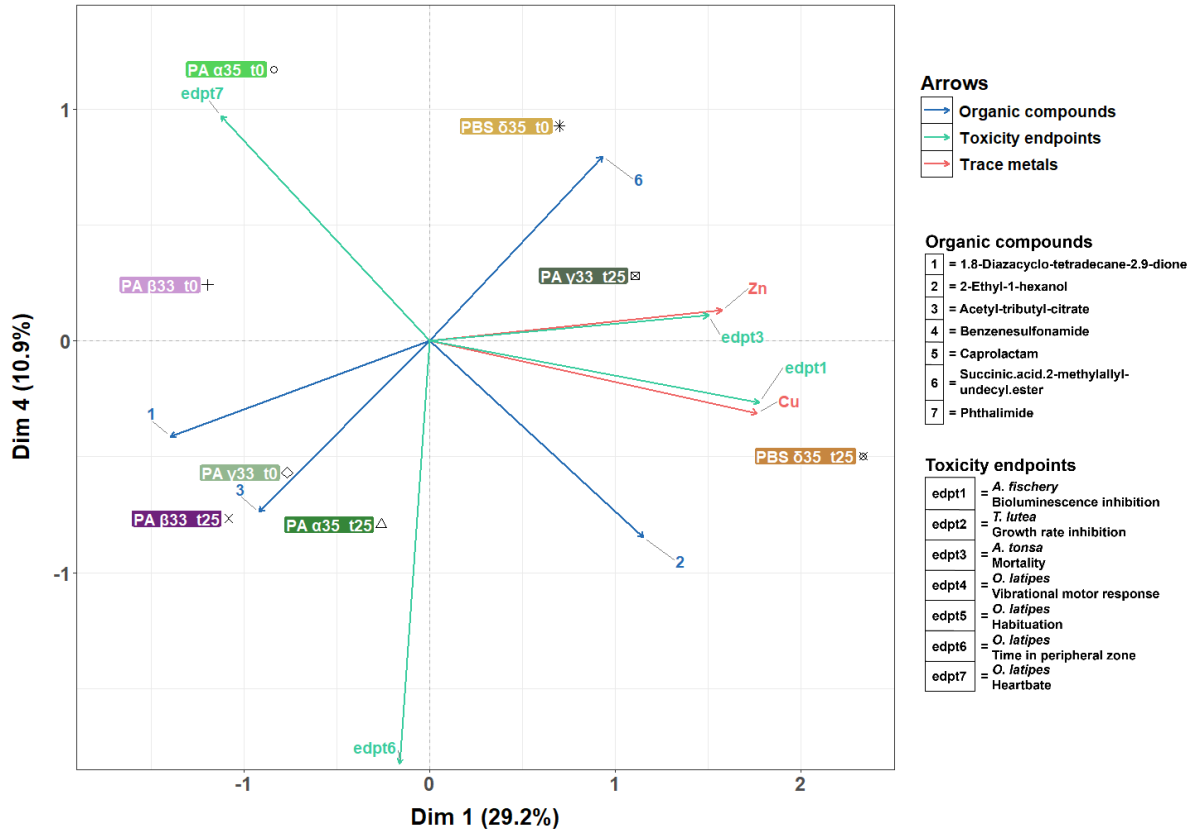

Figure 5.A.3: Representation of the dimension 1 and dimension 4 of the principal component analysis.
The red arrows are the trace metal concentrations. The brown arrow represents MP concentration, and the dark blue arrows are the seven concentrations of organic compounds. The green arrows represent toxicity endpoints. Numbers near the FN names indicate the AA times (0 or 25 days).

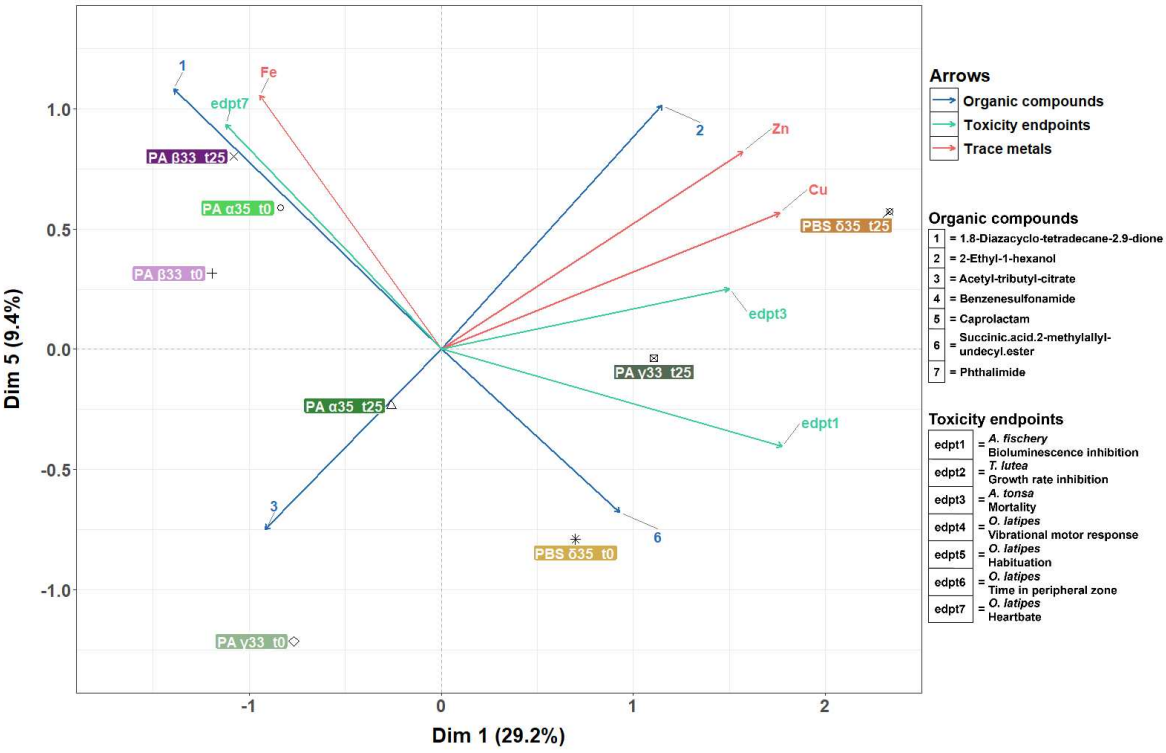

Figure 5.A.4: Representation of the dimension 1 and dimension 5 of the principal component analysis.
The red arrows are the trace metal concentrations. The brown arrow represents MP concentration, and the dark blue arrows are the seven concentrations of organic compounds. The green arrows represent toxicity endpoints. Numbers near the FN names indicate the AA times (0 or 25 days).

References

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Hafez, T. 2021. "Ecotoxicity Effects of Oil Water Accommodated Fractions and Oil Water Accommodated Fractions + Dispersant on Cold Environments: Acarita Tonsa Based Bioassays and Microbial Community Dynamics as Monitoring Tools." University of the Basque Country and University of Pau, Leioa. https://theses.hal.science/tel-03714133v1.

5.7 Highlights, main results and conclusions

Highligths of the chapter:

- Artificially aged gillnets produced a significant amount of MPs with a median size of 23.6 µm.
- Gillnets used in the SE BoB in 2023 produced 2.2 MP/m³ (95% CI: 0-14.8 MP/m^3).
- Oil-based, as well as partially biobased and biodegradable, gillnets both released hazardous chemicals.
- Each gillnet leachate significantly affected at least two out of the four species exposed.
- Oil-based, as well as partially biobased and biodegradable, nets had an absent to slight environmental risk level.

Main results of the chapter:

FNs	IMAGING & SPECTROSCOPY		DEGRADATION			CHEMICAL COMPOUNDS			BIOASSAYS											
	SEM global aspect	ATR-FTIR	Lost mass	LDIR MPs	SEM NPs	POPs		Heavy metals	Marine bacteria (*A. fischeri*)		Microalgae (*T. lutea*)		Adult zooplankton (*A. tonsa*)		Fish (*O. latipes*)					
						Non-Target	Quantif.	Quantif.	Bioluminescence inhibition (%)		Growth inhibition (%)		Survival (%)		Survival (%)		Heart rate (ctl. = 100%)		Behavior (BSI)	
									0d	25d	0d	25d	0d	25d	0d	25d	0d	25d	0d	25d
PA6 α35	✓	✓	✓	✓	✓	20	4/10	Fe, Pb, Zn	12%	16%	4%	0%	47%	**33%**	100%	93%	**82%**	**93%**	**0.5**	**2**
PA6 β33	✓	✓	✓	✓	✓	20	4/10	Fe, Zn	**16%**	14%	10%	0%	70%	50%	95%	78%	**113%**	**92%**	0	**7**
PA6 γ33	✓	✓	✓	✓	✓	20	4/10	Fe, Zn	**16%**	19%	6%	0%	67%	37%	100%	100%	93%	**111%**	**2**	**1.5**
PBS-PBAT δ35	✓	✓	✓	✓	✓	23	5/10	Cu, Fe, Pb, Zn	**20%**	**23%**	0%	0%	**33%**	**30%**	100%	98%	102%	101%	**1**	**1.5**

***Values in red and bold represent significant differences between the endpoint and the control.**

Conclusions:

This chapter revealed that gillnets made of PA contribute to MP pollution in the SE BoB, specifically generating MPs within the smallest size fraction (median 23.6 µm). The substitution of oil-based FNs with partially biobased and biodegradable alternatives has reduced gillnet MP emissions; however, this novel polymeric composition remains inadequate to fully eliminate MP pollution. After investigating the toxicity of the degradation products of these thin gillnets, it was observed that the majority of leachates substantially impacted the exposed species, particularly by decreasing the survival of the copepod *A. tonsa* and disrupting sub-lethal endpoints in two other species (*A. fischeri* bioluminescence; *O. latipes* heart rate and behavior). A significant finding was that despite gillnets being composed of the same polymer, variations between manufacturers, which likely result in differences in chemical composition, led to disparities in toxicities. Concerning the environmental risk of the FNs, the Weight of Evidence approach estimated that the degradation products of gillnets present an absent to slight risk for the marine environment. Nonetheless, this initial risk assessment was based only on acute bioassays. Consequently, it would be valuable to perform further bioassays involving sub-chronic exposure and sub-lethal endpoints at realistic environmental concentrations to more accurately reflect environmental pollution conditions.

Chapter 6 Sub-chronic exposure to environmentally realistic concentrations of leachates of artificially aged FNs affects the reproduction of copepod *Acartia tonsa*

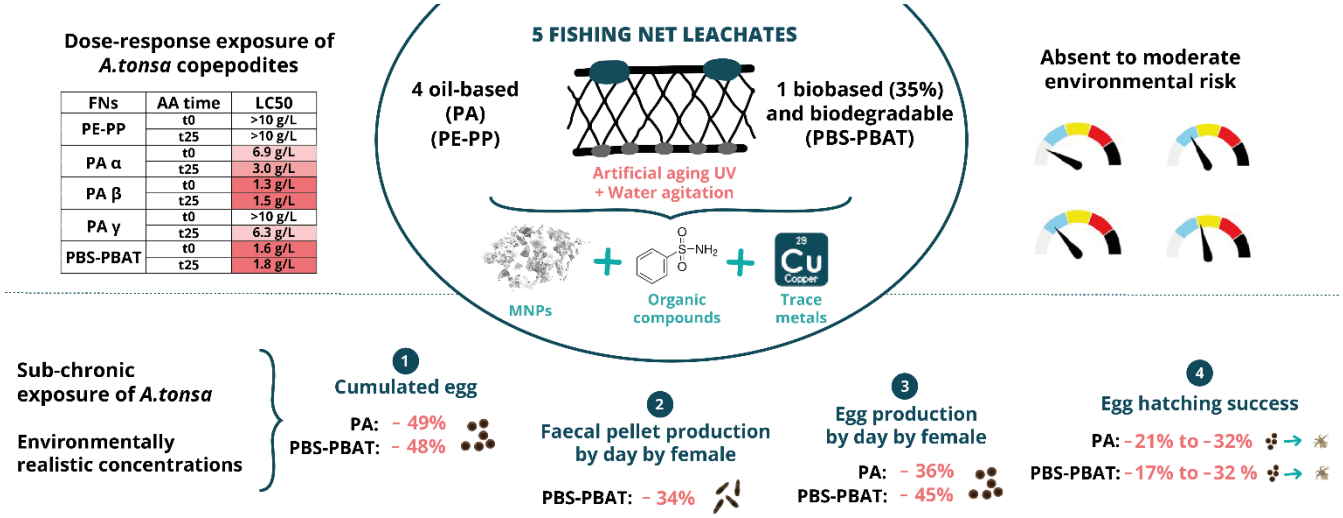
Context:

Results from the previous chapters are of concern, as they highlighted that most of the extracts and leachates of FNs caused acute toxic effects on several aquatic species. However, the toxicity observed represented scenarios of aquatic pollution with high concentrations of MPs, NPs, and plastic chemicals. Therefore, to refine these results, it was essential to expose a key marine species such as *Acartia tonsa* (copepod) to more environmentally realistic concentrations of FN leachate. The copepod clade lends itself very well to this study since these organisms are at the base of the trophic chain and play a major role in the food supply of higher trophic levels. Additionally, these organisms contribute to the regulation of phytoplankton populations and play a substantial role in the functioning of the oceanic carbon pump. Thus, if their exposure to environmental concentrations of FN degradation products over a prolonged period impacts their reproduction, feeding, or development, it could have substantial consequences for the health of coastal marine ecosystems.

Objectives:

To assess the sub-chronic toxic effects on the reproductive outcomes of the copepod species *Acartia tonsa*, resulting from exposure to degradation products derived from an oil-based fishing net, as well as a partially biobased and biodegradable alternative.

The chapter in one illustration:



The content of this chapter reflects a scientific publication that is under preparation to be submitted to the scientific journal "Environmental science & technology" as:

Dusacre, E., Casteli, M., Hafez, T., Cachot, J., & Miren, P. C., n.d. "Sub-chronic exposure to environmentally realistic concentrations of leachates of artificially aged FNs affects the reproduction of copepod Acartia tonsa."

Abstract

Plastic fishing nets (FNs) are widely used worldwide and are vital for feeding hundreds of million people. However, their risk to marine ecosystems required deeper investigations. The aim of this study was to estimate the environmental risk of the artificially aged FN leachates on the marine copeopd *Acartia tonsa.* Copepodites and fertile females were exposed to the leachates of artificially aged oil-based trawl net, gillnets, and to a partially biobased and biodegradable gillnet. Acute survival of the copepodites and the sub-chronic reproduction in the females were studied. Four of the five FNs investigated caused a significant mortality increase of the copepodites at high concentrations. Leachates of the new oil-based gillnet and partially biobased and biodegradable gillnet were the most toxic with LC50 of 34±29 mg/L and 63±120 mg/L respectively. Leachates of these two aged FNs significantly decreased the number of eggs produced (-50%) and egg hatching success of the exposed copepod females (-40%). The biobased and biodegradable gillnet exposure also reduced the production of faecal pellet (-34%). Chemical compounds released during the aging seemed to be the main source of toxicity. Based on risk assessment (Weight of Evidence) the degradation products of gillnets were estimated to present a slight to moderate coastal marine environment risk according to the selected scenario. Therefore, an improvement in the FNs' chemical composition is required necessary to ensure environmental safety while using them.

6.1 Introduction

Zooplankton, particularly copepods, serve as crucial intermediaries in marine trophic webs, facilitating the transfer of energy from primary producers to higher trophic levels. Calanoid copepods dominate planktonic communities, constituting 25 to 80% of total planktonic biomass in marine environments (Mauchline 1998). These organisms exhibit significant ecological functionality, simultaneously exerting top-down control on phytoplankton populations while providing essential nutritional resources for commercially important fish species and other marine consumers, such as cetaceans (Turner 1984; Støttrup 2000). Furthermore, zooplankton play a crucial role in biogeochemical cycling through their contribution to the biological carbon pump, facilitating carbon sequestration through the production of sinking fecal pellets (Turner 2015). However, the environmental exposure of copepods to anthropogenic pollutants, such as plastics, presents substantial concerns for the marine ecosystem. Field and laboratory studies have demonstrated that copepods can ingest roughly all types of microplastics and nanoplastics (MNPs) mainly depending on the species selectivity, particle shape, size and concentrations (Cole et al. 2013; Botterell et al. 2019; Rodríguez-Torres et al. 2024). MNPs were also shown to reduce feeding capacity, impair reproductive output, alter development, and increase mortality rates, while the early life stages exhibit increased vulnerability (Lee et al. 2013; Cole et al. 2015; Botterell et al. 2019; Yu et al. 2020; Shore et al. 2021). Notably, nauplius stages of *Acartia tonsa* have been shown to be up to one order of magnitude more sensitive than adults to certain pollutants including polychlorinated biphenyls, cypermethrin and cadmium (Forget et al. 1998; Medina et al. 2002). The implications of these disruptions extend substantially beyond individual-level effects, potentially cascading ecological consequences throughout marine food web (Shore et al. 2021; Zhao et al. 2024). In contrast, other works also reported minimal toxic effects of MPs on some zooplankton species (Zhao et al. 2024; Botterell et al. 2025). Beiras et al. (2018) showed that polyethylene MPs from commercial powder (1 - 500 µm) did not cause significant acute toxicity on 4 species of zooplankton. Furthermore, Ebbesen et al. (2024) highlighted that at both environmental and high concentrations, weathered MPs (PA, PVS, PP, PS) (<120 µm) did not significantly affect plankton organisms of their mesocosm, including copepods. These opposite results were not unexpected and two main explanations can be proposed. First, it is now known that even for similar polymers or items, the toxicity is rarely linear and similar (Zimmermann et al. 2020, 2021). This phenomenon is mainly due to the fact that two similar plastics items that do not come from the same manufacturer would have a different composition due to different manufacturing processes and plastic chemicals (Hahladakis et al. 2018; Wagner et al. 2024). Moreover, because degradation conditions are rarely homogeneous, degradation by-products will most likely differ in nature, number and concentration (Hermabessiere et al. 2017; Masry et al. 2021; Andrady 2022). Second, in many of the studies that showed significant effects on copepods, the MNP concentrations and the plastics used (size, shape, diversity) were not environmentally realistic (Botterell et al. 2019; Rodríguez-Torres et al. 2024; Zhao et al. 2024). However, with respect to concentrations, there is still a challenge in the definition of environmentally representative MP concentrations. In fact, most of the field-based estimations were for MPs > 330 µm (Botterell et al. 2019; Reineccius & Waniek 2024). This size threshold leads to underestimating the quantity of MNPs

in the marine environment (Paul-Pont et al. 2018; Reineccius & Waniek 2024). Moreover, some studies showed that the concentration of MPs increases while their size decreases. Erni-Cassola et al. (2017) determined that this phenomenon follows a power law with a ratio of concentration of approximately 2.1. Poulain et al. (2019) highlighted that small MPs (25 – 1,000 µm) are 5 to 171 times more abundant than large MPs (1-5 mm). Therefore, these observations raise the following question: "were the concentrations considered environmentally irrelevant, really too high?". A universal answer is not applicable and should be given on a case-by-case basis for each study, taking into account not only the concentration, but also the size, shape and diversity of the MNPs used in the toxicity study (Botterell et al. 2019; Ebbesen et al. 2024). A recent study estimated that the concentration of MP (10 – 5,000 µm) in the ocean was on average $1.5\times10^{5}\pm36.2\times10^{5}$ items/m^3 with a maximum of $1.2\times10^{6}\pm10.6\times10^{6}$ in the waters of the Australasian Mediterranean Sea (Reineccius & Waniek 2024). For the Atlantic European coastal waters, the concentration of MP ranged from 5 to 1603 MP/m^3 items/m^3 (Buhhalko et al. 2025). The authors found 71% of fragments and 29% of fibres. To our knowledge, no studies have been published on the sub-chronic effects of FN leachates on *A. tonsa* at environmentally relevant concentrations. However, Gambardella et al. (2024) highlighted that various species of zooplankton including *A. tonsa* were significantly affected (mortality, development anomalies and behavior disruption) by acute exposure to leachates of beached aquaculture fishing gear and maritime ropes. Therefore, given the ecological importance of copepods, their potential vulnerability to MNP and plastic chemical pollution, and the limited research on sub-chronic effects, we studied the effects of FN leachates on *A. tonsa*. In particular, we hypothesized that MNPs coming from the leachates of artificially aged FNs at environmentally realistic concentrations would have negligible effects on the survival of adult copepods but would affect the reproduction and development of the copepod *A. tonsa*.

6.2 Materials and Methods

6.2.1 Fishing nets

The first net studied was a new cod-end of a trawl, made of a blend of polyethylene and polypropylene. It was structured of two green and yellow braided ropes of 4.25 mm diameter each. In this work, it will be named "PE-PP ε4.25". The four other gear were gillnets. The first named "PA α35" was a straight gillnet made of polyamide 6 (PA). The three others were monofilament inner panels of trammel nets. The PA β33, and the PA γ33, were made of PA and had a diameter of 0.33 mm. Finally, the last one was an inner panel of a trammel net made of polybutylene succinate-polybutylene adipate terephthalate (PBS-PBAT) with a diameter of 0.35 mm. This material was partially biobased at 35% and was biodegradable. Manufacturers and sellers of these FNs were described in our precedent work (Dusacre et al. 2025).

6.2.2 Artificial aging and leachate preparation

The new FNs were artificially aged for 25 days following the method described in (Dusacre et al. 2025). Briefly, pieces of FN (2 to 2.5 cm) were irradiated under ultraviolet light (wavelength 310 nm (25%) and 365 nm (75%), 40 mW/cm^2, 23°C) during 25 days, which was equivalent to 4 years in the environment for the Southeastern Bay of Biscay (SE BoB) (Gewert et al. 2018; Dusacre et al. 2024). Subsequently, pieces of each FN were suspended in deionized water filtrated at 0.22 µm at a concentration of 10 g/L and agitated for 24 h using an orbital shaker set up at 190 rpm (Ika KS 501 digital, radius 15 mm) with 62 glass beads (1.5 mm of diameter). Ultimately, the leachates were filtered with a 1 mm stainless mesh. New FNs have also undergone the leaching step to obtain the leachates of non-aged FNs. Triplicates of aging and leachates were made for each FN and a procedure blank that followed every step without FN was also produced. At the end of the process, leachates were directly frozen at -20°C in closed glass pots. The blank samples served as controls alongside seawater controls in each bioassay.

6.2.3 Culture of the copepod *Acartia tonsa*

Cold-stored eggs of *Acartia tonsa*, kept at 4°C and supplied by Algova® (Germany), were hatched according to the manufacturer's guidelines. Briefly, eggs were separated from the buffer solution using a 41 µm mesh sieve and then rinsed with seawater filtered to 0.2 µm (salinity = 31±1 psu; pH = 8.0±0.1). They were then incubated for 72 hours at 22±1°C in a 2-liter glass decantation funnel at a concentration of 200,000 eggs per litre with filtrated air bubbling to prevent sedimentation. The newly hatched *A. tonsa* nauplii, at the “Nauplius I” stage, were transferred to a 10-litre aquarium in a climate-controlled room at 23±1°C with a 14:10-hour light:dark cycle and gentle filtrated air flow using a glass tube. Microcrustaceans were fed once a day with a combination of two microalgae species, ensuring a daily density of 3,000 cells/mL. During the naupliar stage, only *Isochrysis galbana* was provided, while copepodites and adults received a 1:3 mix of *I. galbana* and *Tetraselmis chuii* (Hafez 2021). Every 1.5 weeks, microcrustaceans were transferred to a clean aquarium with an 80% seawater renewal. An antibiotic treatment (160 µL/L containing 5,000 units/mL of penicillin and 5,000 µg/mL of streptomycin) was added at each water change to prevent bacterial and cyanobacterial growth (Agostini et al. 2016). To obtain F1 or Fx generations of consistent age, nauplii were collected from the aquarium using 100 µm mesh nets and transferred to a different aquarium 3±1 days post hatching (dph).

6.2.4 Dose-response survival assay with *Acartia tonsa* copepodites

Acute toxicity tests followed the ISO 14669 standard (International Organization for Standardization 1999) for determining acute toxicity to marine copepods. Minor modifications of the guideline were made. The vials were maintained in an incubator at 22±1°C, under an illumination of 1,000–1,500 lux and a 14:10 light:dark cycle (Gorbi et al. 2012; Hafez 2021). The exposure was prolonged until 72 h rather than the 48 h recommended. The frozen leachates of non-aged and aged FNs were thawed at 4°C. Exposure mediums were prepared

at six concentrations (0, 1.22, 3.66, 11, 33 and 100%) in artificial seawater (ASW), with salinity adjusted to 31±1 PSU, pH adjusted to 8.0±0.1, concentration of dissolved O_2 concentration >80% and 160 µL/L of antibiotic mixture. The prepared exposure conditions were agitated (vortex mixer for 15 s) and 5 mL of the mixture were transferred into 6 mL glass vials. Three replicates per leachate concentration and FN were prepared, along with blanks, artificial seawater and seawater controls (five replicates with filtered artificial seawater and five with filtered seawater (0.22 µm mesh)). Using stereomicroscope, ten F2 copepodites of 8±2 dph, were randomly assigned to each treatment and controls using a plastic Pasteur pipette. Vials were then covered with small glass petri dishes (ø40 mm) to prevent external contamination. During the assay, copepods were not fed. No distinction between males and females copepodites was done.

Observations of mortality were performed at 24, 48, and 72 hours using an inverted microscope (Nikon Eclipse Ti with a Plan Fluor 4x/0.13 OFN25 PhL DLL objective) to examine the bottom of the vials and a stereomicroscope (Nikon smz 745T) to observe the water column and the surface. Copepodites were considered dead if no movement was observed for 30 seconds. The experiment was repeated twice for statistical robustness (Gorbi et al. 2012). The test was considered valid if mortality in controls did not exceed 30% over 72 hours (International Organization for Standardization 1999; OECD/OCDE 2014).

6.2.5 Sub-chronic exposure of *Acartia tonsa*

The sub-chronic effects (mortality, faecal pellet production, reproduction, nauplii malformations) of the leachates of artificially aged FNs (PA β33, PBS δ35) on *A. tonsa* were assessed using a multiphase design (OECD/OCDE 2014). FNs PA β33 and PBS δ35 were selected based on their higher copepodite LC_{50} compared to the other leachates of FN. Copepods were exposed to $1/10^{th}$ of the lowest calculated LC_{50}, i.e., 0.132 g/L of equivalent mesoplastic concentration (OECD/OCDE 2014). The preparation of leachates followed the procedure described in the section of the dose-response survival assay. The only difference in the methodology was the use of fresh seawater filtered to 0.22 µm instead of artificial seawater. Throughout all steps of the sub-chronic exposure, the incubator was maintained at a temperature of 22±1°C, with illumination levels ranging from 1,000 to 1,500 lux, and a photoperiod set to a 14:10 light to dark cycle (Gorbi et al. 2012; Hafez 2021).

For the first phase of the sub-chronic exposure (7 days) (Appendix 6.A, Figure 6.A.1), three independent replicates, with two technical replicates per FN leachate alongside blanks and seawater controls, were prepared in 50 mL glass beakers. Blanks served as conservative control that took into account all the potential contamination along the preparation of the FN leachates. Using stereomicroscope, 10 fertile females and 2 fertile males were introduced into the beakers (Hafez 2021). Ratio of one male for five females was maintained to ensure good fertility of the females during the exposure. Daily feeding of copepods was carried out to reach a microalgal concentration of 2000 cells/mL of *I. galbana* and 1000 cells/mL of *T. chuii* within the exposure medium. Mortality was recorded daily using both stereomicroscope and inverted microscope, and dead copepods were promptly removed each day. To ensure consistent fertilization, any dead male was replaced with another male of the same culture.

Egg production was counted every 48 h and eggs were removed afterward. Every 48 h, half of the exposure medium was renewed and cleaned to maintain water quality.
At the end of Phase 1 (Appendix 6.A, Figure 6.A.1), 10 healthy females randomly selected from the two technical replicates of each FN leachate were transferred to individual 15 mL petri dishes (ø60 mm) containing fresh exposure medium, resulting in 30 dishes per condition (three independent replicates). Each female was transferred to a new petri dish with fresh exposure medium every 24 hours. The petri dishes containing the 24 h aged leachates, the eggs and the faecal pellets, were directly examined for egg and fecal pellet counts. This process was repeated at day 8, 9, and 10 to ensure precise quantification of the eggs and faecal pellets production. The 8-day petri dishes were retained for egg hatching evaluation.
Hatching success was assessed by incubating the retained petri dishes under the same experimental conditions for 48 hours. Eggs and hatched nauplii were counted under a stereomicroscope (Hafez 2021). For each replicate, 12 h after hatching, five randomly selected petri dishes were retained in a fixative solution (2.5% glutaraldehyde, 1% formaldehyde, 0.2 M sodium cacodylate) and stored at 4°C for later analyses of naupliar malformations (Poulet et al. 1995). Nauplii (10 to 40 by replicate) were observed under an inverted microscope and images were captured for morphological assessment. Malformations were identified based on morphological parameters (missing external appendages, asymmetrical appendages, swollen or emaciated body, excrescences) described by Poulet et al. (1995) and by comparing our nauplii with pictures of known well-developed *A. tonsa* nauplii (Poulet et al. 1995; Alver et al. 2011; Bruno et al. 2012). For greater robustness, three authors were involved in assessing the malformations.

6.2.6 Risk assessment of FN leachates

The environmental risk of the degradation products of both gillnets studied in this work was calculated using the Weight of Evidence approach (WOE) adapted for MPs (Avio et al. 2015; Regoli et al. 2019; Nardi et al. 2024). Two lines of evidence were used. The first one (LOE1) focused on the level of hazard of the presence and bioavailability of MPs in the water column.

Table 6.1: Scenarios of pollution of MP derived from gillnets in the SE BoB.
Equations for the calculation of the concentration of MP per square meter were described in Chapter 5.

Scenarios	Explanations
S1	Prediction of the quantity of MPs produced by the daily operation of gillnets in the SE BoB in 2023
S2	Prediction of the quantity of MPs produced by the daily operation of gillnets in the SE BoB between 1970 and 2023 based on the quantity of FNs used in 2023.
S3	Prediction of the quantity of MPs produced by the gillnets lost in the SE BoB in 2023.
S4	Prediction of the quantity of MPs produced by the gillnets lost in the SE BoB between 1970 and 2023, based on the quantity of gillnets lost in 2023.

The second one (LOE4) was based on the ecotoxicological hazard of the FN degradation products using the different endpoints of the sub-chronic exposure of *A. tonsa*. The equations used to calculate the hazard quotient (HQ) of each LOE and their integration to calculate the general WOE were described in Chapter 5. The data used for the calculations of the LOE1 were based on the scenarios of the contribution of FNs used and lost to the MP pollution in the SE BoB in 2023 (Table 6.1) (Chapter 2, Chapter 5).

6.2.7 Data and statistical analysis

Data treatment and statistical analysis were performed using RStudio (R version 4.3.1, RStudio version 2024.04.0 +735). Conditions of application of statistical tests were carefully checked for each test (density plots, Shapiro–Wilk tests, QQ-plots, Bartlett´s test or Levene´s test). When the requirements were met, Student´s t test was used to independently compare the means of each endpoint of organisms exposed to FN leachates with the values of the blank or seawater controls. It was also used to compare endpoint values of organisms exposed to leachates at different times of artificial aging of FNs. If the homogeneity of variance was not met, a Welch's test was used and if no parametric condition was met a Wilcoxon-Mann-Whitney's test (MWU test) was used. In the case of a dose-response relation, means values of the different concentrations of a same FN leachate were compared using a one-way ANOVA followed by a Tukey´s Honest post-hoc test. A significant difference was considered when p-value < 0.05. Lethal concentrations (LCx) were calculated using the "drm()" function of the "drc" R package (Ritz & Strebig 2016) with a four-parameter log-logistic model. A principal component analysis (PCA) based on centered and scaled data was performed. This analysis aimed to explore the relationships between the concentrations of MPs, organic compounds, and trace metals in the leachates described Chapter 5 and the results of the toxicity in *A. tonsa* of this study. To represent the toxic effects of the leachates in the PCA biplot, the values of cumulative egg production, daily egg production per female, daily fecal pellet production, and hatching success were subtracted from the corresponding values obtained from the controls.

6.3 Results

6.3.1 Acute effects of FN leachates on *Acartia tonsa* survival

Exposure of copepodites of *A. tonsa* to artificially aged FN leachates revealed a different pattern between the non-aged and aged FNs. Indeed, only the leachates of the non-aged PA β33 and PBS δ35 significantly affected copepodite survival (independent one-way ANOVA for each FN: df = 5, p-value < 0.05) (Figure 6.1). The post-hoc test highlighted that for the leachates of the non-aged PA β33, only the highest equivalent mesoplastic concentration (10 g/L) had a significant effect compared to the blank, with a mortality of 48±12 (SE) % (post-hoc test: Tukey HSD, p-value <0.05). While for the leachate of the non-aged PBS δ35, two concentrations (10 g/L and 3.33 g/L) caused a significant mortality of 48±7 (SE) % and 41±12 (SE) %, respectively compared to the blank (Figure 6.1).

Considering the copepodites exposed to leachates of aged FNs, four out of the five FN leachates (PA α35, PA β33, PA γ33, PBS δ35) significantly increased the copepodites mortality compared to the blank (Independent one-way ANOVA for each FN: df = 5, p-value <0.05). Among these leachates only the maximum equivalent mesoplastic concentration (10 g/L) caused a significant mortality ranging from 51±21 (SE) % for PA γ33 leachates to 58±7 (SE) % for PA β33 leachates (post-hoc test: Tukey HSD, p-value <0.05) (Figure 6.1). The statistical comparison of similar concentrations between leachates of non-aged and aged FNs revealed no differences in copepodites mortality attributable to the duration of artificial aging (independent MWU tests: df = 5, p-value > 0.05). The leachates of the non-aged and aged PE-PP ε4.25 FN induced no significant mortality increase compared to the blank.

By estimating the lethal concentration for which 50% of copepodites died (LC_{50}), the leachates of the non-aged and aged PA β33 FNs appeared to be the most toxic ones (Table 6.2). The leachates of the non-aged and aged PBS δ35 FN had the second lowest LC_{50} (Table 6.2). On the contrary, the 3 leachates of non-aged and aged PE-PP ε4.25 and non-aged PA γ33 had a LC50 higher than the maximum equivalent mesoplastic concentration tested (> 10 g/L). Expressed in MP concentration, the LC_{50} of PBS δ35 FN leachates ($1.72*10^4$ MP/L to $4.21*10^4$ MP/L), were found to be one to two orders of magnitude lower than those of the other FNs (except for the non-aged PA β33 FN). Taking into account the quantified weight loss of FNs during the artificial aging process (Appendix 6.A, Table 6.A.1), LC_{50} of non-aged and aged PA β33 FNs were 0.034±0.029 g/L and 0.063±0.12 g/L of degradation products, respectively. Concerning non-aged and aged PBS δ35 FNs, LC_{50} of their leachates were lower with values of 0.006±0.003 g/L and 0.014±0.009 g/L of degradation products, respectively.

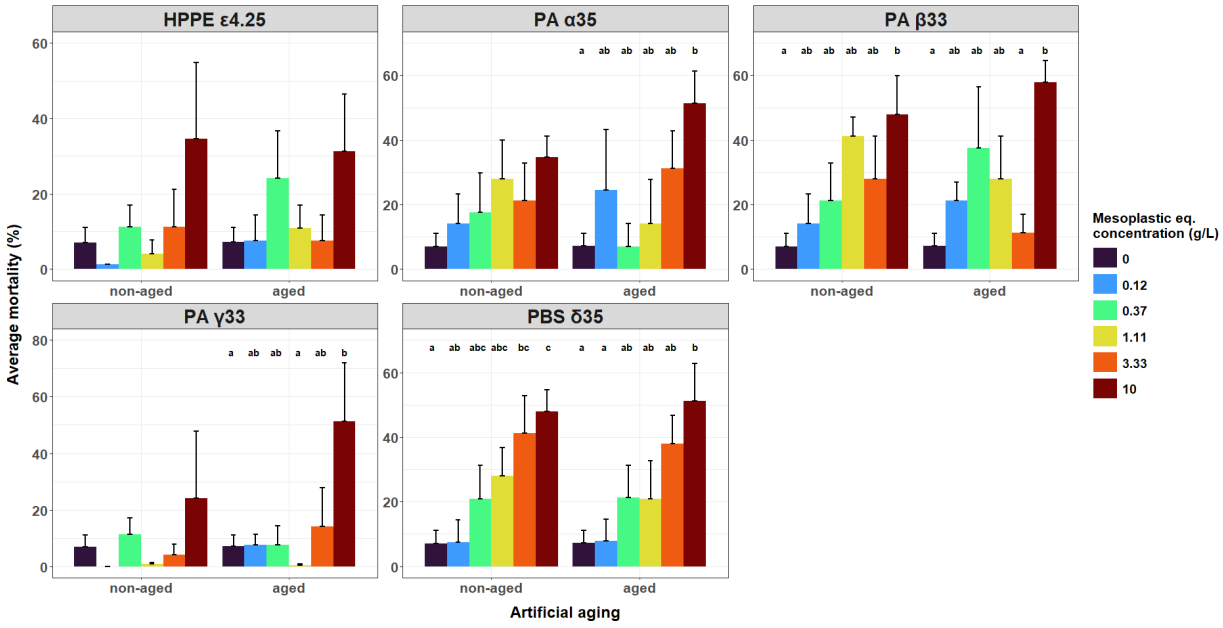


Figure 6.1: Dose-response effects of artificially aged FN leachates on the mortality of *A. tonsa* copepodites.
The top of the colored bar shows the average percentage of mortality. Black lines at the top of the bars represent the standard errors. Letters indicate significant differences between mesoplastic eq. concentration (g/L) for each AA time of each net independently (One-way ANOVA: p-values < 0.05 and post-hoc Tukey HSD). Sample size by concentration n = 6.

Table 6.2: Lethal concentration of FN leachates on the survival of *A. tonsa* copepodites.
Values indicate LC50±SE. ">10 g/L" means that the LC50 were higher than the maximum concentration tested. "AA time" mean the artificial aging time. LC20 and LC10 are available in supplementary materials (Appendix 6.A, Table 6.A.2).

Fishing nets	AA time (day)	LC_{50}±SE (g/L)	LC_{50}±SE (MP/L)
PA6 α35	0	6.94±9.65	$3.18x10^{5}$±$2.10x10^{5}$
	25	2.98±3.25	$1.29x10^{6}$±$1.21x10^{6}$
PA6 β33	0	1.32±1.14	$8.90x10^{4}$±$5.14x10^{4}$
	25	1.51±2.79	$1.71x10^{5}$±$2.87x10^{5}$
PA6 γ33	0	>10	$6.30x10^{5}$±10
	25	6.03±3.3	$4.12x10^{6}$±$1.95x10^{6}$
PBS-PBAT δ35	0	1.55±0.82	$4.21x10^{4}$±$4.44x10^{3}$
	25	1.76±1.15	$1.72x10^{4}$±$5.29x10^{3}$
HPPE-PP ε4.25	0	>10	$1.31x10^{5}$±$1.71x10^{5}$
	25	>10	$7.17x10^{6}$±$2.85x10^{7}$

6.3.2 Sub-chronic effects of fishing nets leachates on *A. tonsa* reproduction

6.3.2.1 Impact of FN leachates on survival and fecundity on A. tonsa females

After 7 days of exposure to leachates of PA β33 and PBS δ35 non-aged and aged FNs, no significant decrease in survival of female *A. tonsa* was observed. This was true compared to the blank or the seawater control (Welch's test, df = 5, p-value > 0.05) (Appendix 6.A, Figure 6.A.2). At environmentally realistic concentration, neither the leachates of the PA β33 (non-aged: $3.4x10^{-3}$±$2.9x10^{-3}$ g/L; aged: $6.3x10^{-3}$±$1.2x10^{-2}$ g/L) and PBS δ35 (non-aged: $6x10^{-4}$±$3x10^{-4}$ g/L; aged $1.4x10^{-3}$±$9x10^{-4}$ g/L) were lethally toxic for *A. tonsa*. Regarding the proportion of fecund females following 10 days of exposure no significant effect was observed (Welch's test, df = 5, p-value > 0.05) (Appendix 6.A, Figure 6.A.3).

6.3.2.2 Impact of FN leachates on egg and faecal pellet production and egg hatching success in A. tonsa females

Overall, exposure of female *A. tonsa* to FN leachates had an effect on egg-related endpoints and on faecal pellet production. Leachates of the non-aged PA β33 and PBS δ35 FNs induced a significant decrease in the cumulated number of eggs produced after 7 days of exposure compared to both blank and seawater controls (Student's t-test, df = 5, p-value < 0.05) (Figure 6.2). Leachates of the non-aged PA β33 FN resulted in a 49% reduction in cumulative egg production (70±8.9 (SE) eggs) compared to the blank (137.3±15.4 (SE) eggs). The leachate of non-aged PBS δ35 FN resulted in a 48% decrease of egg production (71.3±6.6 (SE) eggs). Concerning leachates of aged FNs, they caused no significant difference compared to the blank (Student's t-test, df = 5, p-value > 0.05). Comparing the leachates of non-aged and aged FNs, statistical analysis highlighted that the leachates of the non-aged FNs were significantly more toxic than the ones of corresponding aged FNs (Welch's test, df = 5, p-value < 0.05) (Figure 6.2). These findings were consistent with those observed in daily egg production by individual

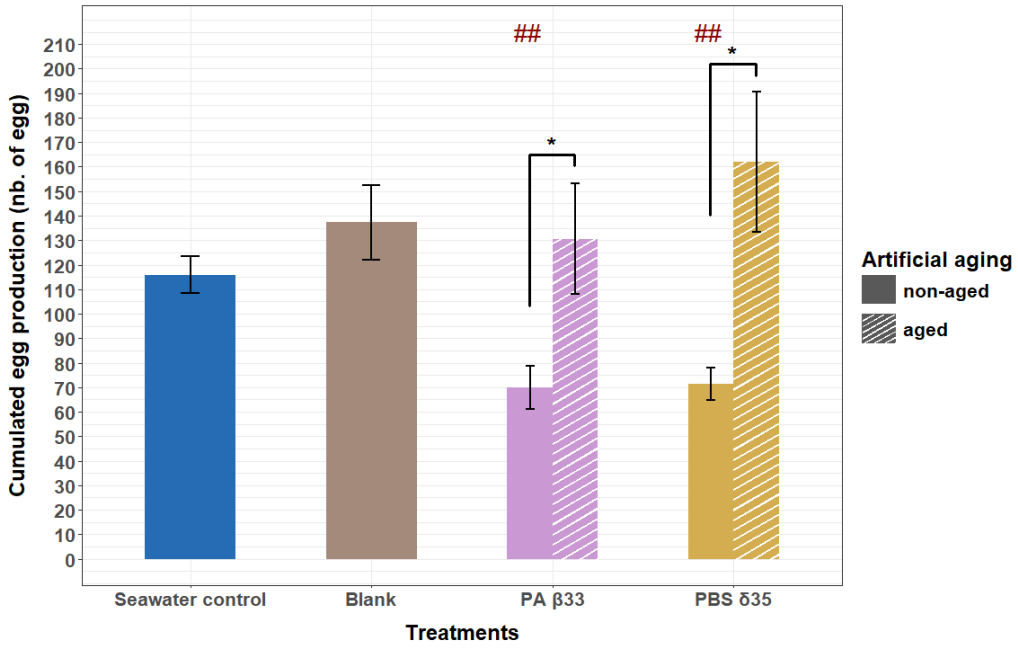


Figure 6.2: Effects of FN leachates on the cumulative egg production of adult female *A. tonsa* exposed for 7 days.
Top of the bars show the average cumulated egg production within 7 days. Error bars represent standard error. Full color bars represent the leachates from new FNs, while the hatched bars represent the leachates of aged FNs. Red sharp signs indicate a significant difference between leachates of FNs compared to the blank and seawater control (Student's t-test: ## = p-value < 0.01). Black brackets highlight a significant difference between leachates of corresponding non-aged and aged FNs (Student's t-test: * = p-value < 0.05). Sample size by treatment: n = 6

females monitored at 8, 9 and 10 days of exposure (Figure 6.3 A). Leachates from new FNs resulted in a significant reduction in egg production rate by female by day (MWU, df = 29, p-value < 0.05). Females exposed to leachates of the new PA β33 FN produced 1.5 times fewer eggs than the blank, while those exposed to the new PBS δ35 FN produced 1.7 times fewer eggs than the blank (Figure 6.3 A). Furthermore, leachates of the new FNs exhibited higher toxicity than those aged for 25 days with respect to daily egg production rate (MWU, df = 29, p-value < 0.05). During the exposure period from day 8 to day 10, leachates of the new PBS δ35 FN significantly reduced the daily fecal pellet production in females (MWU, df = 29, p-value < 0.05), resulting in a 1.5-fold decrease (-34%) (Figure 6.3 B).

Following 48 h of exposure to FN leachates, eggs laid by females previously exposed for 8 days exhibited a decrease in hatching success. Compared to blank (74.5±3.8 (SE)% of egg hatched) only the leachates of the new PA β33 (51.3±6.9 (SE)% of egg hatched) and PBS δ35 (51.7±7.7 (SE)% of egg hatched) FNs led to a significant lower hatching success (MWU test, df = 9, p-value < 0.05) (Figure 6.4). However, compared to the seawater control (83.8±2.8 (SE)% of egg hatched), leachates from non-aged and aged FNs caused a significant decrease of the hatching success (MWU test, df = 9, p-value < 0.05) (aged PA β33: (62.9±5.9 (SE)% and aged PBS δ35: 66.6±6.1 (SE)%) (Figure 6.4). Statistical comparison between the aging times revealed no significant difference (MWU test, df = 9, p-value < 0.05).

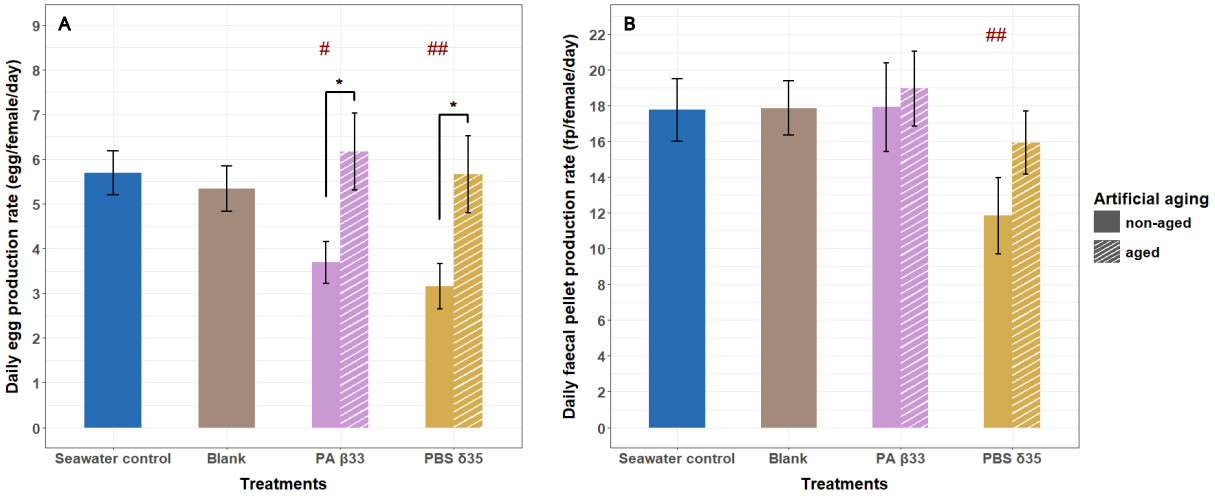


Figure 6.3: Effects of FN leachates on (A) average daily egg production rate and (B) average faecal production rate of female *A. tonsa* individuals exposed for 10 days.
The top of the bars show the daily production by reproductive females. Error bars are standard errors. Full color bars represent the leachates from new FNs, while the hatched bars represent the leachates of aged FNs. Red sharp signs indicate a significant difference between leachates of FNs compared to the blank and seawater control (MWU test: # = p-value < 0.05). Black brackets highlight a significant difference between the leachates of corresponding non-aged and aged FNs (MWU test: * = p-value < 0.05). Sample size by treatment: n = 30.

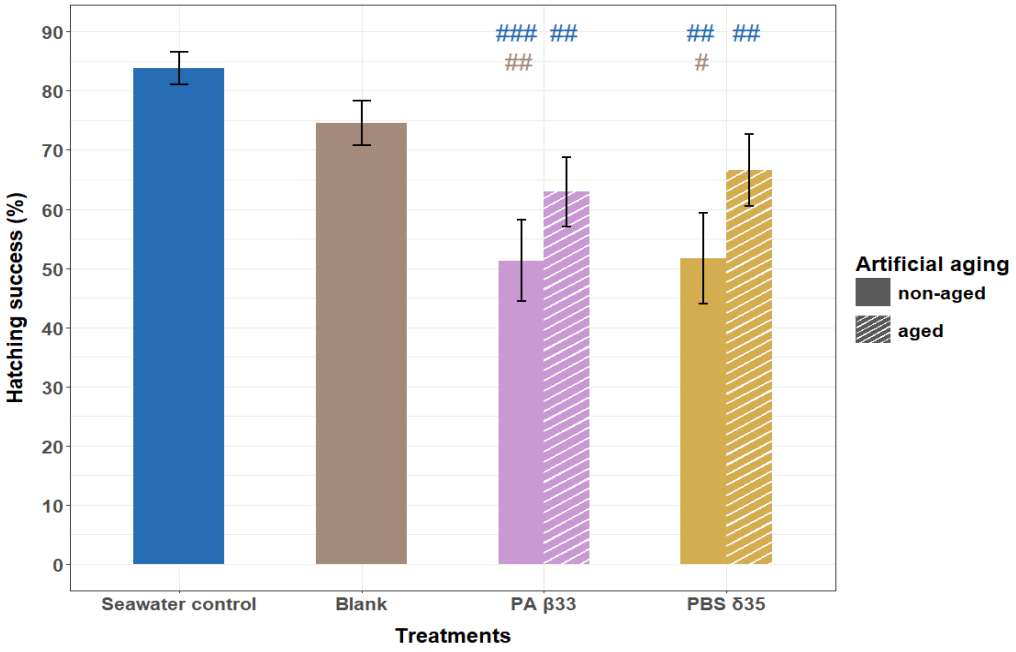


Figure 6.4: Effects of FN leachates on the hatching rate of *A. tonsa* eggs after 48 h of exposure.
The eggs were collected from females exposed up to 8 days to the leachates. Top of the bars show the average hatching percentage. Error bars denote SE. Full color bars represent the leachates non-aged FNs, while the hatched bars represent the leachates of aged FN. Blue sharp signs indicate a significant difference of the leachates of FNs compared to the seawater control, while the brown sharp signs referred to a comparison with the blank (Student's t-test: # = p-value < 0.05, ## = p-value < 0.01, ### = p-value < 0.001). Sample size by treatment: n = 27-50.

6.3.2.3 Impact of FN leachates on malformation in Acartia tonsa nauplii

The observations of nauplii at 12 h post-hatching resulted in the identification of various developmental abnormalities. Most of the malformations observed were emaciated body, swollen and asymmetrical appendages (Figure 6.5). However, no significant differences were observed between leachates of FNs compared to the blank, whatever the aging time of the FNs. Concerning PA β33 FN, leachates of both non-aged and aged FN caused similar percentage of malformation with wide SE (non-aged FNs: 7.8±4.8 (SE)% and aged FNs: 9.1± 6.2 (SE) %) (Figure 6.6). For the PBS δ35 FN, the leachates of the non-aged FN caused the lowest percentage of malformations (2.6±2.6 (SE) %). While the leachate of the aged FN caused the highest percentage of malformations with 12.1±0.8 (SE) % of the nauplii that had a malformation. However, no significant difference was found between the leachates of the non-aged and aged PBS δ35 FNs (MWU test, p-value > 0.05) (Figure 6.6). Nonetheless, it should be emphasized that no significant differences were observed either in comparison with the blank or seawater control (Student's t-test, df = 2, p-value > 0.05).



Figure 6.5: Example of well-developed and malformed nauplii after 8 days of exposure of *A. tonsa* females to leachates of artificially aged fishing nets.
The left column shows well-developed nauplii, while the right column shows malformed ones. Malformed nauplii (A) had a swollen and asymmetric right appendage. Malformed nauplii (B) had an emaciated body. Malformed nauplii (C) had swollen and asymmetric appendages. Malformed nauplii (D) had emaciated appendages and an overall malformation of the body (Poulet et al. 1995; Alver et al. 2011; Bruno et al. 2012).

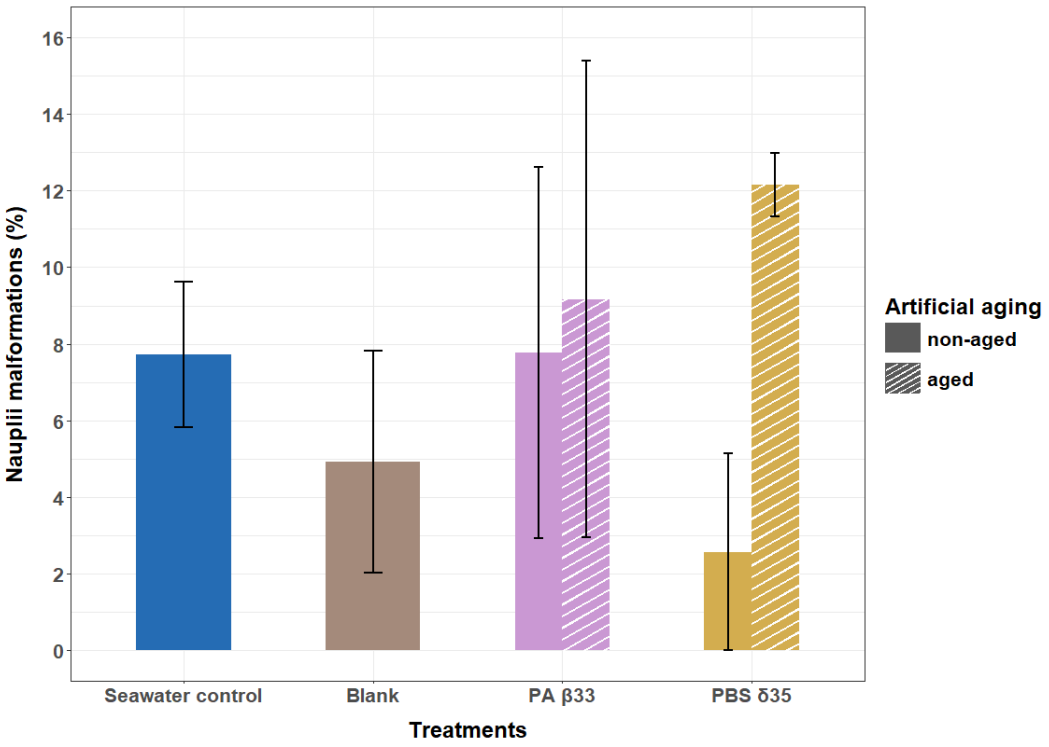


Figure 6.6: Effects of FN leachates on the development of *A. tonsa* nauplii exposed for 24 h.
The top of the bars show the average percentage of malformed nauplius. Error bars are standard errors. Full color bars represent the leachates of non-aged FNs, while the hatched bars represent the leachates of aged FNs. Sample size by treatment: n = 3.

6.3.3 Relations between *A. tonsa* reproduction impairment and the FN degradation products

The first component of the PCA hold 48.9% of the variance of the variables integrated and the second component 37.5%. This makes a total of 86.4% of the variance represented by the first two components of the PCA (Figure 6.7). The first observation derived from the principal component analysis (PCA) indicated that the toxicity endpoints associated with the decrease in egg production and hatching success were not positively related to an explanatory variable (organic compounds, trace metals and microplastic quantifications) (Figure 6.7). Furthermore, while the PCA visually suggested a potential association between nauplii malformations and certain trace metals (Cu, Pb), this relationship should be interpreted with caution. Indeed, the quantitative analysis of variable contributions reveals that edpt5 variance accounts for only 37.1% of the combined first two dimensions, indicating a weak influence on the main components (Figure 6.7). Taking into account the daily production of faecal pellets per female, the PCA demonstrated that this endpoint was positively related with the concentration of succinic-acid-2-methylallyl-undecyl.ester. This latter observation would mean that this organic compound could be an inhibitor of the production of faecal pellets. When examined in the reverse direction, the decrease in the daily production of faecal pellet per female exhibited a negative relationship with iron and MP concentrations. This meant that, potentially, when concentrations of Fe and MNPs in the leachates increased, the production of faecal pellet per female also increased.

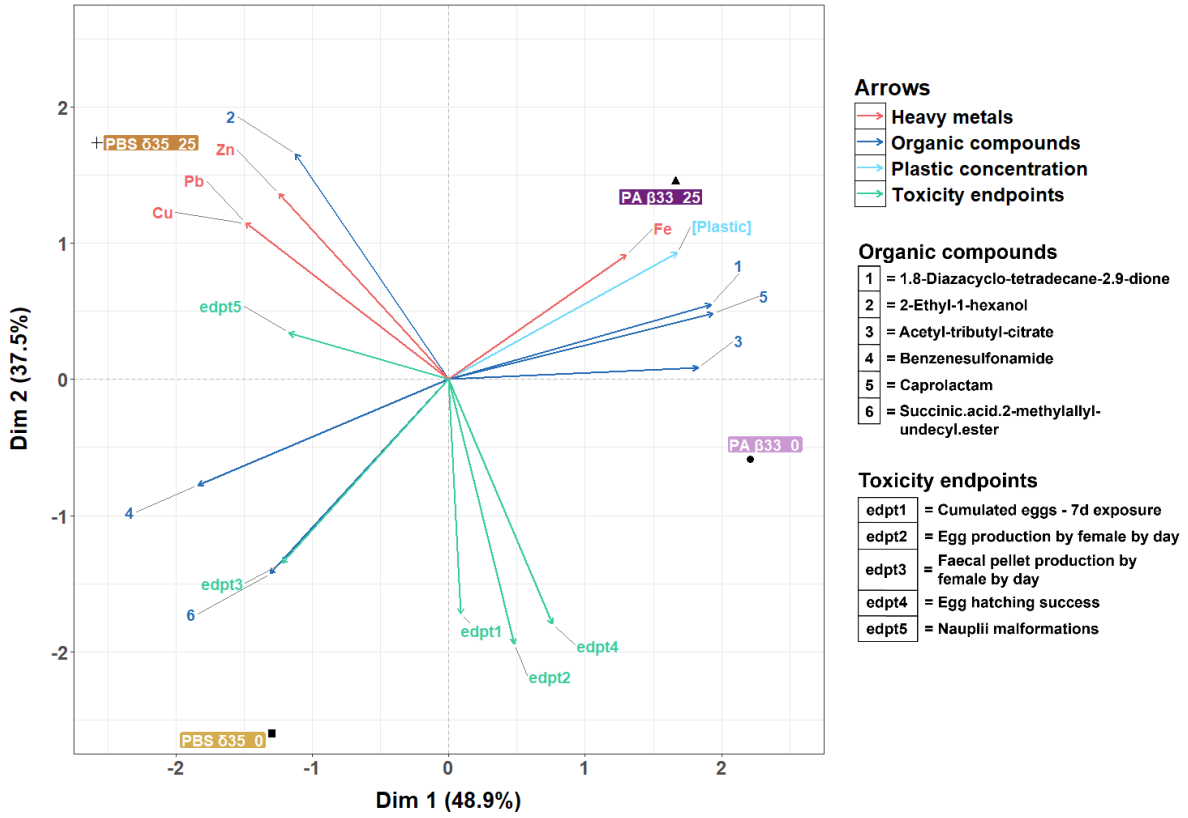


Figure 6.7: Principal component analysis of *A. tonsa* reproduction endpoints, and concentrations of microplastics, organic compounds and trace metals in leachates.
(A) The first and second dimensions explain 86.4 % of the variance. The green arrows represent the reproduction endpoints studied; the sky blue arrow represents the MP concentration, the red arrows are the concentrations of the four trace metals; and the dark blue arrows are the concentrations of the seven organic compounds. Numbers after FN names indicate the artificial aging times (0 = non-aged or 25 = aged).

6.3.4 Risk assessment of the gillnets used in the Southeastern Bay of Biscay

The 92.7 tons of FN used in the SE BoB in 2023 were estimated to cause an emission of 3.6 MP/m^3 (95% CI: 0-17.2 MP/m^3) of new secondary MPs considering that 100% of the gillnets were PA β33. This contribution to MP pollution was estimated to represent a slight level of exposure (LOE1) (Table 6.3). If they had been replaced by the PBS δ35 FN, the emission of new secondary MP would have been lower (1.48 MPs/m^3 (95% CI: 0-3.5 MP/m^3)) but the level of exposure stayed the same as for PA β33 FN (slight) (LOE1). For the PA β33 FN, the aged FN produced more MP per gram of FN (Chapter 5), but because only 6 tons of FNs were lost in 2023, their contribution to the emission of new secondary MP was very low (0.01 MPs/m^3 (95% CI: 0-0.03 MP/m^3)). However, because only a release of 0 MP/m^3 is considered without risk, the level of exposure was estimated slight (LOE1). The scenario of accumulation of secondary MP produced by gillnets during their operation since 1970 (PA β33: 230.3 MP/m^3 (95% CI: 0-663 MP/m^3); PBS δ35: 108 MP/m^3 (95% CI: 31-185 MP/m^3)) resulted in a moderate level of exposure (LOE1) for both gillnets.

In terms of ecotoxicological hazard (LOE4), the non-aged FNs were more hazardous to the copepod *A. tonsa*. In particular, the PBS δ35 caused a moderate level of hazard (HQ = 1.65). On the contrary, the degradation products of PA β33 FN were not hazardous for *A. tonsa*

(Table 6.3). The degradation products of the aged gillnets were estimated to have an absent level of hazard for the copepod *A. tonsa*.

The integration of both LOEs enabled us to estimate an absence of environmental risk of the degradation products of the PA β33 gillnet for the scenario of annual operation in 2023 (S1) and both scenarios of lost gillnets (S3 and S4) (Table 6.3). If the PBS δ35 FN had replaced the gillnets made of PA, the environmental risk of the gillnets used in 2023 in the SE BoB would have increased from absent to slight. In the scenario of accumulation of MP produced by gillnets during their operation since 1970 (S4), the environmental risk of PA β33 FN was estimated as slight. However, long-term operation of PBS δ35 FN was more of concern as a moderate environmental risk was estimated (Table 6.3).

Table 6.3: Risk assessment of the degradation products of the two different gillnets studied, under four exposure scenarios (S1–S4) in the SE BoB in 2023.
Hazard Quotients (HQ) were calculated at two effect levels: LOE1 "Microplastic characterization in abiotic matrices" and LOE4 "ecotoxicological bioassays". Details of the specific HQ for each toxicity response studied are available in the supplementary material (Appendix 6.A, Table 6.A.3).

Scenarios	FNs	LOE1	LOE4	Environmental risk	
S1	PA β33	SLIGHT (2.2)	ABSENT (0.96)	ABSENT	
	PBS δ35	SLIGHT (2.09)	MODERATE (1.65)	SLIGHT	
S2	PA β33	MODERATE (4.95)	ABSENT (0.96)	SLIGHT	
	PBS δ35	MODERATE (4.28)	MODERATE (1.65)	MODERATE	
S3	PA β33	SLIGHT (2)	ABSENT (0.27)	ABSENT	
	PBS δ35	SLIGHT (2)	ABSENT (0.28)	ABSENT	
S4	PA β33	SLIGHT (2.13)	ABSENT (0.27)	ABSENT	
	PBS δ35	SLIGHT (2)	ABSENT (0.28)	ABSENT	

6.4 Discussion

Recent years have seen a significant increase in the study of the toxicity of MPs in copepods (Shore et al. 2021; Almeda et al. 2024; Langenfeld et al. 2024; Rodríguez-Torres et al. 2024; Zhao et al. 2024; Galir et al. 2025). However, few studies focused on the toxicity of the degradation products of commonly used plastic items on copepodites survival and on the reproduction of copepods. Therefore, it was relevant to conduct research on this subject, knowing that degradation products are released when plastic ages in the oceans (Masry et al. 2021; Dimassi et al. 2022). Moreover, the effects on copepod survival and reproduction could

have considerable impacts on the rest of the trophic chain and the carbon pump (Turner 2015; Shore et al. 2021).

In this study we highlighted that degradation products from oil-based, as well as partially biobased and biodegradable gillnets, can substantially diminish the survival of *A. tonsa*'s copepodites and adversely affected the reproductive capabilities of the adult females. More specifically, this study highlighted that the effects on copepodite survival depended on the chemical composition of gillnets, as two (FN PA β33 and FN PBS δ35) of the four FN leachates studied were more toxic than the others. The higher toxicity of the leachates of PA β33 FN (LC_{50} 1.32 g/L of equivalent mesoplastic concentration) did not seem to be explained by the MP concentration estimated in these leachates, as other PA gillnets had a higher concentration of MPs (Chapter 5). Moreover, other studies observed low to no significant effects of MNPs on the acute survival of different copepod species (Lee et al. 2013; Beiras et al. 2018; Langenfeld et al. 2024). Therefore, such toxicity observed could be due to the release of chemicals from the plastic and more particularly two organic compounds (the 1.8-diazacyclo-tetradecane-2.9-dione and the 2-ethyl-1-hexanol) leached during aging of PA β33 FN. Heindler et al. (2017) reported that the di(2-ethylhexyl) phthalate, a plastic plasticizer, exacerbated the reduction in population size of the copepod *Parvocalanus crassirostris*, as compared to exposure solely to PET MPs (5-10 µm). Moreover, the 2-ethyl-1-hexanol quantified in some of our FN leachates was classified as hazardous plastic additives in the PlastChem list (Wagner et al. 2024). Its hazard is due to its carcinogenic, mutagenic and reprotoxic properties. Regarding partially biobased and biodegradable FN (PBS δ35), the toxicity of the leachates might come from the release of 2-ethyl-1-hexanol, succinic.acid.2-methylallyl.undecyl.ester and trace metals. Concerning trace metals quantified in FN leachates, Sunda et al. (1987) showed that cross-exposure of zinc and cupric ions during 96 hours significantly affected survival of adults *A. tonsa* (> 20% of mortality) from $6.5x10^{-4}$ µg/L of cupric ions and 0.65 µg/L of zinc ions. Consequently, since zinc and copper concentrations in the leachates of the FNs were higher than the toxic concentrations reported in Sunda et al. (1987), both metals could be responsible of the mortality observed. This observation was particularly true for the three highest concentrations of PA β33 FN and the four highest concentrations of PBS δ35, since at lower concentrations, zinc levels were below the ecotoxic concentrations reported in Sunda et al. (1987). Moreover, copepodites are known to be more sensitive to pollutants than adults (Sunda et al. 1987; Medina et al. 2002; Langenfeld et al. 2024).

The MP concentrations used for the sub-chronic exposure of *A. tonsa*, ranging from 1,720 MPs/L to 17,100 MPs/L (0.6 mg/L to 6.3 mg/L) can be considered environmentally realistic. Indeed, although most of marine pollution monitoring reported MP concentrations between 0.001 MP/L and tens of MP/L (Lusher et al. 2014; Barrows et al. 2018; Rist et al. 2020; Campillo et al. 2023), these values are probably underestimated due to methodological size thresholds (100 or 330 µm) (Huvet et al. 2016; Reineccius & Waniek 2024). When accounting for the complete MP size range (1-1000 µm), average marine MP concentrations were estimated at approximately 150±362 MPs/L, with extreme concentrations reaching several thousand MPs/L (Dubaish & Liebezeit 2013; Lefebvre et al. 2024; Reineccius & Waniek 2024). Using environmentally realistic MP concentrations, MP sizes and MP shapes (Botterell et al. 2019; Zhao et al. 2024), our results showed that leachates from artificially aged FNs did not

significantly affect survival, female fertility, or nauplii development in the copepod *A. tonsa*. However, the leachates and especially those of non-aged FNs, caused a significant reduction in egg production, faecal pellet production and egg hatching success of copepods. By studying more closely the inhibition of egg production and egg hatching caused by leachates of artificially aged FNs, we identified that non-aged FNs induced more toxic effects than the one aged 25 days. First, it could be hypothesized that the dry irradiation of 25 days could cause the release and degradation of volatile organic compounds (VOCs) which were therefore not released in the leachates during the water agitation. Recent studies confirmed this hypothesis, as Wu et al. (2022, 2023) demonstrated that 51, 33 and 20 VOCs were produced by the UV irradiation aging of PE, PET and PS respectively. Another study showed that straws made of coffee grounds, polylactic acid (some sold as biodegradable) and PP released between 150 and 430 VOCs during aging depending on the analysis technique used (Li et al. 2024). The higher toxicity of the leachates of non-aged FNs could also come from other hazardous organic compounds that we were not able to quantify in the leachates (Dusacre et al. 2025). Indeed, at least 1,572 chemical compounds can be released by plastic items, with many being hazardous or potentially hazardous (Wagner et al. 2024). This potentially incomplete chemical analysis of degradation products may also explain why the PCA failed to identify a relationship between chemical compounds and observed decreases in egg production and hatching success. Nonetheless, based on the degradation products quantified, the significant reduction of faecal pellet production caused by the leachate of the partially biobased and biodegradable FN agitated 24 h in water, was linked to the presence of succinic.acid.2-methylallyl.undecyl.ester and partly to acetyl-tributyl-citrate. Shore et al. (2021) observed similar effects with a mean reduction of 75% of egestion material after a chronic exposure of *A. tonsa* to 6-8 µm PS MPs. The authors also measured an overall decrease of 38% of faecal pellet production after foodborne exposure (6.0 - 8.0 µm PS MPs). The quantity of faecal pellets produced being linearly related to the amount of food ingested (Butler & Dam 1994), it can be assumed that the degradation products of the partially biobased and biodegradable FN have reduced the ingestion of microalgae by female. In addition to chemical effects, the observed feeding decrease could be attributed to selective feeding behavior demonstrated by certain copepod species (i.e., *A. tonsa*, *Acartia clausi*, and *Calanus helgolandicus*), which actively avoid MPs, but may consequently reject microalgae with similar sizes and shapes of MPs (Coppock et al. 2019). Previous studies have reported similar observations after exposure of copepods to MPs (Cole et al. 2013; Coppock et al. 2019; Malinowski et al. 2023; Galir et al. 2025). Finally, the observed feeding decrease could be part of the explanation for the drop in egg production, due to the lack of energy available for reproduction (Cole et al. 2015). The effects on the zooplankton egg production and embryo hatching success were mainly hypothesized to be due to long-term energy depletion due to a lower microalgae consumption, causing a prioritization of energy allocation for other biological functions (Cole et al. 2015; Malinowski et al. 2023; Galir et al. 2025). The decrease in feeding that may have been caused by the degradation products of FNs could resulted in cascade issues in the trophic chain. Indeed, a reduced zooplankton grazing of phytoplankton may lead to algae bloom (Hallegraeff 2010; Liu et al. 2022). Reduced feeding can also cause a depletion of lipid concentrations in copepods, which could impact the subsistence of organisms at higher levels of the trophic chain due to lower caloric transfer (Cole et al. 2015). Furthermore, by causing a

decrease of faecal pellets egestion by copepods, plastic pollution, including FNs, could alter the carbon pump and have a major impact on the health of oceans and climate (Cole et al. 2015; Turner 2015; Shore et al. 2021).

The reprotoxic and potential feeding effects caused by FN leachates on *A. tonsa* at environmentally realistic concentrations raised concerns for marine ecosystems, as zooplankton are key organisms in the food chain (Støttrup 2000; Turner 2015) and *A. tonsa* is a very abundant species in coastal marine water of the SE BoB (David et al. 2007; Cajaraville et al. 2016; Villate et al. 2018). Shore et al. (2021) highlighted that PS microbeads caused a significant reduction in *A. tonsa* diameter and a 15% decrease in reproduction rate. The authors also calculated that this decrease in reproduction rate could cause a 30-fold decrease in the abundance of the copepod population over 1 year. Moreover, in a mesocosm experiment, Langenfeld et al. (2024) showed that exposure to MPs caused a moderate reduction in egg production of the copepod *Tropocyclops extensus*, as well as a decrease in abundance of nauplii. However, in another mesocosm experiment, Ebbesen et al. (2024) did not observe significant effects of MPs from weathered plastic films (PA, PP, PS, PVC) on the population of nauplii, juvenile or adult copepods.

The degradation products of abandoned, lost or otherwise discarded fishing gear (ALDFG) (ropes, filaments, aquaculture material) from the Portuguese and Adriatic coasts were estimated to have moderate to major ecotoxicological hazard (LOE4) (Gambardella et al. 2024; Vilke et al. 2024). However, the leachates of aquaculture ALDFG from SE BoB were estimated to present an absent ecotoxicological hazard (Gambardella et al. 2024). This latter result corresponds with the findings of the current study, as the estimated ecotoxicological hazard (LO4) associated with the degradation products of aged gillnets was estimated as absent. In the contrary, the ecotoxicological hazard (LO4) associated with the degradation products derived from the non-aged PBS δ35 FN was estimated as moderate, aligning it more closely with the hazard levels observed for the ALDFG from the Portuguese and Adriatic coasts. Nardi et al. (2024), highlighted that the ecotoxicological hazard of the MPs was significantly increased with the reduction of size of the MP. The authors estimated a moderate hazard for MPs of PE with a size that ranged from 20 to 250 µm, while larger MP caused slight ecotoxicological hazard. Recently, Goncalves et al. (2025) revealed that NPs of PS (50 nm) also caused a slight to major ecotoxicological hazard based on results of cellular biomarkers battery (superoxide dismutase, catalase, glutathione S-transferase and lipid peroxidation). Particularly the authors showed that, the mixture of NPs and 5-fluorouracil had a synergetic or antagonist effect depending of the time of exposition and the sex of the mussels.

By integrating two indices, the Microplastic Pollution Index and the Environmental Status Index for beach organisms, Rangel-Buitrago et al. (2021) estimated microplastic pollution risks for 23 beaches of the Colombian Central Caribbean. The authors estimated that MP pollution posed a moderate environmental risk to 35% of the studied beaches, a high risk to 44% of them and a very high risk to 22% of them. Several studies calculated the Potential Ecological Risk Index (PERI) (Hakanson 1980) of MPs from sediment or seawater field samples, a risk index with 5 levels, enabling some cautious comparisons with our WOE approach. Khaleel et al. (2023) estimated that across 12 surface seawater samples from the St. Mary's Island (India), three presented a minor ecological risk, seven a medium risk and two a high risk. Compared to the classification of our WOE approach a “minor risk” of the PERI method would be an

absent risk, a “medium risk” would be a slight risk and a “high risk” would be a moderate risk. Wei et al. (2025) using also the PERI, estimated that MP from the sub-surface water of the South China Sea presented a high risk, while the MPs sampled in sediments had a minor ecological risk. Although our environmental risk assessment of the degradation products of gillnets in the SE BoB was based on a distinct methodological approach, it appeared to be consistent with previous risk evaluations as the risk was estimated from absent (minor in PERI approach) to moderate (high in PERI approach).

Based on the results of the present study, the production of MP by the used gillnets made of PA in a long period of time should be considered as a slight risk for the marine environment. Therefore, in addition to make some actions to reduce the ghost fishing of ALDFG (García-Barón et al. 2019; Lively & Good 2019; Roman et al. 2021), MP pollution from the gillnets should also be taken into account to reduce the impact of the fishing activity. Partially biobased and biodegradable gillnets may present a viable solution to mitigate ghost fishing (Le Gué 2024; Wataniyakun et al. 2025) and enhance the management of end-of-life FNs (Kim et al. 2023). Nonetheless, replacing the currently used PA gillnets with this alternative plastic polymer and composition could potentially intensify the issues associated with their degradation products, as a moderate environmental risk was estimated in a scenario involving long-term operation.

Therefore, the composition of these partially biobased and biodegradable materials requires improvement, and thorough ecotoxicological studies must be conducted on these new formulations to ensure a sustainable and long-term solution.

This first environmental risk of degradation products of gillnets based on sub-chronic exposure of *A. tonsa* should be treated with caution, as it is based on several proxy, which could underestimate or overestimate the level of risk. (i) The deterioration of the gillnets was examined under laboratory conditions, which may not accurately reflect the actual environmental conditions (Therias et al. 2021). (ii) Estimations of secondary MPs produced by the operation of gillnets have non-negligible uncertainties due to technical limitations in quantifying the smallest fractions of MPs in heterogeneous samples. (iii) The estimation of MPs accumulation originating from gillnets over a period of 53 years did not account for factors such as sedimentation, degradation into NPs, the transport of certain MPs out of the studied area or the entrance of MPs produced outside of the studied area. (iv) The plastic chemicals were not included in the WOE as there was a lack of environmental or regulatory data (thresholds) on the chemical compounds quantified. (v) NPs were identified within the leachates; however, they were not quantified in the present study and thus were excluded from the WOE analysis.

6.5 Conclusions

Fishing nets (FNs) are a widely used plastic item around the world, supporting the feeding of hundreds of millions of human annually. However, like any other plastics, they needed to be studied in order to determine their hazard and risk to the coastal marine ecosystems. In this context, we focused on the investigation of the toxicity of their degradation products on a key marine species, the copepod *A. tonsa*. The results highlighted in this study are of concern, as

the degradation products from the four different gillnets studied, including MNPs, organic compounds and trace metals, reduced the survival of juvenile copepods at high concentrations. Moreover, these same degradation products, but at environmentally realistic concentrations significantly impaired the faecal pellet egestion, the egg production and the nauplii hatching success of *A. tonsa* females. These findings were obtained for an inner panel of trammel net commonly used in the Bay of Biscay, and also for a prototype inner panel of trammel net made of a partially biobased and biodegradable material. The results further support the claim that the toxicity associated with FNs, predominantly originates from additives, NIAS, and degradation by-products released during their usage or weathering. The environmental risk of degradation products associated to the operation of gillnets in the SE BoB appeared to be absent to slight. However, their accumulation in the SE BoB over years could have caused and can cause in the future a slight to moderate environmental risk. Therefore, we advocate for an improvement in the chemical formulation of FNs, especially for the partially biobased and biodegradable ones currently under development as replacements for PA gillnets, in order to effectively reduce their potential risks to marine ecosystems in the future.

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6.6 Supplementary Materials

6.6.1 Appendix A.6

Table 6.A.1: Estimation of the mass of degradation products in the leachates resulting from the artificial aging of the FNs. Calculation method was described in our previous work (Dusacre et al., 2025). "AA time" mean the artificial aging time. "SD" means standard deviation.

Fishing nets	AA time (day)	Average concentration (± SD)	Unit
PA6 α35	0	0.24±0.01	g/L
	25	0.5±0.05	g/L
PA6 β33	0	0.26±0.03	g/L
	25	0.42±0.02	g/L
PA6 γ33	0	0.21±0.04	g/L
	25	0.34±0.06	g/L
PBS δ35	0	0.04±0.03	g/L
	25	0.08±0.05	g/L
PE-PP ε4.25	0	0.01±0.01	g/L
	25	0.01±0.01	g/L

Table 6.A.2: Lethal concentration of FN leachates on the survival of *A. tonsa* copepodites. Values indicate LC_x±SE. ">10 g/L" means that the LC_x were higher than the maximum concentration tested.

Fishing nets	AA time (day)	LC20±SE (g/L)	LC10±SE (g/L)	LC20±SE (MP/L)	LC10±SE (MP/L)
PA6 α35	0	0.06±0.07	$8.64x10^{-3}$±0.02	$1.62x10^{3}$±$3.91x10^{2}$	$2.41x10^{2}$±89
	25	0.05±0.08	$7.05x10^{-3}$±0.02	$5.37x10^{2}$±$3.95x10^{2}$	71±80
PA6 β33	0	0.01±0.03	$7.80x10^{-4}$±$3.28x10^{-3}$	$8.32x10^{2}$±$1.50x10^{3}$	54±$1.52x10^{2}$
	25	$1x10^{-3}$±0.01	$2.00x10^{-5}$±$2.30x10^{-4}$	$1.37x10^{2}$±$1.04x10^{3}$	2±25
PA6 γ33	0	>10	>10	$6.22x10^{5}$±$7.34x10^{2}$	$6.18x10^{5}$±$1.15x10^{3}$
	25	1.38±2.1	0.59±1.43	$9.37x10^{5}$±$1.21x10^{6}$	$3.94x10^{5}$±$8.22x10^{5}$
PBS-PBAT δ35	0	0.02±0.06	$8.40x10^{-4}$±$4.00x10^{-3}$	$1.10x10^{3}$±$1.43x10^{3}$	40±90
	25	0.06±0.15	$5.65x10^{-3}$±0.02	$2.56x10^{4}$±$5.85x10^{4}$	$2.58x10^{3}$±$9.48x10^{3}$
PE-PP ε4.25	0	0.27±0.48	0.02±0.08	$2.04x10^{3}$±$2.65x10^{3}$	$1.78x10^{2}$±$4.42x10^{2}$
	25	0.02±0.12	$1.60x10^{-4}$±$1.77x10^{-3}$	$1.57x10^{3}$±$6.61x10^{3}$	11±97

Table 6.A.3: Ecotoxicological hazard of the degradation products of two gillnets, under four exposure scenarios (S1–S4) in the Southeastern Bay of Biscay in 2023. Hazard Quotients (HQ) Battery were calculated based on the Specific HQ of each ecotoxicological assay.

Scenario	Sample code	Specie	Endpoint	Specific HQ	$HQ_{Battery}$	Level of hazard for bioassay	Note
Scenario 1	PA β33	*A. tonsa*	Survival	0.03	0.96	ABSENT	Egg production rate Feaecal pellet production rate Egg hatching
			Reproduction	2.68			
			Feeding	0			
			Development	2.71			
Scenario 2	PBS δ35	*A. tonsa*	Survival	0.07	1.65	MODERATE	Egg production rate Feaecal pellet production rate Egg hatching
			Reproduction	3.73			
			Feeding	2.99			
			Development	2.65			
Scenario 3	PA β33	*A. tonsa*	Survival	0	0.27	ABSENT	Egg production rate Feaecal pellet production rate Egg hatching
			Reproduction	0			
			Feeding	0			
			Development	1.06			
Scenario 4	PBS δ35	*A. tonsa*	Survival	0	0.28	ABSENT	Egg production rate Feaecal pellet production rate Egg hatching
			Reproduction	0			
			Feeding	0.72			
			Development	0.71			

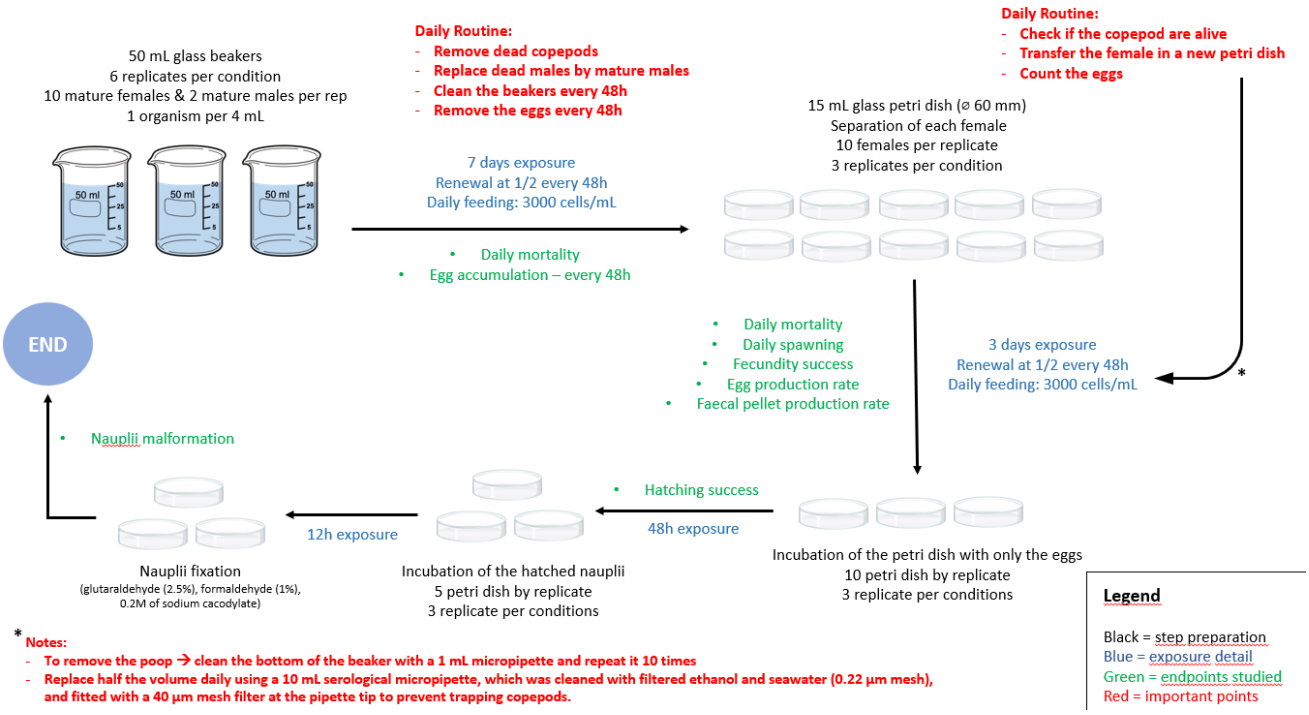

Figure 6.A.1: Schematic explanation of the sub-chronic exposure of the copepod *Acartia tonsa* to fishing net leachate.

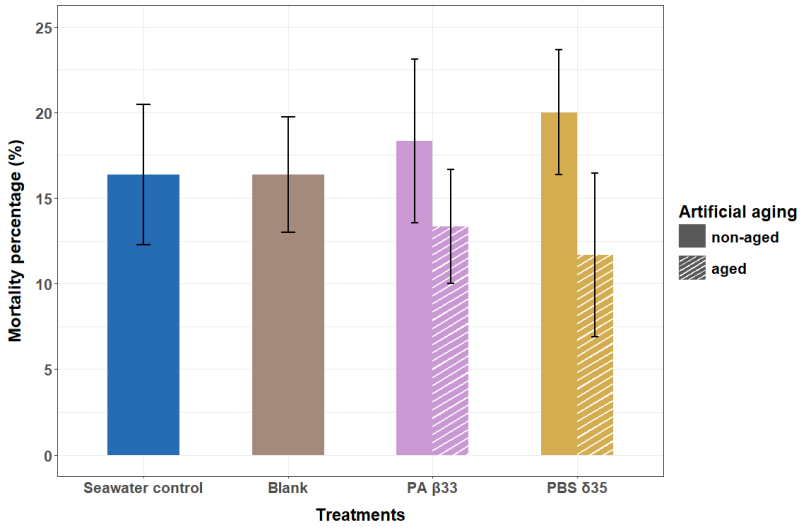

Figure 6.A.2: Effects of artificially aged FN leachates in the mortality of *A. tonsa* females after 7 days of exposure. Top of the colored bar are the average percentage of mortality. Black lines at the top of the bars represent the standard errors. No significant differences were observed between the FN leachates and the blank of seawater control (Welch's test, df = 5, p-value > 0.05). Sample size by concentration = 6.

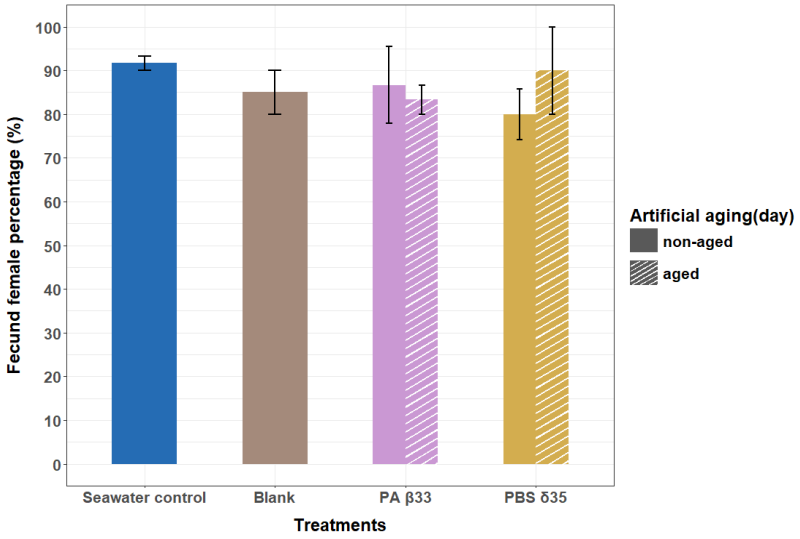

Figure 6.A.3: Effects of artificially aged FN leachates in the percentage of fecund females of *A. tonsa* females after 8 days of exposure. Top of the colored bar are the average percentage of mortality. Black lines at the top of the bars represent the standard errors. No significant differences were observed between the FN leachates and the blank of seawater control (Welch's test, df = 5, p-value > 0.05). Sample size by concentration = 6.

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Highlights, main results and conclusions

Highligths of the chapter:

- The survival rate of copepodites of *A. tonsa* was reduced by up to 57% by fishing net leachates.
- Gillnet leachates significantly affected egg production and hatching in the copepod *A. tonsa.*
- PBS-PBAT FN leachates significantly affected faecal pellet production in the copepod *A. tonsa.*
- The partially biobased and biodegradable gillnet had similar toxicity to the oil-based one in *A. tonsa*.
- Oil-based, as well as partially biobased and biodegradable, FNs pose a slight to moderate environmental risk.

Main results of the chapter:

FNs	BIOASSAYS															
	Zooplankton (*A. tonsa*)															
	Acute exposure Juvenile survival 72h LC_{50} (g/L)		Chronic exposure Survival 7 days (%)		Eggs produced 7 days. (nb. of eggs)		Fecund female (%)		Egg production rate (egg/day/female)		Faecal pellet production rate (fp/day/female)		Hatching success (%)		Nauplii malformation	
	0d	25d	0d	25d	0d	25d			0d	25d	0d	25d	0d	25d	0d	25d
PA6 α35	**6.94**	**2.98**	/	/	/	/	/	/	/	/	/	/	/	/	/	/
PA6 β33	**1.32**	**1.51**	82%	87%	**70**	130	87%	83%	**3.7**	6.2	17.9	18.9	**51%**	**63%**	8%	9%
PA6 γ33	>10	**6.03**	/	/	/	/	/	/	/	/	/	/	/	/	/	/
PBS-PBAT δ35	**1.55**	**1.76**	80%	88%	**71**	162	80%	90%	**3.2**	5.7	**11.8**	15.9	**52%**	**67%**	3%	12%
PE-PP ε4.25	>10	>10	/	/	/	/	/	/	/	/	/	/	/	/	/	/

***Values in red and bold represent significant differences between the endpoint and the control or LC_{50} lower than the highest concentration tested.**

Conclusions:

This chapter highlighted that the degradation products of FNs induced sub-chronic toxic effects on the copepod *A. tonsa*, a key specie to the coastal marine trophic chain. What is particularly concerning it that effects were identified at multiple developmental stages of the species and at environmentally realistic concentrations. Sub-lethal toxicity endpoints were predominantly affected, which underscores the insufficiency of relying only on lethal endpoints to evaluate the overall toxicity of FNs. While sub-lethal effects may initially appear to be of lesser concern, the impact of FN degradation products on the reproductive capabilities of copepods raised significant ecological implications. Indeed, even a minor disruption in copepod population renewal could trigger potential cascading effects throughout the trophic chain. From an environmental perspective concerning the contribution of FNs to MPs in the SE BoB in 2023, the degradation products of gillnets made of PA appeared to be able to cause a slight to moderate hazard for coastal marine ecosystems. Therefore, attention must be paid to the cumulative effects of this FN pollution over the years, as their risk could increase.

GENERAL DISCUSSION

Most of the results presented in the different chapters of this PhD manuscript were already discussed. Therefore, this general discussion will have as objective to integrate the different chapters and to comment on them according to the related existing literature. As a reminder, the general objective of this Ph.D. thesis was to evaluate the production and ecotoxicological impacts of the mixture of microplastics, nanoplastics and plastic chemicals, coming from the degradation of plastic fishing nets (FNs) on four different species (marine bacterium *Allivibrio fischeri*, microalga *Tisochrysis lutea*, copepod *Acartia tonsa*, fish larva *Oryzias latipes*), with a specific focus on the nets predominantly used in the Southeastern Bay of Biscay (SE BoB). Firstly, the contribution of FNs to macroplastic, microplastic, nanoplastic, and chemical pollution in SE BoB will be compared to the other sources of plastic pollution. Secondly, the toxic effects of the different FNs will be discussed. This section then continues with a discussion of the environmental risk of the degradation products of FNs, with a focus on the SE BoB but in the context of a wider picture. The general discussion will conclude with two distinct sub-sections, which will address the available solutions for mitigating the environmental impacts associated with the degradation of FNs, as well as the limitations of this Ph.D. thesis and potential future research perspectives, respectively.

7.1 The contribution of fishing nets to macroplastic, microplastic, nanoplastic and plastic chemical pollution in the SE BoB

7.1.1 The fishing sector as a substantial source of plastic marine litter

The input of new plastic pollution into the world ocean is estimated to be between 1.8 million (Schwarz et al. 2023) and 12.7 millions tons (Jambeck et al. 2015) each year with an average of 7.7±2.5 million tons (Thompson et al. 2024). Considering the contribution of the fishing sector to this pollution, different estimations were published. Schwarz et al. (2023) estimated that the fishing industry could be responsible for 6% of the annual input. However, based on a worldwide estimate of plastic fishing gear lost in 2018 (48,400 tons), the fishing industry was assumed to account for 0.6% of plastic pollution (Kuczenski et al. 2021). This disagreement is most likely due to the fact that Schwarz et al. (2023) included both industrial and small-scale fisheries in their mass flow analysis, while Kuczenski et al. (2021) excluded the small-scale fisheries because they were not equipped with AIS and their efficient fishing activity (in hours) was not available. Concerning the SE BoB, based on the modelization carried out in this Ph.D. thesis (Chapter 1), 6 tons of FNs were estimated to be lost (trawl nets, bottom seines, purse seines, gillnets, longlines) in 2023. Therefore, it could be estimated that the total input of plastic waste in the SE BoB in 2023 was 1,000 tons if the fishing sector contributed for the 0.6% of the emission and 100 tons if it contributed for 6%.

Considering the sampling of fishing related items from eight selected beaches of the SE BoB (Chapter 1), which estimated that 7.6% of the plastic litter was coming from the fishing sector, there is a consistency with the upper estimation of the contribution of the fishing sector to macroplastic pollution based on models (6%). Howerer, taking into account estimations from

other sampling studies (Chapter 1 Table 1.4), the average number of items related to fishing in the different compartments of the BoB (beaches, sea surface, sea floor) was higher than the estimation based on models. Indeed, fishing items were estimated to represent 37±20% of the plastic marine litter with a minimum of 12% (beaches, (Rayon-Viña et al. 2018)) and a maximum of 74% (sea floor, (Lopez-Lopez et al. 2017)). There are three main hypotheses to explain these substantial differences between models and field sampling. First, the estimations based on models and mass flow analysis are limited due to the difficulty to integrate the small-scale fisheries. This is a significant constrained as at least 40% of the fish catches, 90% of the people employed in the fishing sector and 20% of the key nutrients intake (iron, zinc, calcium, selenium, vitamin A and omega-3 fatty acid) of 2.3 billion humans depend on the small-scale fisheries worldwide (FAO et al. 2023; Basurto et al. 2025). Furthermore, in the SE BoB, small-scale fishery represented almost 1/3 (371 vessels) of the total number of vessels and 1/8 (34,673 KW) of the total engine power of the fleet (Chapter 2).

The second hypothesis assumes that the disparity can be attributed to the accumulation of fishing gear in marine litter hotspots, such as the SE BoB or the North Pacific Subtropical Gyre, in comparison to other types of plastic waste (Lebreton et al. 2018; Declerck et al. 2019; Rodríguez-Díaz et al. 2020). Lebreton et al. (2022) showed that floating plastics from fishing activities were nearly twice as likely to reach oceanic gyre waters compared to plastics originating from rivers when considering a 24-day beaching timescale and more than ten times more likely under a 2-day beaching scenario. This difference was attributed to the fact that 81% of plastic litter originating from rivers spent more than 10 days near the coasts before reaching accumulation zones and 26% spent more than 100 days near the coast. On the contrary, 21% of trawl pieces and 15% of fixed gear pieces (gillnets, set longlines, pots and traps) spent one day or less near the coast. Consequently, given 1 kg of fishing-related items lost compared to 1 kg of plastic litter from rivers, 0.58 kg of fishing-related gear versus 0.32 kg of other plastic debris would be expected to reach the accumulation zone (Lebreton et al. 2022). Furthermore, the resistance of fishing-related debris to weathering and fragmentation compared to films or bottles, could further enhance their probability of reaching an accumulation area and staying there for long periods (Ruiz et al. 2020; Lebreton 2022; Lebreton et al. 2022). Moreover, others plastic debris are often too fragmented to be recognized and are not classified in a specific activity sector (Ruiz et al. 2020; Alfaro-Ortega et al. 2025).

The third hypothesis to explain the descripancy between estimations based on models and field sampling is the limits of field sampling methods used. First the classifications to identify the FG related items are often not enough specific which could lead to an overestimation of these items. This Ph.D. thesis evaluated the origin of plastic waste on eight beaches in the SE BoB and estimated, according to the OSPAR classification (OSPAR Commission 2010), that 15.1% of the plastic debris originated from the fishing sector. However, by employing the ID-key specifically designed to identify FG, proportion of the FG in the plastic litter decreased to 9.3% when maritime ropes were included and to 7.6% when they were excluded. Second, the high amount of the ALDFG estimated on the sea surface can also be explained by the sampling method used, that is mainly based on trawling transects (Ruiz et al. 2020; Gérigny et al. 2022; Lebreton et al. 2022). Indeed, larger and more rigid plastics, such as fishing gear are more likely to be caught in trawls than smaller and smoother plastics (Watters et al. 2010).

7.1.2 Degradation kinetics of the fishing nets

It is well established that plastic degradation and fragmentation are governed by a complex interaction of intrinsic plastic properties (polymer type, molecular weight, crystallinity, additive composition and surface geometry) and extrinsic environmental drivers such as UV radiation, thermal fluctuations, oxygen availability, mechanical stress, salinity gradients and biocolonization (Chamas et al. 2020; Masry et al. 2021; Landrigan et al. 2023). Therefore, it can be assumed that degradation processes begin as soon as plastic is produced, because plastics are immediately subjected to chemical, mechanical, and biological processes, even before being considered as waste or before their entrance in the environment (Le Gué et al. 2024). This lifecycle perspective reframes plastic pollution as a continuum, where release of secondary MPs, NPs, and plastic additives occurs not only when they are lost or discarded but also during use phases. This phenomenon is particularly applicable to FNs, which are used in environments where these degradation factors are frequently combined, leading to their progressive degradation. (Brakstad et al. 2022; Napper et al. 2022; Le Gué 2024).

The degradation kinetics of plastic is difficult to predict because of the multitude of factors involved in plastic aging. For instance, degradation of PP revealed a linear mass loss (0.075–0.083% daily) under UV irradiation but a sigmoidal dissolved organic carbon accumulation kinetics, indicating decoupled oxidation and desorption or fragmentation mechanisms (Tuttle et al. 2024). Furthermore, the energy needed to activate the degradation varies depending on the polymers and aging time. For instance, the energy needed to activate HDPE (206–209 $kJ \cdot mol^{-1}$ for virgin; 195–198 $kJ \cdot mol^{-1}$ for aged) and PP degradation decreases with plastic aging (211–214 $kJ \cdot mol^{-1}$ virgin; 183–186 $kJ \cdot mol^{-1}$ aged), while it increases for PS (144–163 $kJ \cdot mol^{-1}$ virgin; 159–167 $kJ \cdot mol^{-1}$ aged). Plastic additives and especially plasticizers and antioxidants introduce additional complexity, as they can reduce activation energy and create heterogeneous oxidation fronts, leading to unpredictable leaching and degradation patterns (Hahladakis et al. 2018; Masry et al. 2021; Singh et al. 2024).

These polymer-dependent and multi-factorial degradation kinetics agreed with the findings of the UV-exposure of FNs at ambient temperatures (23°C) (during 25 days equivalent to 4 years in the environmental), which exhibited non-linear degradation patterns (Chapter 4). Considering that oil-based and non-biodegradable plastic FNs are slow degradation plastics (Min et al. 2020) and have half-lives between 12 and > 2,500 years (Chamas et al. 2020; Lebreton et al. 2022), it was not suprising that the FNs studied under these degradation conditions showed only slight surface changes. Nevertheless, for the FN made of 50% biobased and biodegradable PBS-PBAT, the limited degradation observed at 23°C highlighted uncertainties regarding its environmental fate. After the weathering of a monofilament made of PBS-PBAT in daily renewed seawater at a similar temperature (25 °C), Le Gué et al. (2025) observed heterogeneous deterioration of the filament surface, with the presence of holes of size from a few microns to several tens of microns after three years of exposure. According to mechanical testing, this degradation resulted in a significant reduction in both the load and strain at the point of failure. Under the same weathering conditions but at 15 °C, fewer and smaller holes (measuring several microns) were identified on the monofilament. The loss of mechanical properties was less pronounced than when the filament was weathered at 25°C. At 4 °C no signs of degradation were observed after three years. Kim et al. (2016), reported

that the degradation rate of PBS-PBAT gillnets was higher during summer, when surface water temperatures of the Yellow Sea reached 30 °C, than during winter, when temperatures dropped to 0.4 °C. In that work, the initial appearance of holes in the gillnet filaments was observed after two years of submersion. Both studies attributed the enhanced degradation rate of FN filaments at elevated temperatures primarily to increased microbial activity (Kim et al. 2016; Le Gué et al. 2025). In the case of this Ph.D. thesis, the artificial aging of the PBS-PBAT FN biobased at 50% to extreme aging conditions characterized by intense UV irradiation and temperatures above 60°C, resulted in substantial filament degradation and the generation of a significant concentration of small MPs (median size: 16 µm). In environment with limited microorganism concentration, the higher degradation rate observed at elevated temperatures compared to temperatures below 25°C can be explained by the fact that high temperatures activated the photooxidation of the plastic surface by accelerating chain scission rates (Chamas et al. 2020; Masry et al. 2021). Multiple studies have shown that the degradation of PBS-PBAT plastics is particularly sensitive to temperature, with notable increases observed between 25°C and 40°C (Min et al. 2020; Le Gué 2024; Le Gué et al. 2025). For plastics made of PE, PP, and PA, the initial physico-chemical changes appeared above 50 °C (Gonçalves et al. 2007; Therias et al. 2021), although the earliest effects for PA and PP were detectable at 40 °C (Marek & Lerch 1965; Audouin et al. 1998).
Therefore, at moderate to high marine water temperatures, FNs made of partially biobased and biodegradable PBS-PBAT are expected to degrade more rapidly than conventional oil-based PA and PE-PP FNs, which could help mitigate the problem of ghost fishing. However, at lower temperatures characteristic of the SE BoB (Michel et al. 2009), existing research does not provide sufficient evidence to conclude that PBS-PBAT nets would degrade more quickly than those made of oil-based, and non-biodegradable polymers.

The investigation of the degradation of the cod end trawl net made of PE-PP and the outer panels of trammel nets made of PA and 50% biobased PBS-PBAT (Chapter 4) showed that the leaching of plastic chemicals varied according to the FN polymer type but tend to increase with prolonged aging. Concentrations of five out of seven organic compounds quantified in the leachates of the PBS-PBAT FN increased from 0 to 12 days of artificial aging and subsequently declined between 12 and 25 days. A primary hypothesis for the decrease in these compounds after 12 days of aging is the substantial reduction in crystallinity observed in PBS-PBAT filaments between 6 and 12 days of degradation. This decline of crystallinity was not observed for the two other FNs. Enhanced crystallinity in plastics, due to chemi-crystallization, is known to alter degradation kinetics, as more crystalline regions restrict water diffusion and limit access to hydrolytically labile bonds, thus reducing the potential release of plastic chemicals (Bejgarn et al. 2015; Masry et al. 2021; Nakatani et al. 2024; Tsai & Lo 2025). A second hypothesis to account for the reduced concentrations of these plastic chemicals in the leachates of the PBS-PBAT FN after 12 days of artificial aging is that these substances may either have volatilized (Wu et al. 2022, 2023; Nzimande et al. 2024) or have undergone photodegradation (Wang et al. 2019; Tang et al. 2024) during the UV irradiation at dry.

The findings on degradation kinetics presented in this doctoral research, when integrated with existing literature, lead to three novel interpretations concerning the degradation of FNs. First, as for many other plastics, the aging of FNs should be studied independently for each fishing

métier due to non-linear degradation kinetics that vary with the polymers. Second, FNs should be stored in conditions not exceeding 40°C and with no UV irradiation to minimize their degradation and potential release of MPs and plastic chemicals during their subsequent operation (Hingant et al. 2023). Third, for the FG lost and especially for those beached, environmental conditions with high degradation potential (strong UV irradiation, high temperatures and abrasion) would result in a high degradation rate of the ALDFG, thus causing a substantial production of MPs, NPs and plastic chemicals.

7.1.3 Fishing net contribution to the microplastic and chemical pollution of the oceans

Whatever the methods used for artificially aging of FNs and to quantify the MPs produced, it appeared that the cod end trawl net made of PE-PP did not produce a significant amount of MPs (Chapter 4). However, this observation is probably not realistic because bottom trawling is an active fishing activity in which the cod end can touch the sea floor and suffer abrasion. This assumption was corroborated by the stereomicroscope and SEM images of the cod-end used for three years in sandy bottom (Chapter 3). Indeed, these images showed substantial degradation of the filament composing the braided rope of the net, with numerous cracks and long and thin filaments that could correspond to fibers (Liu et al. 2019). However, this does not confirm that trawls could produce fibers since there is a non-negligible probability that the observed fibers, which were still attached to the main filament, degrade into fragments (Napper et al. 2022; Song et al. 2025). Syversen et al. (2022) also confirmed these assumptions showing that the weighted dragline (FAO 2024a) of bottom Danish seine, made of PE or PE-PP, lost between 9 and 12.8% of their mass of plastic during each fishing operation. This is probably because the dragline drags along the sea floor to drive benthic fish in the cod-end and stretch the wings of the seine (Figure 7.1). The second explanation is the abrasion of the dragline in the winch and pulleys of fishing vessels (Napper et al. 2022; Syversen et al. 2022). In total, the authors estimated that wear of draglines from the bottom seine fishery in Norway released between 77 and 97 tons of plastic per year. Therefore, albeit the cod-ends of the bottom trawl do not drag the sea floor as the dragline, their regular contacts with the sea floor most likely produces plastic microfragments or microfibers.



Figure 7.1: Draglines of Danish bottom seines. (A) new dragline. (B) and (C) used draglines from two different fishing vessels. Images taken from Syversen et al. 2022.

Set gillnets, mostly made of polyamide, are passive fishing gear that are set above the sea floor. Except the bottom rope of set gillnets which can be mechanically damaged by friction with the sea floor, the net panels have little contact with the sea floor (Kim et al. 2016; FAO 2024a). Therefore, the main drivers of degradation during the operation are elongation during the haul, the variable temperatures and pressures between operations and storage conditions, in particular UV exposure during storage (Le Gué 2024). Thus, it may be assumed that the artificial aging method employed in this Ph.D. was a relevant approach to simulate the degradation of the gillnets during their operational period. In this context, the results of this Ph.D. thesis showed that gillnets contribute to the smallest size fraction of MP pollution of the oceans, with an estimated production of $4.05x10^4 \pm 8.76x10^4$ (SD) MP/g of PA-made gillnets. For gillnets made of PBS-PBAT this value was lower, with $2.72x10^4 \pm 2.46x10^4$ (SD) MP/g of FN. It is crucial to acknowledge that due to inherent uncertainties within the various steps of the methodology used, the 95% confidence interval (95% CI) of number of MPs produced per gram of gillnet was notably wide, extending from 0 to $3.2x10^5$ MP/g of FN non-aged in the case of the gillnet PA β33. The uncertainities of the estimation of the number of MPs produced per gram of gillnet made of PBS-PBAT and non-aged were smaller compared to the gillnets made of PA, with a 95% CI extending from $1.5x10^3$ to $6.8x10^4$. In the case of gillnets aged for four years, the production of MPs per gram of gillnet was significantly higher in two of the three PA gillnets studied. The estimated mean number of MPs produced per gram of aged gillnets ranged from 1.1×10^5 to 8.3×10^5 MPs/FN. Based on comparisons with other studies, averages of MPs produced per gram of gillnet found in this work were in the upper range of the quantification of secondary MNPs produced by other types of plastic items (Table 7.1). The main explanation for the higher values of MPs produced from gillnets is that the size quantification limit (QL) was 5 µm in the case of the present work, while most of the size-QL were not mentioned or were greater than 20 µm in other studies. Studying the degradation of PA braided nets, Montarsolo et al. (2018) measured a production ranging from 167±121 (SD) to 299±232 (SD) MP/g of FN depending on the braided net, which was one to three orders of magnitude lower than the mean number of MPs produced by the gillnets studied in this Ph.D thesis. However, their method of quantification, based on scanning electron microscopy (SEM) at x250 was certainly not able to quantify small MPs due to the low magnification and the heterogeneous surface of the PVDF membranes used (Caldwell et al. 2022).

Taking into consideration the quantity of MPs produced by gram of FN and the volume of water in the SE BoB, it was possible to estimate that the gillnets used in 2023 in the area contributed to the low fraction size of MP pollution (median size 23.6 µm), with an average of 2.2 MP/m^3 (95% CI: 0-14.8 MP/m^3). Compared to a recent field quantification performed in the same area (2 km offshore of Arcachon Bay, France) (Lefebvre et al. 2023), the MPs produced by the operation of gillnets in one year represented between 0.26±0.57 (SD) % and 0.39±0.85 (SD) % of the MP pollution of the water column. Taking into account only the MPs from 10 to 500 µm in the water column (64.4 to 70.5 %), MPs from gillnets would represent between 0.37±0.8 % and 0.63±1.38 % of the MP pollution of the water column. Focusing specifically on the MPs with a fragment shape quantified by these authors, the MPs produced

Table 7.1: Estimates of the amount of secondary MNPs generated by various plastic items.
"Size QL" means the MP size quantification limit. References indicated by an asterisk (*) denote that the values of MNPs produced per gram of plastic were inferred from the data available in the publications due to the use of differing units.

Plastic item	Polymer	Degradation conditions	Quantification methodology	Size QL (µm)	MNPs produced by g of plastic (MNP/g)	References
Sweat-shirt	Polyester-cotton	Washing machine (1h15, 1400 rpm, 30-40°C)	Stereomicroscope & SEM (x2500)	na	23	(Napper & Thompson 2016)
	Polyester				82	
	Acrylic				121	
Braided net	PA	Linitest equipment (15-20°C)	SEM (x250)	na	167±121 (SD)	(Montarsolo et al. 2018)
					299±232 (SD)	
Pellets with plastic additives	PE	2 months mechanical abrasion (MA) (37 rpm & sediments)	Nile red staining & fluorescence microscopy (x50)	20 µm	339±103 (SE)	*(Song et al. 2017)
	PP				11±1 (SE)	
	PS				4220±33 (SE)	
	PE	12 months UV irradiation + MA			826±343 (SE)	
	PP				$3.51*10^5±6.1*10^4$ (SE)	
	PS				$1.5*10^7±2.6^6*10^4$ (SE)	
Garbage bags	LDPE	Inclined (40°) mixer with natural marine pebble (ø 4.5-6 cm) without blade (24 h)	na	500 µm	125	*(Efimova et al. 2018)
Single-use cup	PP				5	
Single-use plate	PS solid				5750	
Foam plate	PS foam				400	
New ropes	PP	Custom-designed hauler system	Semi-automatized FTIR	25 µm	129	*(Song et al. 2025)
2 years aged rope					6510	
12 textiles	Polyester	Washing machine (3h, 40 rpm, 40°C)	Nanoparticle tracking analysis	~0.1 µm (max 0.6 µm)	4.6×10^{10} to $8.9*10^{11}$	(Yang et al. 2024)
Pressed pellet	Poly(ε-caprolactone)	Enzymatic hydrolysis in aqueous buffer (40°C, 200 rpm, 6 days)	Stereomicroscope & SEM (x200)	0.09 µm	$1*10^7$	(Wei et al. 2022)
Pellets	PBS	21 days UV irradiation (40°C) + agitation 150 rpm every 4h	Nile red staining & fluorescence microscopy (x100)	1 µm	2187±20	(Tong et al. 2022)
	PLA				665.8±60	
	PBAT				197.5±14.4	
	PE				92.5±0	
	PS				247.5±20	
	PVC				375±0.1	

by gillnets during one year of operation would represent between 6.52±14 (SD) % and 10.88±24 (SD) % of the fragment-shaped MPs present in the offshore water column of the Arcachon Bay. Furthermore, based on the polymer composition of the MP pollution in this area (PET, PA, PA-cellulose, PS, PE, PP) (Lefebvre et al. 2023), PA gillnets would contribute between 4.07±22 % and 10±24 % to MP pollution made of PA. A contribution of gillnets to MP pollution of the offshore water column of the Arcachon Bay in the 10–500 µm size range, estimated between 0.37%±0.8% and 0.63%±1.38%, could be interpreted as relatively low. However, a substantial fraction of the MPs quantified by Lefebvre et al. (2023) were most probably part of the historical MP pollution, which lead to an underestimation of the contribution of the gillnets to the new emissions of secondary MPs annually. Second, the value of 2.2±4.76 MPs/m^3 represents only the gillnets used by the fleet equipped with AIS, and thus

does not take into account the substantial quantity of FNs used by small-scale fishing fleet. It is important to note that these estimates consider the SE BoB as a closed system without taking into consideration the potential input of MPs from outside and those leaving the area, even if the SE BoB is a plastic debris accumulation zone from which less than 20% of the particles leave (Ruiz et al. 2022).

In the future, oil-based gillnets will most certainly be replaced by biobased and biodegradable ones (Kim et al. 2016, 2023a; Grimaldo et al. 2018; Deroiné et al. 2019; Cerbule et al. 2022, 2023; Park et al. 2023; Yu et al. 2023; Le Gué 2024; Savina et al. 2024). For this, these new gillnets would require a similar fishing yield efficiency and similar prices. At the same time, in order to reduce their ghost-fishing, they will have to be able to degrade faster than oil-based non-biodegradable FNs when lost. Biodegradable FNs will also have to be efficiently composted at the industrial level. Further, they will need to comply with the above requirements without releasing toxic MPs, NPs and plastic chemicals (Paul-Pont et al. 2023; Scientists' Coalition for an Effective Plastics Treaty 2023b). Currently used biodegradable FNs and those under development seem to be able to achieve suitable fishing efficiency, and their price will probably fall with supply and demand (Grimaldo et al. 2018, 2020; Cerbule et al. 2022; Le Gué 2024). Regarding their degradation when lost, although uncertainties persist about their longevity in marine environments, it is anticipated that their ghost fishing will be diminished compared to that of oil-based nets because of their faster degradation. However, as to the production of MPs, NPs and plastic chemicals, the results of this Ph.D. work indicate that partially biobased and biodegradable trammel nets made of PBS-PBAT would contribute up to 1.48 MPs/m^3 (95% CI: 0-3.5 MP/m^3) annually to MP pollution in the SE BoB if they replaced oil-based FNs. NPs were also identified in leachates of artificially aged FNs made of PBS-PBAT.

Considering the production of MPs and NPs by the biobased and biodegradable FNs, questions remained about their environmental persistence. Lara-Topete et al. (2024) observed a significant variability in the degradation rates of biodegradable polymers among studies investigating this topic. Under industrial thermophilic composting conditions (55–66 °C), degradation of items made of polylactic acid (PLA) ranged from 30% (60 days of composting) to 100% (28 days), while the degradation of items made of polyhydroxyalkanoates (PHAs) ranged from 30% (60 days) to 91% (78 days). For the plastic items made of 100% polybutylene succinate (PBS) and PBS blends (75%/25% with switchgrass, canola meal, gluten meal, or soy meal), their degradation under industrial thermophilic composting conditions (55–66 °C) reached 90% within 160 days for 100% PBS, and within 70 to 100 days for the various PBS blends. Anaerobic digestion, which could give first insights of biodegradation of biodegradable plastic in deep-sea sediments, revealed reduced efficiency compared to industrial thermophilic composting conditions. Indeed, under industrial mesophilic (25–40°C) anaerobic digestion conditions the degradation of items: made of PHAs ranged from 55% (55 days) to 90% (30 to 175 days depending of the plastic item), made of PLA ranged from 12% (60 days) to 25% (50 days), made of PBS ranged from 2% to 36%, and made of polybutylene adipate terephthalate (PBAT) reached 2% after 126 days (Lara-Topete et al. 2024). In seawater the degradation percentages of biodegradable plastics were heterogeneous between polymers. For a plastic made of PHA the degradation reached 8% after 365 days. The degradation ranged

from 30% (90 days) to 99% (49 days) for a plastic made of polyhydroxybutyrate (PHB) and 69% after 236 days for a plastic made of Mater-Bi (Lara-Topete et al. 2024). The authors warned that incomplete biodegradation may lead to the generation of substantial quantities of MPs, which could further degrade into NPs and remain persistent in the environment for extended periods before full mineralization. Wei et al. (2022) showed that polymer hydrolysis produced over one million MPs from 0.1 g of LDPE and PBAT films. Additionally, after 10 weeks in seawater with sediments and microorganisms, secondary MPs derived from these films were found (Wei et al. 2021).

In the Yellow River (China), PBS MPs were in the top three of the MPs most found in surface waters and in samples of sediments, both during the dry or the wet seasons (Qian et al. 2023). PBAT MPs was also found in all the compartments studied of the river but at lower quantities. In sediments from the inner part of the Venice Lagoon (Italy), Vianello et al. (2013) quantified the polyvinyl alcohol polymer at a concentration of 61±61 particles/kg dry sediment. In comparison, at the same site, 1,505±404 particles of PE/kg of sediment were quantified. In the Arctic sea ice, PLA MPs were also identified at a percentage lower than 1 % (Peeken et al. 2018). Consequently, although uncertainties persist regarding the fate of MPs and NPs originating from biobased and biodegradable plastics (Wang et al. 2021; Payanthoth et al. 2024), it appeared that under environmental conditions characterized by low temperatures, limited nutrient availability, reduced organic matter presence, and low microbial activity, MPs and NPs derived from biobased and biodegradable plastics can be persistente (Lara-Topete et al. 2024).

In addition to the ability of producing MPs and NPs FNs were also able to release more than 70 chemical compounds during operation and aging according to the results of this Ph.D. work and several other studies (Turner 2017; Chaihad et al. 2020; Brakstad et al. 2022; Amariei et al. 2023; Vilke et al. 2024). These chemicals included intentionally added plastic additives, NIAS, and degradation by-products (Brakstad et al. 2022; Vilke et al. 2024). Trace metals were often identified and quantified in leachates and extracts of FNs, with concentrations of lead chromate pigments (Pigment Yellow 34 and Pigment Orange 21) reaching > 1,000 mg/L in PE-PP and PP ropes and in PE nets (Amariei et al. 2023). This high amount of lead in FNs was also found in the gillnet leachates studied in this Ph.D. work and particularly in the leachates of the PBS-PBAT FNs. Zinc, copper, chromium, bromine, iron, and antimony were also quantified in the mending pieces and filaments of ALDFG that beached on coastlines, exhibiting concentrations that posed environmental concerns (Turner 2017; Vilke et al. 2024).
Organic additives intentionally added to FNs were also identified in the leachates of new FNs, EOL-FNs and ALDFGs, highlighting their weak physicochemical binding to the polymeric matrix. In both this Ph.D. thesis and Brakstad et al. (2022), biobased and biodegradable FNs (non-aged and aged) leached in general a higher number of organic plastic additives at similar or higher concentrations than oil-based and non-biodegradable FNs. Based on the different studies focused on FNs, the plastic additives leached were mainly plasticizers, dyes, flame retardants and antioxidants (Chaihad et al. 2020; Jang et al. 2021; Brakstad et al. 2022; Vilke et al. 2024). NIAS and degradation by-products, originating from manufacturing residues, contamination, or chemical reactions occurring during the manufacture, use, or degradation of plastics (Hahladakis et al. 2018; Wagner et al. 2024), were also identified in the leachates

of FNs. Jang et al. (2021) showed that aging of PA FNs in seawater for five months caused their structural degradation, leading to the production of a novel organic compound, the 5-amino-pentanol. Other NIAS, such as antimicrobial agents used to clean factory production lines, flavoring agents, and production intermediates, were also found in fishing gear (FG) leachates (Vilke et al. 2024). The identified degradation by-products from FNs (PA and PBS-PBAT) were mainly succinic acid-2-dimethylallyl undecyl ester, 1,8-diazacyclotetradecane-2,9-dione, 7butyl-3,4,5,6(2H)-tetrahydrozepine, 2-methyl-6-tridecyl-6-piperideine and 1-(3,4,5,6-tetrahydro-2H-azepin-7-yl)-2-azepanone (Chaihad et al. 2020). However, the random and uncontrolled formation of these degradation products makes them very difficult chemicals to identify and quantify.

Recently, from the 4,184 plastic chemical compounds identified as of concern for the environment, the persistence and bioaccumulation criteria were studied only for 5.66 % (237) of them. Of these 237 compounds studied, 94.5 % were classified as persitent and bioaccumulative in the environment (Wagner et al. 2024). From these persistent chemicals, phthalate plasticizers demonstrated moderate persistence in aquatic environments with half-lives ranging from 26 to 79 days (Paluselli et al. 2019; Carstens et al. 2020). However, their continuous release from plastic materials ensure persistent environmental contamination by phthalates (Oehlmann et al. 2009; Souaf et al. 2023). Furthermore, other organic compounds such as brominated flame retardants, e.g., polybrominated diphenyl ethers (PBDEs) and hexabromobenzene (HBB), exhibited very long persistence with half-lives ranging from minutes to > 224 days under sunlight and up to 617 days in soils (Hermabessiere et al. 2017; Xiong et al. 2019; Petrlik et al. 2021). The persistence of NIAS and degradation by-products are very versatile and are very difficult to predict (Wagner et al. 2024).

This Ph.D. thesis work, by extending and complementing already existing knowledge, has demonstrated that FNs used in the SE BoB in 2023 contributed to the pollution of the oceans by secondary MPs, NPs and plastic chemicals.

7.2 Comparative toxic effects of different fishing nets

The leachates and DMSO extracts of FNs containing a mixture of degradation products resulted in significant toxicity to the five aquatic species selected. After examining the integrated toxicity index derived from the acute toxicity assessments of leachates of non-aged FNs (Figure 7.2), it was determined that the biobased and biodegradable FN composed of PBS-PBAT and the gillnet PA α35 caused the highest toxic effects. On the basis of the PCAs performed in the different chapters, the higher toxicity of these two FNs was predominantly due to higher concentrations of plastic chemicals in the leachates. In particular, according to the PCAs perfomed in Chapters 4, 5, 6, the higher concentrations of succinic acid 2-methylallyl undecyl ester, lead, copper, and zinc in PBS-PBAT δ35 FN leachates had a substantial role in explaining the higher toxicity. For PA α35, the only plastic chemical quantified that could explain its higher toxicity was the neurotoxic chemical N-butyl benzenesulfonamide (Strong et al. 1991). Considering the leachates of aged FNs, the PA β33 and the PA γ33 gillnets were the most toxic (Figure 7.2). Overall, the toxicity of all FN leachates increased significantly with artificial aging time, but not to the same extent according to the different FNs. Indeed, while the leachate from the non-aged PA γ33 gillnet induced lower toxicity compared to the others

leachates of non-aged FNs, it was the FN that experienced the highest increase in toxicity with aging (+8.71 points = IBR index of aged FN – IBR index of non-aged FN). In contrast, although the toxicity of the FNs PA α35, PBS-PBAT δ35 and PE-PP ε4.25 also increased with aging time, this was less pronounced (+2.19, +2.53 and +0.48 points, respectively). In particular, the leachates from both non-aged and aged PE-PP ε4.25 FNs exhibited one of the lowest IBR indexes, and aging had little effect on their toxicity. In a similar manner, Quade et al. (2023) documented a non-uniform increase in toxicity to *Paracentrotus lividus* embryos associated with the UV aging on various types of plastic bags. The authors showed that leachates of PE bags did not cause toxicity regardless of the UV aging time and intensity, while biobased and biodegrable plastic bags caused different patterns of toxicity. The leachates derived from the industrial compostable bag made of polylactic acid-polybutylene adipate-co-terephthalate exhibited greater toxicity (4.41 toxic units (TU)), under conditions of intensive UV aging, compared to those derived from new materials (2.65 TU). Conversely, leachates from another industrial compostable bag made of PLA caused a higher toxicity after a 24-hour leaching process (3.00 TU) as opposed to bags subject to subsequent exposure to UV aging for a duration of 21 days (1.98 TU) (Quade et al. 2023).

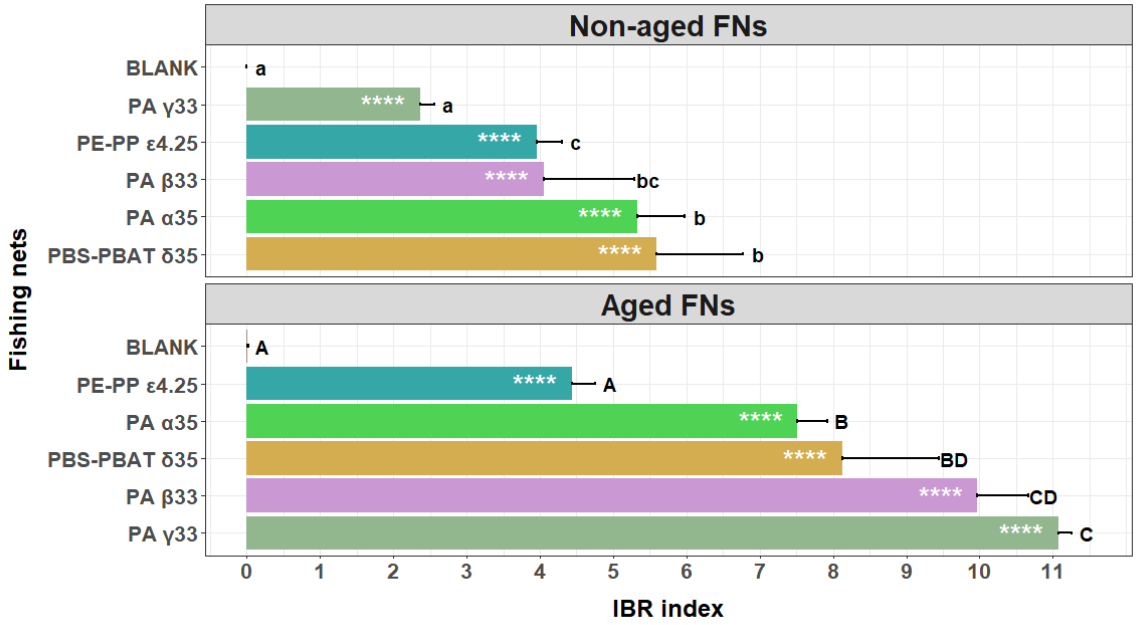

Figure 7.2: Integrated Biological Response (IBR) index (Devin et al., 2014) quantifying acute toxicity of leachates of non-aged FNs (24 h of agitation) and aged FNs (25 days UV-irradiation + 24 h agitation) across five endpoints (bacteria bioluminescence, copepodite survival, copepod survival, fish heart rate, fish behavior stress index).
Values represent mean±SD calculated from 120 geometric permutations (120 = factorial of 5). Tested species: *Aliivibrio fischeri*, *Acartia tonsa*, and *Oryzias latipes*. Statistics: independent Kruskal-Wallis's tests between artificial aging time (non-aged FNs: n = 144, chi^2 = 117, df = 5, p-value < 0.00001; aged FNs: n = 144, chi^2 = 133, df = 5, p-value < 0.00001). The post-hoc Dunn's test was executed with Holm's correction. Different letters mean significant differences between FN leachates. Independent MWU tests were also performed to study significant differences between the aging times of the same nets. All comparisons resulted in significant differences (n = 48, W = 56:96, p-values < 0.00001). Significant differences between the IBR indexes of non-aged and aged fishing nets (FNs) are indicated by white asterisks ("****") inside the bars.

The concentrations of chemicals quantified in the leachates of aged PA β33 and PA γ33 FNs were not enough to explain their increased toxicity compared to that of the other FNs. Therefore, the increased toxicity of aged PA β33 and PA γ33 gillnets may be attributed to the concomitant increase in MP concentration and chemical compounds present in the leachate. Zhang et al. (2022a) demonstrated that aged PA MPs exhibited an increased negative surface

charge (zeta potential) compared to non-aged ones, which enhanced their bioavailability and cellular interactions with aquatic organisms. This surface charge modification, combined with the concurrent production of MPs, would create dual exposure scenario in which both particulates and chemical compounds contributed to the enhanced toxicity. In the case of the fish *Danio rerio*, its exposure to aged MPs of PA resulted in higher growth inhibition, intestinal damage, and lipid malabsorption compared to new ones (Zhang et al. 2022a). Additionally, PA MPs were also reported to enhance the bioavailability and toxicity of the flame retardant Tris (1,3-dichloro-2-propyl) phosphate in *D. rerio* (Zhang et al. 2022b). Therefore, the combination of increased MP production and chemical leaching from aged PA FNs could likely intensified their toxic effects. However, even if this estimation seems plausible, the presence of unidentified plastic chemicals in the leachates could be an additional reason.

The diameter of the FN and therefore the surface of exchange did not seem to be a major driver of toxicity between FNs. Indeed, toxicity differed between PA γ33 and PA γ60 or between PBS-PBAT δ35 and PBS-PBAT δ60, but differences in toxic effects did not follow a linear pattern with the increase in surface exchange. To make an effective assessment of the diameter effect and especially to try to verify that high surface exchange cause a higher rate of degradation, a higher release of plastic chemicals and a higher environmental pollutant sorption capacities, comparison of FNs with the same chemical composition would be required (Trotter et al. 2019; Zimmermann et al. 2020; Liang et al. 2023). In this Ph.D. thesis, even if the inner panels and outer panels of the PA gamma FNs and PBS-PBAT FNs came from the same manufacturer, their chemical composition was different. For the PBS-PBAT FNs, the inner and outer panels did not have the same composition since the inner one (thinner with a diameter of 0.35 mm) was biobased only at 35% and the outer one (thicker with a diameter of 0.60 mm) at 50%. For the PA γ33 and PA γ60 net panels, the former was green whereas the later was translucent without color. Therefore, PA γ33 FN had pigments not present in PA γ60 net. It is interesting to note that both after artificial aging or after weathering in the ports of Pasaia or Arcachon (Lefebvre et al. 2025), the colored gillnets lost some intensity and shine in their color, suggesting that the pigments may have leached or photo-degraded. This observation is of concern as some dyes used in the plastic industry are known to be hazardous to aquatic species. Indeed, colorants can be azo dyes or anthraquinone that can cause acute lethal and sub-lethal toxicity to several species such as *Daphnia magna* (Bae Jin- Seok et al. 2006; Wang et al. 2009), *A. fischeri*, *Raphidocelis subcapitata* (Novotný et al. 2006) or *Silurana tropicalis* (Soriano et al. 2014). Moreover, it was recently highlighted that 39.3 % of azo dyes are hazardous for the environment and are included in the list of plastic chemicals of concern that must be regulated (Wagner et al. 2024).

When the sub-chronic toxicity endpoints (egg production rate, faecal production rate and egg hatching success) of the copepod *A. tonsa* were included in the integrated biological response (IBR) index, a different toxicity pattern was evident. The leachates from non-aged FNs had an IBR index approximately 2 times higher than the leachates from aged FNs (Figure 7.3). Several hypotheses can explain this inverse relationship. First, based on the PCA of the chapter 6, it was possible to identify a negative relation between copper and zinc concentrations in the leachates and the effects on the quantity of eggs produced. This observation supported the hypothesis that the copper and zinc present in higher concentrations in the leachates of aged

FNs could have stimulated egg production in *A. tonsa* through hormetic dose-response mechanisms (Fitzer et al. 2013). In the copepod *N. conifer*, exposure to copper showed clear hormetic responses with egg production increasing from an average of 5.67 eggs/female under control conditions to 11.2 eggs at 0.8 µg Cu/L before declining at higher concentrations (Fitzer et al. 2013). The copper and zinc concentrations measured in leachates at the different aging times used for the subchronic exposure fall within the range where this hormetic response could happen. The stimulation of the egg production by these two metals could also have limited the inhibitory effect of other chemicals present in leachates, resulting in no significant effects on this endpoint compared to the controls. This phenomenon led to the second hypothesis, which is that potential antagonistic interactions among plastic chemicals could occur and therefore change the patterns of toxicity. Zeng et al. (2023) demonstrated that complex contaminant mixtures of cosmetic and plastic chemicals exhibited predominantly antagonistic toxicity interactions under sub-acute exposure conditions of the bacterium *V. qinghaiensis*. These results suggest that FNs undergoing aging processes could release various degradation products that could interact in an antagonistic manner at sub-lethal concentrations, reducing the toxicity of aged leachates compared to non-aged ones through competitive inhibition mechanisms or changes of bioavailability. Conversely, the leachates of non-aged FNs could have simpler chemical profiles and lack the complex mixture dynamics necessary for antagonistic interactions, allowing individual toxic compounds to exert unmitigated toxicity.

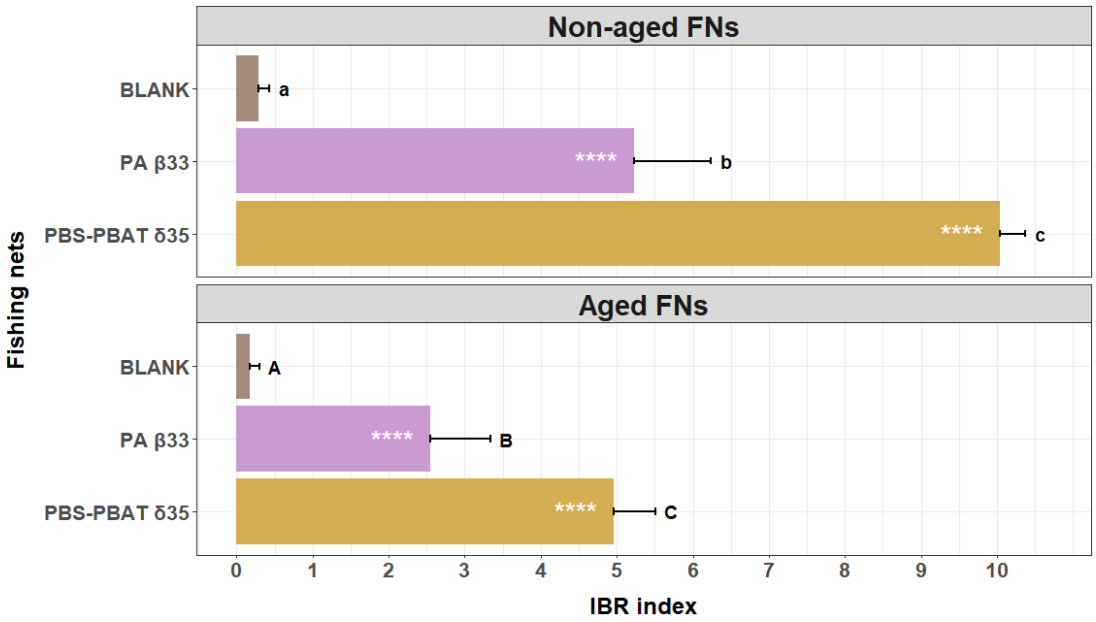

Figure 7.3: Integrated Biological Response index (Devin et al., 2014) quantifying acute and chronic toxicity of leachates of non-aged FNs (24 h of agitation) and aged FNs (25 days UV-irradiation + 24 h agitation) across eight endpoints (bacteria bioluminescence, copepodite survival, copepod survival, copepod egg rate production, copepod faecal pellet production, copepod egg hatching success, fish heart rate, fish behavior stress index).
Values represent mean±SD calculated from 40,320 geometric permutations (40,320 = factorial of 8). Tested species: *Aliivibrio fischeri*, *Acartia tonsa*, and *Oryzias latipes*. Statistics: independent Kruskal-Wallis's tests between artificial aging times (Non-aged FNs: n = 15,120, chi2 = 13,440, df = 2, p-value < 0.00001; Aged FNs: n = 15,120, chi2 = 13,376, df = 2, p-value < 0.00001). The post-hoc Dunn's test was executed with Holm's correction. Different letters mean significant differences between FN leachates. Independent MWU tests were also performed to study significant differences between the aging times of the same nets. All comparisons resulted in significant differences (n = 10,080, W = ~2x106, p-values < 0.00001). Significant differences between the IBR indexes of non-aged and aged fishing nets (FNs) are indicated by white asterisks ("****") inside the bars.

Except for Vilke et al. (2024) and Chen et al. (2023), who provided initial insights on ALDFG and rope filament toxicity discussed in different chapters, to the best of our knowledge no other studies are available for comparison with the results of this Ph.D. thesis. Nonetheless, one study showed that leachates from beached ropes and aquaculture materials from the Adriatic Sea had significant effects on *A. tonsa* mortality (1 g/L) (Gambardella et al. 2024). For the three other species of holozooplankton or merozooplankton (*P. lividus*, *Brachionus plicatilis*, *Amphibalanus amphitrite*) exposed to these leachates, the authors reported developmental abnormalities or behavioral dirsuption (Gambardella et al. 2024). Interestingly, similar plastics from beaches of the BoB caused lower mortality and behavioral disruption, but higher developmental anomalies of *P. lividus* larvae (Gambardella et al. 2024). Furthermore, leachates from beached plastic debris from aquaculture in the BoB significantly disrupted the cardiac activity of *O. latipes*, whereas no effect was found with leachates of the ALDFG from aquaculture in the Adriatic Sea. These differences in the toxicity of ALDFG from two different regions could be explained by the chemical composition of leachates, with three times more chemicals in the leachates of the ALDFG sampled from the Adriatic sea coast than in the leachates of ALDFG from the BoB coast. In both cases, 100 % of the chemicals found in the leachates were classified in the Red List of the PlastChem report (Wagner et al. 2024), which means that they are hazardous and must be regulated. Gambardella et al. (2024) mentioned that the potential presence of different concentrations of unfiltered NPs (< 800 nm) in the leachates of the ALDFG of the two different regions could also explain some of the difference of toxicity observed. Zhou et al. (2023) revealed that chronic exposure (3 months) to PS NPs (100 nm) caused significant mortality of *O. latipes* at concentrations ≥ 10^4 NPs/L. At lower concentrations, the authors identified significant modifications of several enzymatic activities including trypsin, lypase, chymotrypsin, amylase, catalase, superoxide dismutase and immune activity markers (lysozyme, alkaline phosphatase) of digestive tract cells. The authors also highlighted that the highest IBR index was reached for the fish exposed to the medium concentration of NPs (10^4 NPs/L), while it decreased at 10^6 NPs/L and 10^1 NPs/L (Zhou et al. 2023).

Although not derived from FNs, Khosrovyan et al. (2022) reported that chronic exposure to both virgin and artificially aged PA MPs (80 to 180 µm) at a concentration of 180 mg/L did not have a significant impact on the survival and reproductive success of *D. magna*, despite the confirmed ingestion of the MPs. Thus, these results do not agree with the findings of this doctoral thesis, which showed that sub-chronic exposure to leachates of PA and PBS-PBAT led to significant effects on the reproduction of *A. tonsa* at environmentally realistic concentrations. The most probable hypothesis to explain this difference is that Khosrovyan et al. (2022) used a PA MP powder that probably did not contain plastic chemicals. Moreover, the MPs used were approximately four times larger than the MPs quantified in the leachates of the FNs made of PA (Botterell et al. 2019; Wang & Wang 2023). Although there are very few studies on the toxicity of PBS and PBAT, and even fewer on the blend, Wen et al. (2025) highlighted that PBS MPs (average size: 3.6 µm) and PBAT MPs (average size: 872 nm), provoked a significant decrease in human cells viability (LO2, Caco-2, THP-1, and HUVECs) at concentrations of 1, 25, and 100 mg/L. Their results are consistent with our results about the

decrease in cell viability of a trout liver cell line caused by the DMSO extracts of one of the two FNs made of PBS-PBAT (Chapter 3).

Comparing the effects caused by the leachates of the FNs on the different species, the bioluminescence assay with *A. fischeri* was a simple and rapid test which was useful for initial screening of the toxicity of plastic degradation products and which turned out to be often consistent with results obtained with other species (Miyashiro & Ruby 2012; Zimmermann et al. 2019; Piccardo et al. 2021). In terms of acute lethal effects, the copepod *A. tonsa* was the most sensitive species to the degradation products of FNs. This result was consistent with studies demonstrating that *A. tonsa* and similar other copepods serve as important bioindicator species in ecotoxicological studies due to their often higher sensitivity to pollutants including plastics, than other species (Raisuddin et al. 2007; Rotolo et al. 2021; Bisinicu et al. 2024; Ebbesen et al. 2024; Vitiello et al. 2024). This ecologically relevant group facilitates chronic exposure studies due to its rapid life cycle, thus allowing for the analysis of numerous endpoints that span from molecular to trophic and ecological levels (Gorbi et al. 2012; OECD/OCDE 2014; Langenfeld et al. 2024; Botterell et al. 2025). On the contrary, the microalga *T. lutea* exhibited low sensitivity, as no change in cell density was observed after 72 hours of exposure to the degradation products of FNs. Antonopoulou et al. (2022) showed different sensitivities among microalgae species after an exposure of 72 h to the flame retardant tris(chloropropyl) phosphate. The freshwater microalga *Chlorococcum sp.* (EC_{50} = 0.7 mg/L) and the seawater microalga *Dunaliella tertiolecta* (EC_{50} = 1.6 mg/L) were more sensitive than the seawater species *Tisochrysis lutea* (EC_{50} = 21.5 mg/L) and the freshwater species *Scenedesmus rubescens* (EC_{50} = 58.4 mg/L). Several studies reviewed by Parsai et al. (2022) attributed the differences of sentivity among species to (i) the morphology of the microalgae and particularly their size; and (ii) the presence (*Tetraselmis chuii*) or abscence (*Dunaliella tertiolecta*) of a cell wall and the structure of the cell wall (e.g., dense layer of thin calcified scales, silicate cell wall, polysaccharidic cell wall). Even if there are fewer knowledge on the effects of NPs on microalgae compared to the effects of MPs, available studies showed NPs had a higher risk for microalgae (Schiavo et al. 2017; Parsai et al. 2022). Survival of *O. latipes* fish larvae were rarely affected by FNs leachates, as documented in previous research showing that exposure to MPs of the early life stages of fishes resulted in minimal lethal effects (Foley et al. 2018; Cormier et al. 2022; Qiu et al. 2022; Kim et al. 2023b). However, the disturbance of sub-lethal endpoints such as heart rate and behavior proved to be highly informative, as they frequently anticipate higher level effects such as the improper allocation of energy within fish, or eventual long-term mortality (Schweizer et al. 2017, 2022; Krzykwa et al. 2018; Ricardo et al. 2024). Furthermore, the fish behavioral assay facilitates the identification of potential ecological consequences such as effects on social interaction with congeners, foraging, predator-prey interactions, and impacts at the population level (Easter & Nicola 1996; Champagne et al. 2010; Schnörr et al. 2012; Cormier 2020; Bedrossiantz et al. 2023; Alves et al. 2024).

7.3 Environmental risk of the degradation products of fishing nets

Assessing the environmental risks associated with plastic pollution is challenging due to the heterogeneous presence of MPs in the environment, the extensive diversity of plastic materials, their non-linear degradation, and the considerable variability in their toxicity spectrum across different species (Backhaus & Wagner 2020; Fauser et al. 2022). Nevertheless, it is imperative for researchers to improve the integration of their findings regarding the occurrence of MPs, NPs and plastic chemicals in the environment with toxicity results to evaluate the risks posed by this pollution, both at the global level and individually for different types of plastic. Indeed, from an evidence-based perspective or a precautionary approach, precise risk assessment is essential to effectively target actions aimed at mitigating plastic pollution in a complex socio-economic context marked by strong dependencies of our modern societies on plastic (Chalmin 2019; Backhaus & Wagner 2020; Bolo & Préville 2020).

The most used methods to establish the risk of MPs, NPs and plastic chemicals are the Risk Quotient (RQ), the Pollution Load Index (PLI), the Polymer Hazard Index (PHI) and the Potential Ecological Risk Index (PERI) (de Ruijter et al. 2020; Ranjani et al. 2021; Fauser et al. 2022). However, these methods, even if they are essential to bring first environmental risks of plastic pollution (Fauser et al. 2020; Morales-Espinoza et al. 2025), suffered from fundamental challenges in MP, NP and plastic chemical research, namely: (i) These methods are based on toxicity threshold values for pollutants in the environment, below which the compound in question is not considered to be hazardous for the environment and for which mitigation is not needed (Ranjani et al. 2021; Raimondo & Forbes 2022). However, these thresholds have not yet been established in the regulations for MPs, NPs and most plastic additives (Wagner et al. 2024). (ii) The equations do not account for the chemical composition of the plastics, thus omitting the toxic effects from leached plastic chemicals and the adsorption and release of environmental pollutants. (iii) These methods take very little account of the potential chronic effects of chemicals at low concentrations (Backhaus & Wagner 2020; Patra & Baitharu 2024). (iv) None of these methods is complete enough to take into account all the following criteria: the different polymer types, the plastic chemical composition, the effect of particle size and shape on the toxicity, their role as vectors of environmental hazardous chemicals, the heterogeneity of their concentration in the environment and the sensitivity of the different spieces and different endpoints (de Ruijter et al. 2020; Morales-Espinoza et al. 2025). Therefore, a specific method that takes into account these different variables is needed to ensure a reliable assessment of the environmental risk of MP, NP and plastic chemicals. In this context, a “Weight of Evidence” (WOE) approach (Romero-Franco et al. 2017; Gouin et al. 2019; Koelmans et al. 2021; Raimondo & Forbes 2022) with a software-assisted model (Sediqualsoft) was developed to integrate high amounts of heterogeneous data based on four different lines of evidence (LOE) (Avio et al. 2015; Regoli et al. 2019). The first LOE was focused on “microplastic characterization and quantification in abiotic matrices”. The second line was focused on “microplastic ingestion”, the third on “sublethal effects using biomarkers” and the fourth on “ecotoxicological bioassays”.

Based on the third LOE "sublethal effects using biomarkers", Vilke et al. (2024) established that leachates derived from ALDFG beached on the coastline of Portugal had "moderate" to "major" ecotoxicological hazard on bivalve mussles *Mytilus galloprovincialis*. In particular, they showed that the level of ecotoxicological hazard was the highest when mussels were exposed for 21 and 28 days to 10 mg/L of equivalent mesoplastic concentration, compared to shorter exposures (3, 7 and 14 days) and to other concentrations (1 mg/L and 100 mg/L). Overall, the different studies that used the WOE approach with a battery of biomarkers in mussels *M. galloprovincialis* gave similar results, with "moderate" to "major" level hazard after prolonged exposure to MPs, NPs, MNP mix and environmental pollutants. PS MPs tended to present a higher risk for *M. galloprovincialis* than PE or LDPE (Avio et al. 2015; Pittura et al. 2018; Gonçalves et al. 2023, 2025). Moreover, results revealed that the level of hazard was higher for MPs pertaining to size ranges [20-50[µm, [50-100[µm and [100-250[µm compared to upper size ranges (Nardi et al. 2024). Considering the LOE4 and working with seven different aquatic species (*A. fischeri, Aurelia sp., P. lividus, B. plicatilis, A. amphitrite, A. tonsa and O. latipes*), Gambardella et al. (2024) highligthed different levels of ecotoxicological hazard from "absent" to "moderate" for leachates of plastic beached on the Adriatic or BoB coastlines. Hard plastics (mix of five polymers: PP, PE, PET, PS, PVC) from the BoB, and ALDFG (PP, PE, ethylene propylene diene monomer) from the Adriatic Sea had a moderate ecotoxicological hazard, while the hard plastic (PE, PP) from the Adriatic Sea were "slightly" hazardous. Other plastic categories (Pellets, Rapido Trawling Rubber and plastic bottles) had "absent" ecotoxicological hazard. The findings of the LOE4 in this doctoral thesis (Chapter 5 and 6) indicated that the ecotoxicological hazard associated with the leachates from non-aged gillnets ranged from absent to moderate. The moderate risk was particularly identified for leachates from one of the partially biobased and biodegradable PBS-PBAT nets.

Taking into account the different scenarios of exposure to MPs derived from the FNs (LOE1) and the multi-species acute toxicity of the leachates (LOE4), the results of the WOE revealed that the gillnets used and lost in the SE BoB in 2023 had an absent to slight ecotoxicological hazard. Nevertheless, considering the potential accumulation of degradation products from the used FNs over an extended period, the level of hazard increased to a non-negligible degree. Indeed, based on the sub-chronic exposure of the copepod *A. tonsa*, the degradation products of the gillnet made of the 35 % biobased PBS-PBAT were estimated to have a moderate hazard for the marine environment. These results bring new arguments indicating that degradation products of plastic gillnets did not appear to be an environmental risk within a short-term perspective, as discussed by Backhaus and Wagner (2020). However, with a continuous and long-term exposure at low concentrations due to the persistence of MPs, NPs and plastic chemicals, the environmental risk of plastic FN pollution become moderate. Given current trends indicating that MP, NP, and plastic chemical pollution from FNs will continue to increase in the future (Geyer 2020; Schwarz et al. 2023; FAO 2024b), it is highly probable that the environmental risks associated with such pollution will also intensify (Backhaus & Wagner 2020; Geyer 2020; Schwarz et al. 2023).

Unfortunately, to the best of our knowledge, apart from the risk assessment using PHI, PLI and PERI already discussed in chapter 5 and 6, there was no published risk assessment integrating plastic FN degradation products in the environment and associated ecotoxicological results.

Concerning the risk assessment of plastic additives in the marine environment based on Risk Quotients, their hazards were estimated to be from very low (bisphenol A, triclosan, octyl-dimethyl-para-aminobenzoic acid (OD-PABA), metals, monomers, di(2-ethylhexyl) phthalate, nonylphenol, octylphenol) to moderate (di(2-ethylhexyl) phthalate, octachlor (OC), 4-tert-octylphenol, nonylphenol, copper) and high (nonylphenol, 4-nonylphenol, pentabromodiphenyl ether, tributyltin, hexabromocyclododecane, 6PPD-quinone) depending on the species and compartments studied (Fauser et al. 2022). However, from this precedent list reported by Fauser et al. (2022), only two (OD-PABA and OC) were based on bioassays results.

In 2023, the fishing fleet that used 211 tons of FG in the 70,000 km^2 of the SE BoB was the 7th marine area with the highest efficient fishing activity (in hours) and the 10th in terms of the number of boats compared to 25 other fishing fleets of different marine regions of the world (Chapter 1, Table 1.1). Therefore, based on the results of this Ph.D. thesis and keeping in mind that several proxies made, we can anticipate that other fisheries of the world would also participate to the MP, NP and plastic chemical pollution. Marine regions exhibiting efficient fishing activities comparable to or exceeding those of the SE BoB, coupled with significant accumulation of plastic litter (e.g., Barent Sea, Adriatic Sea, Gulf of Thailand, South China Sea, and Yellow Sea) may also experience slight to moderate impacts due to the degradation products of the used FNs. The case of the Yellow Sea draws particular attention, as it is one of the most important fishing area in the world (GFW 2024) (Chapter 1, Table 1.1), where high concentrations of MPs have been reported (Zhao et al. 2018; Zhu et al. 2018; Jiang et al. 2022; Reineccius & Waniek 2024), and since 2007 the Republic of Korea government is one of the only nation that has implemented measures to increase the operation of fishing gear made of PBS and PBS-PBAT (Kim et al. 2016, 2020; Park et al. 2019). Albeit the biobased and biodegradable FG favoured by the Republic of Korea government could improve the management of the EOL FNs (Kim et al. 2023a) and decrease ghost fishing (Kim et al. 2016, 2020), to the best of our knowledge, no peer-reviewed publication on the toxicity of the degradation products of these FNs is available to ensure their sustainability. Although the partially biobased and biodegradable gillnets tested in this Ph.D. thesis were most likely different to the ones used by the fishermen of the Republic of Korea, the results of the risk assessment of their long-term operation (slight to moderate) should raise some concerns and initiate research projects into the toxicity of degradation products of similar biobased and biodegradable gillnets.

7.4 From challenges to solutions: reducing fishing gear contributions to plastic pollution

In view of the results of this Ph.D. thesis and the current literature, action must be taken to mitigate the contributions of the fishing sector to macroplastic, MP, NP and plastic chemical pollution of the oceans. Indeed, plastic FG can be classified as essential plastic products (Scientists' Coalition for an Effective Plastics Treaty 2024) because millions of people rely on them for their survival (FAO et al. 2023; FAO 2024b) and therefore they can not be just

eliminated. To prevent secondary MP, NP and plastic chemical emissions while simultaneously addressing ghost fishing caused by ALDFG, abandonment and loss of fishing gear must be reduced. Effective prevention measures include the implementation of mandatory gear marking systems to establish ownership and promote the retrieval of lost equipment (Gilman et al. 2023). The establishment of comprehensive reporting and quick retrieval programs represent critical steps, where fishers are required to report lost gear and to participate in coordinated recovery efforts led by fisheries authorities (Richardson et al. 2021; Drinkwin 2022). Port reception facilities must be developed to provide adequate infrastructure for the proper disposal and recycling of end-of-life fishing gear, preventing improper disposal at sea and at ports (Richardson et al. 2021; Drinkwin 2022). Fishing activity should be regulated in an area specific manner, particularly in complex marine environments such as rocky shores or areas with strong currents, or during complex meteorological periods where gear damage, degradation and loss rates are significantly higher (Macfadyen et al. 2009; Richardson et al. 2018; Gilman et al. 2023). Concerning mending pieces, standardized mending procedures should be implemented to ensure the proper handling and storage of repair materials during fishing operations or gear preparation on port docks. Dedicated storage systems must be established onboard and, in the ports, to store all mending materials and prevent their accidental discharge overboard or runoff into the surrounding marine environment of ports. Training programs for fishing crews should emphasize the importance of proper net repair techniques and waste management practices (OCEANOPLASTIC 2023).

On the other hand, to minimize secondary MP, NP and plastic chemical emissions during operational use of FNs, the lifetime of components of fishing gear must be shortened, particularly for ropes, bottom trawls and draglines (Napper et al. 2022; Syversen et al. 2022). Regular replacement schedules should be implemented based on usage intensity and environmental exposure rather than on equipment failure (Napper et al. 2022; Basurko et al. 2023). Then, to reduce the toxicity of the degradation products of the FNs the chemical composition of FG must be adapted. To do so, the chemical composition of the FG must be transparent, at least for researchers and for agencies (e.g., ECHA) that are competent to regulate hazardous compounds. Second, known hazardous plastic chemicals must be phased out. Third, a positive list of chemicals should be presented to manufacturers to help them improve the compositions of FG (Scientists' Coalition for an Effective Plastics Treaty 2023a; Wagner et al. 2024). Finally, because non-biodegradable FNs will most probably not address all the above-mentioned challenges (to reduce ghost-fishing, to imptove end-of-life FG management, to decrease MP, NP and plastic chemical emissions, to not be hazardous) biodegradble FNs could be an effective alternative. It remains essential for these nets to be degraded promptly in the environment if they are lost, ensuring they maintain adequate fishing efficiency, and that their degradation products achieve complete mineralization in both aquatic environments and industrial composting. However, this Ph.D. thesis highlighted that the partially biobased and biodegradable nets studied were not a viable alternative with their actual composition as they presented a slight to moderate environmental risk for the aquatic environment. Therefore, the future biodegradable FNs that will be marketed must also fullfill the requirments of avoiding toxic chemicals and substituting them with non-toxic ones and full mineralization tests must become mandatory. It is important to keep in mind

that the development of new biobased and biodegradable FN formulations by private companies does not guarantee that these materials will be ecotoxicologically safe for marine environments. Therefore, even if the nets tested in this study are not marketed, those that will be marketed in the future must also undergo a thorough toxicity study, even if this is not currently mandatory. This can only be a positive process and will demonstrate the transparent and ready-to-action position of the fishing industry (Dauvergne 2023; Scientists' Coalition for an Effective Plastics Treaty 2024).

7.5 Limitation of the study and perspectives

To address the main hypotheses of this doctoral thesis, certain proxies were necessary. These proxies represent limitations that must be considered when interpreting and communicating the results. Additionally, constraints related to available resources, whether humans, social, technological, or financial, also contributed to these limitations. The aim of this section is to outline the principal limitations of the study and to propose future perspectives for overcoming some of them. This approach will help improve understanding of the occurence and toxicity of degradation products from FNs, a topic that was largely underexplored prior to this Ph.D. research.

The downscaling of the numerical and statistical models (Kuczenski et al. 2021; Richardson et al. 2022) used to estimate the quantity of used and lost FNs enabled us to produce the first data for the SE BoB. However, these estimations were time-consuming to generate, and inconsistencies in the data have resulted in estimates with non-negligible uncertainties. The main uncertainties derived from: (i) the inconsistencies between the fishing *métier* classification of fishing vessels in the Global Fishing Watch (GFW) platform (GFW 2024) and those in the national and European Union fishing registers; (ii) the catch and landing data extracted from the European Union database (European Commission & Eurostat 2024), which were sometimes incomplete and often had a low geographic resolution that made it difficult to align them accurately with the boundaries of the study area; and (iii) the inability to fully integrate the small-scale fishery of the SE BoB, because a significant proportion of boats under 12 meters in length were not equipped with AIS. Therefore, it would be highly beneficial for future research projects, as well as for environmental management agencies and fishermen, to automate the process and to use higher-quality data. An intuitive and easy-to-use software with a direct API connection to the GFW platform (GFW 2024) and direct data flow from national institutions responsible for recording fishing catches would provide an efficient novel tool. This would support sustainable fishing management, enhance the development of plastic FG collection and recycling programs, mitigate the loss of FG, and help develop strategies for the recovery of abandoned, lost, or discarded FG. In this chapter of the Ph.D. thesis (Chapter 2), we found a significant negative correlation between the estimated quantity of lost FG and the quantity of FG beached in the SE BoB in 2023. The primary reason for this could be that, unlike PE-PP bottom trawl net mending pieces or panels, gillnets and longlines are fixed fishing *métiers*, are often weighted, and are mainly made of PA, which has a higher density than seawater and therefore FG made of PA sink when lost. Consequently, the results of this Ph.D.

thesis should be complemented with sea floor and deep sea observations. With this additional data, a large-scale transport model should be built to verify our loss estimates and to better understand the fate of lost nets such as gillnets and longlines.

The artificial aging of FNs was based on UV irradiation under dry conditions, with two different temperatures and agitation in deionized water. it would have been beneficial to perform the artificial aging in salt water or even in the environment. Environmental weathering bears higher realism and allows to determine the effects of microorganisms on the degradation of the FNs and especially the partially biobased and biodegradable ones. In this context, between 2023 and 2024 we conducted a one-year environmental weathering of six FNs (cod end trawl net made of PE-PP and gillnets made of PA and PBS-PBAT) in two different ports of the SE BoB (Arcachon and Pasaia) (Lefebvre et al. 2025). The biocolonization of the FNs by microorganisms was also studied. However, in this experiment it was not possible to quantify the MPs and NPs produced by the FNs during the weathering process. Therefore, another experiment could be set up to reproduce fishing operation situations with successive immersions and solar irradiation, without losing the small-sized fraction of MPs and NPs, and the plastic chemicals. Conducting this latter experiment over years could also generate new knowledge concerning the persistence of MPs, NPs, and plastic chemicals produced by FNs made of biobased and biodegradable polymers. Regarding bottom trawls and bottom seines, an experiment simulating the active abrasion of the nets should be conducted to better estimate their contribution to pollution from MPs, NPs and plastic chemicals. Machines such as the Mila 200 Wet (Buraschi Italia srl, Milan, Italy) could be suitable equipment to conduct this type of experiment.

To reduce the uncertainties associated with the estimation of the average quantity of MPs produced per gram of FN, it would have been preferable to analyse the leachate replicates of each artificially aged FN multiple times, rather than relying on a single pooled sample from the three replicates of each FN. However, quantifying the small size fraction of MPs (1 µm to 10 µm) for the numerous different FNs and aging times studied, using the laser direct infrared chemical imaging system was time consuming. Thus, it was not possible to analyse three replicates per FN and per aging time, in addition to the controls. Moreover, as explained in Chapter 4 and 5, the laser direct infrared chemical imaging system also had its own limitations. Therefore, alternative methods such as Pyrolysis-Gas Chromatography-Mass Spectrometry (GC-MS) or thermal desorption-proton transfer reaction-mass spectrometry could be used in the future, although these methods are destructive and do not allow for characterization of the size and other characteristics of particles on the same samples. On the other hand, automated micro-Raman analysis could be used to improve estimations of the amounts of MPs and NPs produced by FNs.

The quantification of plastic chemicals in the leachates of FNs enabled us to identify 27 compounds and to quantify 10 of them. However, based on the literature, it is likely that many other organic compounds were also present in the leachates, such as dyes, NIAS, or degradation by-products. Therefore, other methodologies, such as high-performance liquid chromatography coupled with mass spectrometry or pyrolysis coupled with GC/MS, would allow for a more exhaustive characterization of the chemical degradation products of FNs. Moreover, since the chemical composition of FNs is not transparently provided by

manufacturers, it would be useful to characterize the full chemical composition of the FNs using a depolymerization protocol and GC/MS or LC/MS.

The ecotoxicological findings of this Ph.D. thesis are among the first results addressing the toxicity of degradation products from new, artificially aged, and end-of-life FNs. Therefore, they contributed to bring new knowledge to the scientific community but require further investigations are required. Specifically, given the significant toxicity of FN degradation products observed in copepods, and considering the ecological importance of this clade, additional chronic exposure studies on copepods should be conducted. Mesocosm experiments using both non-biodegradable and biodegradable FNs could be an effective approach for better understanding their effects on copepod life cycles, copepod populations, and impacts on other trophically connected species. In such mesocosm studies, it would also be valuable to include a control consisting of a parallel exposure to natural particles similar in size and shape to MPs and NPs produced by FNs, in order to distinguish effects of natural particles from those of synthetic ones.

The sub-lethal effects of leachates of FNs observed on fish larvae are another cause of concern, as they could provoke cascading impacts throughout the trophic chain. Further studies on commercial and other wild fish species inhabiting the SE BoB are needed to determine the potential large-scale toxicity of FN degradation products. This research would also facilitate assessment of whether fish stocks available for commercial fishing in the SE BoB are affected by fishing net degradation products.

Furthermore, it would have been useful to study additional endpoints, particularly molecular, cellular and tissue level biomarkers, to elucidate the mechanisms of toxicity associated with the degradation products of FNs. In particular, biomarker studies would be valuable to better understand the 24-hour growth delay observed in the alga *Tisochrysis lutea* and to further investigate the sublethal effects that well-dispersed and stable NPs derived from FN degradation may have on microalgae, copepods and fish. Indeed, NPs resulting from the degradation of FNs could directly trigger toxicity at the cellular level through membrane translocation, and may also act as vectors for plastic chemicals within tissues (Trojan horse concept). Finally, long-term effects on growth and reproduction of target sentinel species is also required for a proper environmental risk assessment.

Moreover, now that we showed that MPs, NPs, and plastic chemicals derived from FNs cause deleterious effects on various species, it would be valuable to conduct further experiments to determine toxicity threshold concentrations. This could be done either using mixtures of degradation products or individual FN chemical compounds registered in databases as persistent, accumulative or/and toxic. This information could help, on the one hand, to identify the most hazardous chemical compounds and, on the other hand, to replace them with less harmful alternatives. This is particularly important, as the PCA and correlation matrix analyses revealed that toxicity was mainly driven by the plastic chemicals released from FNs.

Finally, the environmental risk assessment of FN degradation products should be further refined. Firstly, the scenarios for MP pollution levels derived from the degradation of FNs should be improved to integrate processes such as MP sedimentation, the export of MPs from the area, and the influx of MPs generated by FN operations outside the SE BoB. Secondly, the

quantification of produced NPs and released plastic chemicals needs to be integrated into the first line of evidence, which requires the establishment of risk thresholds for these compounds. However, setting such thresholds remains challenging, as some of the identified plastic chemicals are poorly studied and current knowledge is insufficient. Moreover, broadening the range of species and biological endpoints investigated would enhance the robustness and ecological relevance of future risk assessments.

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CONCLUSIONS & THESIS

8.1 Conclusions

The present Ph.D. thesis aimed to evaluate the production and ecotoxicological impacts of the mixture of microplastics, nanoplastics and plastic chemicals, coming from the degradation of plastic fishing nets (FNs) on four different species, with a specific focus on the Southeastern of the Bay of Biscay (SE BoB). The first step of this doctoral thesis which aimed to gain a better understanding of the fishing sector in the SE BoB indicated that in the last 40 years of the 20th century, plastic materials replaced natural fibres in FG manufacture. Currently in the SE BoB nearly all the nets used in the professional fishing sector are made of plastic. Regarding the degradation of FNs, limited knowledge is available concerning the production of MPs, NPs and plastic chemicals. In general, although the ecotoxicological risks posed by macroplastics, mesoplastics, and microplastics to aquatic organisms have been widely studied across various species, the scientific data acquired remain heterogeneous and do not consistently exhibit a uniform pattern. Regarding the toxicity of degradation products from plastic FNs, only a limited number of studies have been published. However, in the past three years, there were some researches published, particularly focusing on the toxicity of abandoned, lost, or otherwise discarded FG.

In the second step of the Ph.D. thesis, we estimated that 211 tons of FNs were used and 6 tons were lost in the SE BoB in 2023. Gillnets, purse seines and longlines were the most used and lost FG in amount. Based on the plastic litter samples collected in 8 beaches, it was estimated that 3 tons of abandoned, lots or otherwise discarded FG were beached on the SE BoB coast in 2023 and that they represented 7.6% of plastic litter found in these beaches. The main FG found by number of items were bottom trawl and bottom seine mending pieces (92%).

Then, the toxicity of the dimethyl sulfoxide extracts of eight new and end-of-life oil-based FNs used in the SE BoB, as well as of two biobased and biodegradable FNs was determined. Seven trace metals were quantified in the extracts of new and end-of-life (EOL) FNs. The chemicals and potentially the MNPs extracted caused different patterns of toxicity depending on the FNs and species studied. Five of the eight extracts of EOL FNs were more toxic than the extracts of the new FNs. The contrary was true for two other. For one FN made of PA a general low toxicity was observed, which was not significantly different between the new and the EOL state. Regardless of whether the extracts originated from new or EOL FNs, at least one of the two partially biobased and biodegradable nets was among the two most toxic FN extracts for the three aquatic models examined (*Aliivibrio fischeri*, RTL-W1 cell line and *Oryzias latipes*), compared to the other FNs.

The next step of this doctoral thesis enabled us to identify 27 plastic organic chemicals in the leachates of three artificially aged FNs with different polymer compositions and fishing *métiers*. Among these chemicals, 10 organic compounds including plastic additives, NIAS and degradation by-products were identified and quantified. The artificial aging of FNs produced MPs and NPs, particularly under extreme conditions (high UV-irradiation at 60°C). The degradation products of the three FNs studied significantly disrupted the behavior of larvae of the fish *O. latipes*. The degradation products of the 50% partially biobased and biodegradable FN were the most hazardous and were the only ones to cause a significant

bioluminescence inhibition of the bacterium *A. fischeri*. The toxicity observed was mainly related to the organic plastic chemicals quantified in the leachates. As artificial aging time increased, the amount and toxicity of the degradation products of the FNs generally rose, although this increase was not linear over time or consistent across the different FNs. The cod end trawl net made of PE-PP was identified as the FN that generated the lowest amount of degradation products and caused a minimal toxicity to the two aquatic species examined (*Aliivibrio fischeri* and *Oryzias latipes*), compared to the two gillnets made of PA and PBS-PBAT.

Subsequently, a first risk assessment was established based on the toxicity caused by the degradation products of three FNs made of PA but with different chemical composition and of one partially biobased and biodegradable FN made of PBS-PBAT. PA gillnets could contribute to MP pollution by releasing $4.05x10^4$ MP/g of FN (95% CI: 0-$8.3x10^5$ MP/g of FN). Based on the quantity of MPs produced by gram of FN and the quantity of gillnets used, it was estimated that gillnets made of PA released an average of 2.2 MPs/m^3 of water (95% CI: $4.0x10^{-3}$-6.5 MPs/m^3) in the SE BoB in 2023. The PBS-PBAT FN, made of 35% of partially biobased and biodegradable polymer, produced significantly less MPs compared to the gillnets made of PA. Seven organic plastic chemicals and four trace metals were identified in the leachates from all FNs. The three gillnets made of PA but with different chemical composition caused different patterns of toxicity depending on the species. One of them (PA β33) was significantly more toxic for the *O. latipes larvae*. Overall, the 35% partially biobased and biodegradable gillnet caused a higher ecotoxicological hazard than the three PA FNs. The plastic chemicals quantified in the leachates of FNs were the main cause of the toxic effects observed in three of the aquatic species studied (*Aliivibrio fischeri*, *Acartia tonsa* and *Oryzias latipes*). The impact of acute exposure to FN degradation products on the microalga *Tisochrysis lutea* was not significant after 72 h of exposure. Based on all the acute toxicity results, the weight of evidence approach estimated that the risk assessment of the MPs produced by the FNs used and lost in the SE BoB was absent to slight.

Finally, the sub-chronic toxic effects of the leachates of artificially aged FNs on the reproductive outcomes of the copepod species *Acartia tonsa* were investigated. The degradation products of gillnets made of PA and of PBS-PBAT significantly impacted the quantity of eggs and faecal pellets produced by female copepods. Moreover, the hatching success of the nauplii was also significantly reduced when the females and eggs were exposed to the leachates of these two FNs. Overall, the leachates of non-aged gillnets were more toxic for *A. tonsa* than the leachates of FNs artificially aged for 25 days. Using the Weight of Evidence approach, we estimated that the MPs produced by the operation and loss of the gillnets in the SE BoB in 2023 posed an absent to slight environmental risk. However, a continuous and long-term exposure of the copepods to the MPs produced by the gillnets used in the SE BoB could cause a slight to moderate environmental risk. Strikingly, it was the 35% partially biobased and biodegradable gillnet that could cause a moderate environmental risk.

8.2 Thesis

The interdisciplinary investigation into the degradation of conventionally used and under development fishing nets revealed their non-negligible contributions to microplastic, nanoplastic and plastic chemical pollution of the oceans. The increased time of artificial aging of fishing nets enhanced both fishing nets degradation and toxic effects, despite displaying a non-linear pattern over time. Furthermore, the degradation products of fishing nets were toxic to three aquatic species, including two species that inhabit the Southeastern Bay of Biscay. In addition, the plastic chemicals released during the degradation of FNs were identified as the main contributors to the observed toxicity, even though only MPs and not NPs were quantified. The acute environmental risk of the MPs derived from the gillnets studied was estimated to be absent to slight. However, considering sub-chronic exposure, the environmental risk of gillnets could become slight to moderate. Finally, although partially biobased and biodegradable fishing nets offer potential in mitigating ghost fishing and marine habitat deterioration caused by lost non-biodegradable nets, as well as the inadequate management of end-of-life fishing nets, their polymeric and chemical composition need to be improved to ensure that they constitute a truly ecotoxicologically safe alternative for the marine environment.

ANNEXES

Annex A. Developed protocols

A.1 Fishing net artificial aging

This protocol was developed following a series of tests under the supervision of Ph.D. Student Edgar Dusacre, assisted by two trainee students, Coralie Le Picard and Faith Ekoja. It was validated by Dr. Fabienne Lagarde, Pr. Jérôme cachot, Pr. Miren P. Cajaraville. This protocol is inspired by the standardized test "ISO 4892-3: Plastics - Methods of exposure to laboratory light sources - Part 3: Fluorescent UV lamps" (International Organization for Standardization 2016).

Material

-Microbiological safety cabinet (MSC) with laminar flux

-UV chambre (Dusacre et al. 2024, 2025)

-Incubator (> 30°C)

-Filtration unit with pump

-Methyl-cellulose filters (0.22 µm mesh)

-Laboratory gloves

-Filtered ethanol (70% EtOH) (0.22 µm)

-Filtered deionized water (0.22 µm)

-Inox plate (30x20 cm) – to cut the fishing nets (FNs)

-Thick (2 to 3 mm) glass plate – to cut the FNs

-Chirurgical scissors – to cut the FNs

-Glass pots with metal cover – to store FNs pieces

-Inox squeezer

-1 mm mesh stainless filter

-Quartz pots

-Quartz pot cover (plates)

-Glass agitation pots with a watertight cap, avoiding plastic (https://www.boboco.fr/pots-et-bocaux-en-verre-vides/139-verrine-conique-236cl-3597380086931.html)

-Storing glass pots with a watertight cap, avoiding plastic (https://www.boboco.fr/pots-et-bocaux-en-verre-vides/120-pot-standard-cylindrique-212cl.html)

-Glass petri dish (60 mm)

-Glass beads (1.5 mm)

-Glass Syringe

FN preparation

-Under an MSC:

-Cut the fishing net into pieces of 2 to 2.5 cm (select randomly the pieces of the net).

Dry the pieces at 30°C in the dark until a constant mass (not a higher temperature could start to degrade the fishing net (Therias et al. 2021).

-Weight 0.6 g of the FN. Be careful to accurately select the number of pieces with node for each type of FN (0.6g is a good weight because a larger quantity will increase the time and different layers of pieces of FNs will superimpose during UV irradiation, resulting in heterogeneous irradiation).

- According to the FN type (ratio of number of nodes according to the size of the meshes) and the weight of FN pieces used, place a good number of nodes. The R script available in this GitHub repository was made for this purpose: https://github.com/EdgarDusacre/Plasfito_public/blob/29f74f69b7b0b170b0c67c572fe04abf2951df29/Protocols/Artificial%20aging/Number%20of%20nodes%20and%20glass%20beads.R

-Insert 0.6 g of FN pieces in a quartz pot and cover with a quartz plate.

-Randomly sample the location of the quartz pots in the UV chamber. A R script was also made to do it: https://github.com/EdgarDusacre/Plasfito_public/blob/3ee385a54163107da8f8127761dbea642045d3b8/Protocols/Artificial%20aging/Number%20of%20nodes%20artificial%20aging.R

UV irradiation

-Wear UV protective glasses.

-Place the quartz pots inside the UV chamber according to the random sampling of the locations.

-Close the UV chamber.

-Switch on the UV chamber (UV-A 360-370 nm and UV-B 280-350 nm) and set the irradiation to 401 W/m^2 (20% on the dimmer). If you want a different intensity, adjust the chamber as required, avoiding prolonged irradiation in excess of 50% of the chamber's intensity to avoid damaging it.

-Every 3,135 days, switch off the UV chamber, turn over the pots, use a new location plan (randomly sample), place the pots, and switch on the UV chamber. To know the exact dates and hours for the location change, please use the following R script:

https://github.com/EdgarDusacre/Plasfito_public/blob/29f74f69b7b0b170b0c67c572fe04abf2951df29/Protocols/Artificial%20aging/Aging%20time%20curve.Rmd

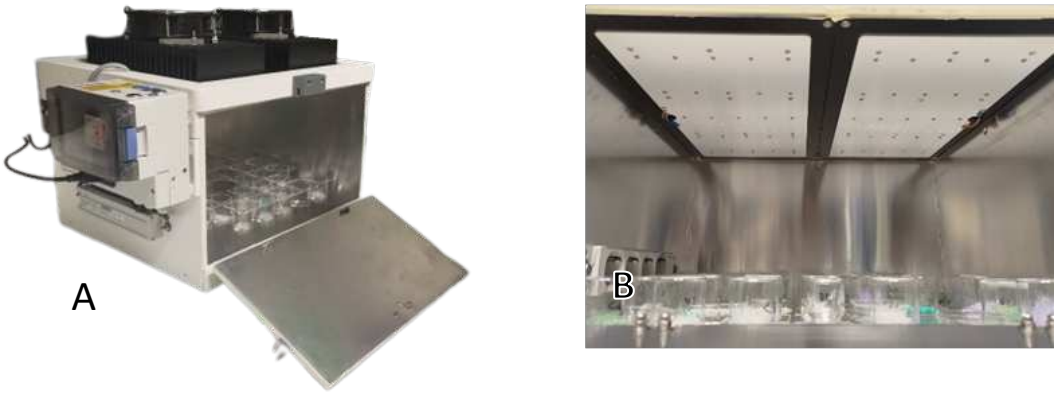

Figure 1: Pictures of the UV chambre ready to be used (A), with its UVA UVB led panel and the quartz pots with FN pieces inside (B)

-Repeat the process until the end of the UV exposition.

-Turn off the UV chamber.

-Place the quartz pots in a dark and dry environment until the next steps.

Leaching process:

-Under an MSC:

- Case of UV irradiated FN pieces.
  - Clean the agitation pots three times with filtered (0.22 µm) 70% ethanol and filtered (0.22 µm) deionized water. Carefully transfer the net fishing pieces from the quartz pot to the agitation pots.
  - Rinse the quartz cover plate two times with 2.5 mL.
  - Rinse the quartz pot three times with 5 ml of filtered (0.22 µm) deionized water using a syringe for pressure.
  - Add 10 mL of filtered (0.22 µm) deionized water to the agitation pot. (The final concentration will be 0.6g/60 mL: 10 mL added in this step + 20 mL of rinsing water from the quartz pot + 30 mL of agitation pot and rinsing water from the mesopieces)
  - Clean the glass beads.
    - In a 1 mm sieve:
      - Rinse the glass beads for 5 minutes with tap water
      - Rinse the glass beads with 1L of ethanol
      - Rinse the glass beads for 5 minutes with deionized water
      - Under an MSC, rinse the glass beads with 1L of filtrated ethanol (0.22 µm)
      - Under an MSC, rinse the glass beads with 1L of filtrated deionized water (0.22 µm)

- Add the glass beads in the agitation pots. Same weight of glass beads as FN. To know the number of glass beads, please use the R srcipt made for this purpose: https://github.com/EdgarDusacre/Plasfito_public/blob/29f74f69b7b0b170b0c67c572fe04abf2951df29/Protocols/Artificial%20aging/Number%20of%20nodes%20and%20glass%20beads.R
- Close the agitation pots.

• Case of non-irradiated FN pieces or in situ protocol.

- Clean the agitation pots three times with filtered (0.22 µm) 70% ethanol and filtered (0.22 µm) deionized water. Add the FN pieces previously weighed.
- Add 30 mL of filtered (0.22 µm) deionized water to the agitation pot. (The final concentration will be: 0.6g / 60 ml (30 ml added in this step + 30 ml later during the agitation pot filtration and rinsing of the macropieces)
- Clean the glass beads.
  - In a 1 mm sieve:
    - Rinse the glass beads for 5 minutes with tap water
    - Rinse the glass beads with 1L of ethanol
    - Rinse the glass beads for 5 minutes with deionized water
    - Under an MSC, rinse the glass beads with 1L of filtrated ethanol (0.22 µm)
    - Under an MSC, rinse the glass beads with 1L of filtrated deionized water (0.22 µm)
- Add the glass beads in the agitation pots. Same weight of glass beads than FN. To know the number of glass beads please use the R srcipt made for this purpose: https://github.com/EdgarDusacre/Plasfito_public/blob/29f74f69b7b0b170b0c67c572fe04abf2951df29/Protocols/Artificial%20aging/Number%20of%20nodes%20and%20glass%20beads.R
- Close the agitation pots.

-Wrap the pots with aluminium.

-Set the agitation pots on the orbital shaker.

-Set the room temperature to 21°C.

-Set the rotation speed at 190 rpm (radius 1.5 cm)

-The conversion from cm/s to rpm has been done using the formula "r" being the orbital shaker radius (0.015m in EPOC, EA, B2)) and "v" the rotation speed ot the orbital shaker.

$$N_{(rpm)} = = \frac{60}{2\pi * r} * v_{(m/s)}$$

-Start the agitation for a duration of 24 h.

-Stop the agitation.

-Take a picture of each pot using a stereomicroscope with a 6.3 zoom. (optional)

-Under a MSC:

- Filtrate the content of the containers using a 1 mm mesh stainless sieve in another glass container (which has previously been cleaned three times with filtered ethanol and three times with filtered deionized water (0.22 µm)).
- Rinse the pot two times with 3 mL of filtered (0.22 µm) deionized water by pouring the contents through the sieve and repeat the process a last time, but with 4 mL of filtered (0.22 µm) deionized water.
- Rinse the sieve two times (with nets inside) with 3 mL of filtered (0.22 µm) deionized water and repeat the process a last time with 4 mL.
- Remove the pieces of FNs and put them in an annotated clean glass Petri dish
- Put the glass Petri in the oven at 35°C until it reaches a constant mass.
- Rinse the sieve two times (with glass beads inside) with 3 mL of filtered (0.22 µm) deionized and repeat the process a last time with 4 mL.
- Use a glass syringe to throw air on the glass beads in the sieve
- Remove the glass bead
- Throw air directly into the sieve
- Close the pot full of 60 mL
- Agitate the pot with a vortex
- Agitate another time the pot with the vortex.
- Take 2 mL with a glass syringe in a glass tube of 4 mL for the MNPs quantification.
- Agitate a last time the pot with the vortex.
- Aliquot in clean glass vials the volumes you need for the different bioassays.
- Keep the rest of the liquid in the pot and close it well.
- Store the leachates at -20°C.

Fishing net mesopieces treatment

-Put the glass Petri dish with the FN pieces > 1 mm in an incubator for them to dry until constant weight (30°C)

-When the nets to be dried are of constant mass, weight them.

-Keep three random pieces apart with one node for FTIR and store them in a dry and dark space. Maximum temperature 30°C.

-Keep three random pieces apart with one node for SEM and store them in a dry and dark space. Maximum temperature 30°C.

-Keep all other pieces of FN in an aluminum pillbox at -20°C for future experiments (optional).

References

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A.2 Microplastics quantification using the LDIR 8700

This protocol was developed thanks to a series of tests under the supervision of Ph.D. Student Edgar Dusacre and the Dr. Nagore Gonzalez-Soto, assisted by one trainee student, Asadulla Hil Galib. It was validated by Dr. Sophie Lecomte, Pr. Jérôme cachot, Pr. Miren P. Cajaraville. This protocol is inspired by the Agilent protocol that markets the LDIR 8700 (Agilent Technologies, 2023).

Creation of a Library of spectra

Sample Preparation in a Kevley Slide for IR library creation.

-Micronize fishing net samples into smaller particles (<=0.5mm) using a file.

-Collect the particles from the file in small glass vials.

-Add 500µL of Pure filtrated Ethanol (96%) to each vial (to facilitate the evaporation).

-Take a clean Kevley slide and mark three section areas with a marker. The idea is to run multiple samples within a single analysis.

-Place a drop of ethanol suspension in the designated area of the kevley slide with a glass pipette.

-Repeat the same steps for the other samples.

-Place the slide in the particular sample holder and keep it under a petri dish to avoid contamination and let the ethanol evaporate. Allow some time to dry the sample before taking it to the LDIR.

LDIR Spectra Library

-Insert the sample into the 8700 LDIR.

- Click the *Ejection* icon on the software to move the sample stage to the starting position.
- Remove the previous sample if it is present.
- Click the *loading* icon to start loading the sample. Follow the on-screen prompts.
- Enter the sample name and any additional comments.
- Slide the sample holder into the instrument and then close the door. Always hold the sample holder with the plastic grip at the top of the holder.
- Click *Continue*.
- The software will automatically load the sample, profile the height, adjust the contrast, build a visible image, and open a new analysis window.

-Focus the IR laser on the sample and check sample flatness.

- *Double-click on the sample* to focus the infrared light.

- *Click + Select Area*
- Draw a box on the sample to choose the area to check the flatness of the sample.
- Click on a large pixel size.
    - Note: If the sample is not flat, use the Line Profile tool to perform a survey (Automated Workflow) on the whole particle or eject the holder, slice the sample further to try to get a flat surface, and put back the holder.

-Add the constituent spectrum to an existing or new library (Manual Method)

- Start with the IR image of the sample.
- Creation of a *Baseline*:
    - *Double-click on an empty area* near the point of interest (particle) to acquire a spectrum that will act as a Baseline.
    - If the Icon that just appeared is **orange**, it will indicate that a baseline was already collected. The point that is just collected is compared with that previously collected baseline.
    - To take a new baseline, *unselect the Orange Icon* and *double-click beside* to select the new Baseline for this particle analysis. The newly selected baseline should indicate that the reflective surface/Reflectance is 100%, a key criterion.
    - Now, any new point taken on the sample will be ratioed against the new baseline.
- *Double-click on several (5 or 6) points of interest* in the sample to acquire an average spectrum of the material.
- Click *Add the spectrum* to the spectral viewer.
- *Open the Spectral Viewer*
- Double-click on the spectrum's name field and enter the material's name.
- *Press ENTER* on the keyboard.
- Elect the material spectrums by *clicking on their names* in the list. (*Ctrl + Select*)
- *Click the Libraries button*

-Add the constituent spectrum to an existing or new library (Automatic Method)

- Add to existing library: *Click the blue lock icon* next to the desired library to unlock the library.
- *Select the box* to the left of the library
- Add to a new library: *click on the new library icon*
- *Write the name* of a new library.
- *Click +* to create and add to the new library.
- The material is added to the library. If the spectrum has been well added to the new library, this one will go up the library list.
- Repeat the process for every sample.

Quantification of MPs and characterization of polymer in samples

Experimental Setup

-Sample preparation/filtration to be performed in a laminar workflow (fume hood). The air flow in the lab must be controlled to maximize air purity and minimize airborne contaminants levels.

-Clean the fume hood and all the glass material used for filtration with filtrated ethanol and Mili Q water three times and let them Air dry.

-The filtration stem supporting material is coarse (fritted glass). To avoid trapping silica particles underneath the filter, wipe the surface with EtOH before use.

-Gold-coated filters are delicate, so careful handling of the filters is required. Use the supplied tweezers to transfer the filters.

-Clean the filter by immersion in new filtered ethanol three times (each time in a new beaker) inside a fume hood. (For each filter, replace the ethanol.)

-Carefully place the filter in the recommended supporting material (fritted glass).

Figure 1.A

-Allow the filter to air dry for a minute.

-Place the funnel and secure the vacuum filtration assembly with the clamp. **FIGURE:1.B**

- **!! Note: Do these red steps just before the filtration of your sample. The filter must stay a minimum of time in the filtration structure!!**



A



B

Figure 1: Illustrations of how (A) set the filter in the filtration unit and (B) use the clamp to block the filter column

Sample preparation

-Take the vials containing microplastic suspension from the Freezer and allow them to thaw at room temperature or use a bath in water at room temperature (<25°C).

-Take 12 mL of 96% filtrated ethanol in a clean glass beaker (already rinsed three times with filtrated ethanol).

-Vortex the sample vial to mix correctly.

-Take 500 µL of the replicate samples with a glass pipette and add them to the glass beaker containing 96% filtrated ethanol.

-Take 500 µL of ethanol in the glass pipette to rinse any remaining microplastics and pour them into the glass beaker.

Repeat for the two other replicates. Finally, you will have 1,500 µL of samples, 1,500 µL of 96% filtrated ethanol used to rinse the pipette, and 12 mL of 96% filtrated ethanol. The final concentration will be 111µL of sample / mL of 96% filtrated ethanol.

-Using a 4mL glass pipet sample in a single time, filter the 4mL using the experimental setup and a vacuum pump with a pressure of 80 mbar (recommended maximum pressure 700 mbar) for 30 seconds (Figure 2.A).

-Rinse the pipette with 4 mL of 96% filtrated ethanol.

-Once the sample filtration has finished, turn off the vacuum, remove the funnel, and let the filter dry for 2/3 minutes.

-After the filter has dried sufficiently, transfer it to the filter holder using the following steps:

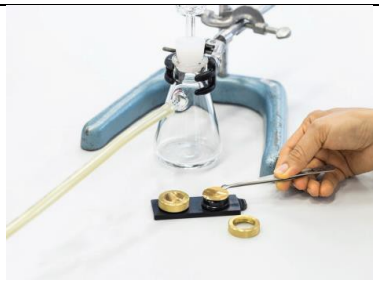
- Remove the brass retaining ring from the filter holder.
- Clean the raised platform of the filter holder with EtOH to remove any particles present on the stage that may create a hump in the filter, affecting the flatness of the sample.
- Keep the filter holder next to the filter (Figure 2.B).
- Carefully remove the filter from the glassware using tweezers and gently place/slide the filter on top of the raised platform. The filter can be centered with the tweezers.
    - **!! Note: Avoid any useless movement with the filter. It must stay flat !!**
    - 2nd Note: The filter holder is clearly labeled I and II. If only one filter is being measured, it should be placed in position I, not II. If two filters are being measured, the two filters should be made from identical materials or with a Blank. Gold-coated and silver filters should not be placed in the same filter holder.
- Gently and straightly thread the brass retaining ring back onto the holder

(Figure 2.C).

- Tighten the brass to secure the filter and keep it flat.
- Place the support with the filter in a clean petri dish.
- Repeat the process if you want two filters in the holding structure.
    - **Note: Even if it is the same sample, clean everything (filtration structure, pipette, tweezer, etc.) three times with filtrated ethanol.**
- Transport the petri dish close to the LDIR without any brutal movement and keep the petri dish very flat.



A



B



C

```
# Random sampling of the ten and three smaller squares

library(dplyr)
library(writexl)

n_random <- sample(0:1000, 1)
n_random

# Prompt the user to enter a sample name
sample_name <- readline(prompt = "Enter your sample name: ")

set.seed(n_random) # Setting a seed for reproducibility
pool <- 1:25
squares_10 <- sample(pool, 10, replace = FALSE)
squares_10

set.seed(n_random) # Setting a seed for reproducibility
pool <- 1:25
squares_10 <- sample(pool, 10, replace = F)
set.seed(n_random)
squares_3 <- c(sample(squares_10, 3), rep(".", 7))
squares_3

# Create a data frame with the squares_10 values, sample_name, and n_random
data <- data.frame(sample_name = sample_name, n_random = n_random, squares_10
= squares_10, squares_3 = squares_3) %>% arrange(squares_10)
data

# Write the data frame to an Excel file
file_name <- file.path(Sys.getenv("USERPROFILE"), "Desktop",
paste0(sample_name, ".xlsx"))
write_xlsx(data, path = file_name)
```

Figure 2: Illustrations of how (A) filter the sample, (B) set the filter on the LDIR support and (C) tighten the clamping ring

Quantification of MPs and characterization of polymers with LDIR

-The Filter particle analysis method will be used to quantify the representative number of particles. This method will use a large square area in the filter from a specific coordinate for **the largest particle (> 100 µm)**. Then, **for the smallest particles (< 100 µm)**, seven randomly determined smaller squares will be used out of a matrix of 25 squares (5x5) (power statistics have been used to determine the number). These 7 smaller squares previously drawn will be used for the real image morphology and the polymer analysis.

- *The area of the large square = 180 mm^2 (13.42mm×13.42mm)*
- *The area of the smaller squares = 7.20 mm^2 (2.684mm × 2.684mm)*
- *Approximate coordinates for Filter holder I (62.5, 5.5); Filter holder II (22.4, 5.5)*

-For the random sampling, please use the Rstudio Software code and the script below.

- Note: Make a new random sampling of the ten and three smaller squares for each new filter analyzed.

Quantification of particles > 100 µm

-Click the *Ejection icon* on the software to move the sample stage to the starting position.

-Remove the previous sample if present.

-Click the *loading icon* to start loading the sample. Follow the on-screen prompts.

-*Enter the sample name* and any additional comments.

-Slide the sample holder into the instrument and then close the door. Always hold the sample holder by the metallic part.

-Click *Continue*.

-The software will automatically load the sample, profile the height, adjust the contrast, build a visible image, and open a new analysis window.

-*Double-click* on the center to focus infrared light equipment.

-Click on *add a new analysis*.

-Click *particle Analysis*.

-Click *the drop-down menu* and select *Library Plasfito Library 2023*

- Note: For any other project, choose the library that fits your objectives the best.

-Click + to create a new method.

-Deselect *Auto scan*.

-Deselect *Collect Visible Image*

-Adjust the criteria for **Quantification** as follows: (Figure:)

- Particle sensitivity: *Max*
- Classification Range: 0.2-> 0.5-> 0.75
- Particle diameter setting: *100-1000*
- Scan Classification Ranges: 0-150; *100-200; 200-300; 300-400; 400-500; 500-600; 600-1000*

-Rename the method as *Quantification_sup_100µm*.

-Click + *Select Area*.

-*Draw the larger* 180 mm^2 *square* (13.42mm×13.42mm) in the sample area with the particle to be analyzed. The square must have the following coordinates:

- **Screw I: 62.4 ; 5.5**
- **Screw II: 22.4 ; 5.5**

-*Click on* ▶ *play*

-Note that the particle number is showed in a new tab after the completion of scanning

-To save the particle data, *start the particle identification* process and *stop after a minute*.

-Export particle data in .csv format.

Quantification of particles between 0µm and 100 µm using real-image analysis

-*Double-click on the center* of the sample to focus the infrared light equipment.

-Click *add a new analysis*.

-Click *particle Analysis*.

-Click *the drop-down menu* and select *Library Plasfito Library 2023*

-Click + to create a new method.

-Select *Auto scan*.

-Select *Collect Visible Image*

-Adjust the criteria for the **IR Scan** as follows:

- Particle sensitivity: Max
- Particle diameter setting: 0 – 1000
- Classification Range: 0.75-> 0.85-> 0.95
- Rename the method as *Quantification_inf_100µm*.
- Scan Classification Ranges: 0-150; *150-300; 300-450; 450-600; 600-750, 750-1000*

-Use the mouse wheel to zoom in and click and drag to adjust the view

-Click + *Select Area*

-*Draw ONE box* on the sample area following the coordinates of the random smpling matrix of the three squares (Figure 3).

- ***!! Note: according to the Screw holder, be careful to use the good coordinates !!***

-*Double-click in the middle* of the box to focus

-Click on *play* ▶

-*Note the particle number* shown in a new tab after the completion of scanning.

-Launch *the IR scanning mode.*

-Repeat the same steps for the other six random squares.

-Export the particle data in .csv

- Note: Each area will have a code from A to G.

SCREW I

1 62.5 ; 5.5	2 62.5 ; 8.1	3 62.5 ; 10.9	4 62.5 ; 13.6	5 62.5 ; 16.2
6 59.7 ; 5.5	7 59.7 ; 8.1	8 59.7 ; 10.9	9 59.7 ; 13.6	10 59.7 ; 16.2
11 56.8 ; 5.5	12 56.8 ; 8.1	13 56.8 ; 10.9	14 56.8 ; 13.6	15 56.8 ; 16.2
16 54.3 ; 5.5	17 54.3 ; 8.1	18 54.3 ; 10.9	19 54.3 ; 13.6	20 54.3 ; 16.2
21 51.7 ; 5.5	22 51.7 ; 8.1	23 51.7 ; 10.9	24 51.7 ; 13.6	25 51.7 ; 16.2

SCREW II

1 22.4 ; 5.5	2 22.4 ; 8.1	3 22.4 ; 10.9	4 22.4 ; 13.6	5 22.4 ; 16.2
6 19.7 ; 5.5	7 19.7 ; 8.1	8 19.7 ; 10.9	9 19.7 ; 13.6	10 19.7 ; 16.2
11 17 ; 5.5	12 17 ; 8.1	13 17 ; 10.9	14 17 ; 13.6	15 17 ; 16.2
16 14.3 ; 5.5	17 14.3 ; 8.1	18 14.3 ; 10.9	19 14.3 ; 13.6	20 14.3 ; 16.2
21 11,6 ; 5.5	22 11,6 ; 8.1	23 11,6 ; 10.9	24 11,6 ; 13.6	25 11,6 ; 16.2

Figure 3: Matrix of the smaller squares with the ID number of each square (in the center) and the coordinates of each square (at the bottom)

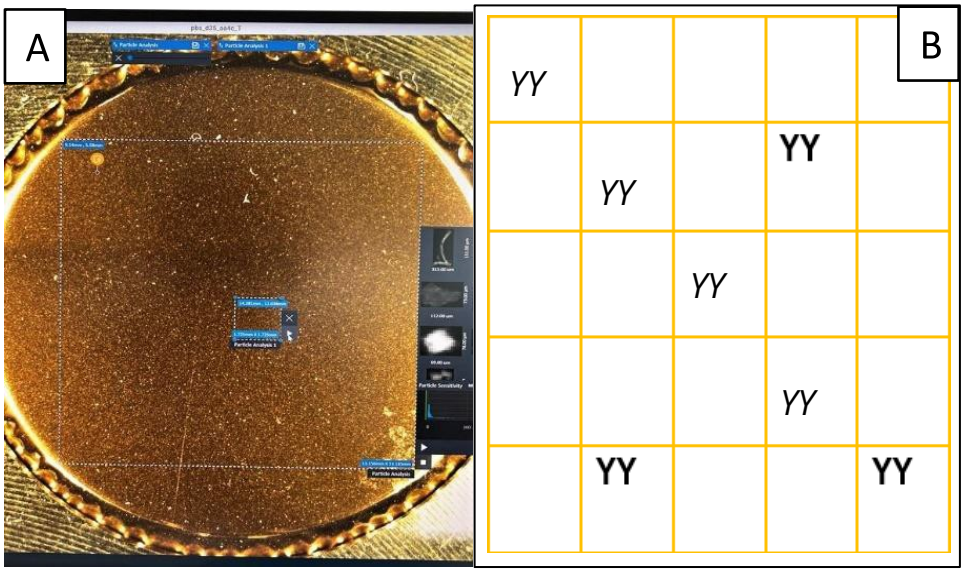


Figure 4: (A) Real image of the software with the large square in the center of a smaller court. (B) Seven random sampling squares for visible image particle analysis and IR characterization

References

Agilent Technologies. 2023. Best Practice for On-Filter Analysis of Microplastics Using Agilent 8700 Laser Direct Infrared Chemical Imaging System. 5994-5467EN, USA. www.agilent.com/chem/8700-ldir

A.3 Microalgae bioassay

This protocol was developed under the supervision of the Ph.D. Student Edgar Dusacre. It was validated by Pr. Jérôme cachot, Pr. Miren P. Cajaraville. This protocol is inspired by the OECD Guideline N°201 (OCED/OCDE, 2011).

Material

-Beckman Coulter Z2 Cell and Particle Counter (Beckman Coulter, Inc, USA)

-Incubator (Pol-Eko ST 2)

-5,000 µL tips filter

-1,000 µL tips with filters

-200/100 µL tips with filters

-Eppendorf 1.5 mL

-Rounded Eppendorf 2 mL

-Long tail glass beakers (25 mL)

-Falcon 50 mL

-Supports for Eppendorf and Falcons

-96-well plastic microplate with black walls

-Neubauer chamber

-25mL, 10 mL and 5 mL volumetric pipettes

-Conical flask 20 mL and 10 mL

-Glass petri dish base (Ø 35 mm)

-Guillard's (F/2) Marine water Enrichment Solution (50x) (G9903-500mL).

-Refractometer for salinity (Zuzi HPF012 or HPF005)

-pH paper scale 1 pt and 0.2 pt

-Micro pH sensor (Hanna instruments HI1330B)

-1L and 250 mL glass media bottle

-Incubator with temperature control and light.

-Orbital Shaker.

-Round LED Light: 20.5 W, 220-240V, 50/60Hz, 540 mA, 2,600 lumen, Ø 205 mm, 4,000 kelvin, light angle 110°, intensity controller (20%).

Cleaning method

-All material that contains solutions, microalgae (µA) culture, water, and seawater or artificial seawater must be cleaned with the following process.

1. Cleaned with a bottle brush and rinse with tap water.
2. Rinse three times with ethanol
3. Soak the material for at least 2 hours in a sodium hypochlorite solution (40 g Cl/L).
    - Add 0.25 mL of Bleach per liter of Elix Water to a plastic bucket
    - Soak the material. Be careful that all materials are filled with the solution without air bubbles inside.
    - Aerate everything with the air network for 10 minutes.
    - Remove the aeration and cover the bucket with a tap or cellophane.
    - Let soak for at least 2 hours.
4. Empty the bucket and the material
5. Under a fume column, rinse the material with Hydrochloric acid (37%, 10.2 mol/L). Rinse all the walls of the glass vials thoroughly and leave for a few minutes for the acid to take effect.
6. Rinse the material 4 times with Elix water.
7. Cover the material with a cap or with aluminum paper.
8. Autoclave the material (120°C for 30 min).
9. Dry the material in a drying oven (minimum 60°C)
10. Store the material in a dry and clean site.

-The incubator and orbital shaker that will be used must be cleaned with ethanol (70%) and Bleach.

Incubator setting

-The incubator must be set at 22°C. Be careful that the air temperature is often higher than the water temperature (+/- 2°C difference).
-Place the incubator at 24°C to get water at 22°C.
-LED light must be installed to obtain an average surface power of 96±3 µmol/s/m^2.

- This value is obtained by measuring the power flux density at the orbital shaker plate at different points (minimum of 9 equidistant points).
- In the case of the plastifo research project, 4 round LED lights have been installed. Some areas of the orbital shaker plate were condemned because the surface power was too strong or weak.

Incubator setting

-Elix water used for the microlaga culture and the artificial seawater preparation must be previously filtered with a mixed cellulose ester membrane (MCE) filter (mesh 0.22µm).

-The pH must then be buffered between 7.3 and 7.8 with HCL or NaOH, previously autoclaved.

-Finally, it must be autoclaved and stored at 4°C This prepared Elix water will be called treated Elix water (tEx-W).

Beckman Coulter Z2 Cell and Particle Counter: settings and use

-Counting spherical microalgae cells with this method takes around 1h for 18 samples.

-Turn ON the machine.

-If the machine has not been used for a long time or was used by another person before you, clean or rinse the stirrer, the glass pump, and the metallic piece.

-Fill the machine recipient (glass in the shape of a cup) with fresh filtered seawater (Ø 0.22 µm).

-Place the cup on the support and immerse the stirrer, the glass pump, and the metallic piece in it.

-In the “fonction” settings, choose “Flush aperture” and start flushing one or two times.

-In the “Set-up” settings, set the analyzed volume at 1 mL or 0.5 mL, the upper size at 6.5 µm and the lower size at 2.5µm.

-Press the “Start/Stop” button to start the first analysis of the particles. The background particle number should not exceed 500 particles.

-Replace the cup with your sample.

The best is to analyze large volumes to be able to activate the stirring.

-The cell concentration should not exceed $1x10^6$ cells /mL; otherwise, the machine could display “a too high concentration”. If you expect a high sample concentration, take a dilution step beforehand.

-If you cannot work with huge volumes, you can work with smaller volumes (minimum 15 mL) without using the stirring. If so:

- Purchase or ask for 25 mL long tailed glass beakers or other containers suitable for working with a minimum of 15 mL and large enough for the glass pump and metal piece to fit inside.
- Make a dilution of your µA culture to obtain a final volume of 15 mL. Use fresh filtered (Ø 0.22 µm) seawater for the dilution. The cell concentration of the dilution should not exceed 200 000 C/mL to avoid machine precision errors. Concentrations below 100,000 C/mL are recommended.
- Make a seawater control to measure the particles between 2.5 µm and 6 µm intrinsic to seawater, even if it has been filtered (background contamination).

- Realize three or four measures of the seawater control. The mean value should be subtracted from the treatment's values.
- Mix the solution using circular movements.
- Place the beaker on the support so that the glass pump and the metal piece fit into the beaker.
- Start the analysis. The volume of the solution cannot be below the pump notch.
- Make three measures for each sample. Up and down the support 3 times between each measure to mix the solution.
- Between each sample, rinse the glass pump and the metal piece with clean seawater.

Microalgae culture

Artificial seawater preparation

-Prepare a saturated artificial seawater (ASW) solution.

- Purchase or ask for Aquamedic Reef Salt
- Prepare 200 mL of a solution between 340 psu in a 250 ml media bottle.
    - Use the tEx-W
    - Add reef salt to the stored water heating at 230°C
    - Allow the salt to dissolve for 15 minutes.
    - Filter the solution with a glass fiber filter or Mixed Cellulose Ester membrane (MCE) filter (mesh 2.7 µm).
    - Measure the salinity with the refractometer. (If the solution exceeds the material possibilities, dilute the solution).
    - Generally, the salinity will be lower than expected by the calculation.
    - Repeat the process as needed by adding the new calculated quantity of reef salt.
    - Once the expected salinity has been reached, close the bottle and autoclave it. The autoclave step will tend to increase the salinity slightly.
    - Measure the salinity (under a fume column).
    - Filter the solution with a glass fiber filter or Mixed Cellulose Ester membrane (MCE) filter (mesh 2.7 µm), then at 0.7 µm, and finally at 0.2 µm.
    - Measure the salinity and write the final salinity.

-Once the Saturated ASW has been made, the ASW solutions can be prepared by dilution.

Initial microalgae culture

-Purchase or ask for a colleague a culture of *Tisochrysis lutea* or *Isochrysis galbana* microlagae. The origin, purchase date, and species of microlagae (molecular identification) must be known.

-Observe a drop of the microlagae culture with an inverted microscope (x400).

- Bacteria must only contaminate the culture in a very small amount, and contamination with other organisms with pigments must be noted. If the culture contains some

organisms that are superior to the µA size, filtration with a filter > 10 µm is recommended. However, axenic culture is not needed.

-Dilute the culture if it suits expectations.

-Keep two or three aliquots of the purchased cultured. The best is to store one in the incubator next to the work cultures but closed and very little handled, and another or two others in different sites in case of contamination of one site.

-Measure the cell concentration using a Neubauer chamber or counter particle (Beckman Coulter Z2 Cell and Particle Counter) 1.4.

-In a 20 mL conical flask, dilute the culture to obtain a concentration between $1x10^4$ and $1x10^5$ cells /mL.

-Use tEx-W, saturated ASW and F/2 medium. 20 µL of F/2 medium must be added by mL of µA culture.

-Every 24 h, measure the Cell concentration until reaching the growth plate.

-Define the concentration for which the growth plate starts.

-Verify that the Cell concentration doubles every 24 hours without a lag phase between t0 and t24. If this is not the case, repeat the process until validating these requirements.

Initial microalgae culture

-Once the microalgae culture is adapted to the incubator settings, the acute toxicity test (OECD N°201 guideline) can be run.
-Favorise the cell count using the particle counter, but fluorescence is an accepted surrogate.
-Fluorescence is measured using the "BioTek Cytation 5 Cell Imaging Multimode Reader".

- Settings: excitation wavelength 470 nm, emission wavelength 680 nm.

-If fluorescence is used, make the regression curve of the cell concentration measured by the Neubauer chamber and fluorescence:

- Start the Cytation 5 (15 minutes before to use it at least). Set the Read temperature at 22°C. Set the Read mode in fluorescence (excitation 470 nm, emission 680 nm).
- Sample 10 mL of the adapted µA culture at the end of the exponential phase (often between $1x10^6$ and $2x10^6$).
- Measure the cell concentration of this initial culture using the Neubauer chamber.
- Prepare 12 rounded Eppendorfs of 2 mL.
- Add 2 mL of the µA culture in the first Eppendorf.
- Fill the 11 others with 1 mL of ASW (34 psu)
- Make a cascade dilution (1/2). At each dilution, make at least seven up and down to mix it.
- Add 200 µL of each dilution in the 96-well microplate. Each dilution must be measured three times (3 wells).
- Measure the Fluorescence

- Using the R software (or another software), perform a linear regression of the concentration as a function of dilution. This will give a linear value for each subsequent fluorescence measurement. Linear regression must have a regression coefficient> 0.98.

-Before starting an experiment, the culture should undergo a preliminary test to perform a positive control and ensure that it reacts well. It could be done with dimethyl sulfoxide (DMSO), Hydrogen peroxide (H2O2), and Potassium dichromate (K2Cr2O7) and with a minimum of 5 different concentrations.

-Start the experiment:

- Control the pH of plastic leachate's or other contaminant with the micro pH sensor. It should be between 7.3 and 7.8.
- An experiment must have at least 3 negative controls (5 recommended) and 3 blanks if working with plastic leachates.
- Measure the cell concentration of the initial µA culture. If you work with fluorescence, use the Neubauer chamber. In contrast, if you use the particle counter, use it directly.
- Prepare each treatment in 10 mL conical flasks. The final volume should be 5 mL (to leave enough space for effective agitation). To work with plastic leachates, starting with a maximum concentration of leachate of 80% and a µA concentration of $8x10^4$ cell/mL is recommended. In this case:
- Add 4,000 µL of leachate or 4,000 µL of tEx-W for the controls.
- Add 500 µL of saturated ASW
- Add 110 µL (100 µL + 1 drop to favor growth) of F/2 medium.
- Add the calculated µA to get a final concentration of $8x10^4$ cells /mL.
- Fill with tEx-W to reach 5,000 µL.
- Mix the treatment by gently shaking the conical flask
- Cover the conical flasks with the base of the Glass petri dish (Ø 35 mm).
- Place all conical conical flasks in the orbital shaker in the incubator.

-Cell concentration measure: measure the cell concentration daily at the same hour.

- By fluorescence:
    - Start the Cytation 5 (15 minutes before at least). Set the Read temperature at 22°C. Set the Read mode in fluorescence (excitation 470 nm, emission 680 nm).
    - In a black 96-well microplate, insert 200 µL of each treatment by well. Three wells by treatments to obtain three values and calculate a mean value.
    - Insert the microplate in the Cytation 5.
    - Start reading.
    - Save the Excel file in your folder.
    - To facilitate data treatment: 1) copy and paste the result matrix below and replace the value with the sample name. 2) Copy and paste the result matrix directly into a new sheet.
    - At the end of the measures, inspect the wells using the Cytation 5 microscope.
    - Verify everything goes well.

- By particle counting:
    - For each treatment, prepare a 15 ml long tail beaker following the method described in the part titled “Beckman Coulter Z2 Cell and Particle Counter: settings and use” of this protocol.
    - If you work with plastic leachate, take the measure of the plastic particles in each leachate to subtract them from your cell count values.

-Data management:

- According to the method used to count the cells, manage the data to get the daily concentration. The means should be used for the three measures of each treatment. However, the mean of the replicate should be done in the end when calculating the growth rate. Use the (OECD/OCDE, 2011) to know the requirements and the formula for growth rate and growth inhibition.

References

OECD/OCDE. 2011. OECD GUIDELINES FOR THE TESTING OF CHEMICALS. Freshwater Alga and Cyanobacteria, Growth Inhibition Test.

A.4 Fish larvae bioassay

This protocol was developed under the supervision of the Ph.D. Student Edgar Dusacre, assisted by the research ingenior Christelle Clerendeau. It was validated by Pr. Jérôme cachot, Pr. Miren P. Cajaraville. This protocol is inspired by the work of Bedrossiantz et al. (2023).

Material

-DanioVision System (Noldus, Wageningen, The Netherlands): DanioVision observation chamber equipped with a near-infrared camera, a panel of LED for light simulation, a vibrational stimulus mechanism (tapping), a Temperature Control Unit, and a computer running the analysis software EthoVision XT (version 17.0.1630)

-Near-infrared camera (Basler acA1300–60gm) mounted on a stereomicroscope (Olympus SZX2-TR30)

-Open-source software Open Broadcaster Software Studio

-DanioScope™ software Version 1.2 (Noldus, Wageningen, The Netherlands)

-Climate chamber (Snidjers Scientific, Tilburg, The Netherlands)

-Filtration unit with pump

-Methyl-cellulose filters (0.22 µm mesh)

-Laboratory gloves

-Filtered ethanol (70% EtOH) (0.22 µm)

-Filtered deionized water (0.22 µm)

-Beakers (5 mL, 50 mL, 100 mL)

-Glass pipette (10 mL and 25 mL)

-Petri dish (70 mm)

-Orbital shaker

-Thermometers

-ERS (Egg rearing Solution) 100X medium

-ISO borosilicate glass bottles 1 L

-Plastic falcons (50 mL and 15 mL)

-Plastic well-paltes (48 wells and 12 wells)

Ordering Oryzias latipes fish larvae

-Make an order with INRAE in Rennes by contacting Mr. Gourmelen (Guillaume.Gourmelen@inrae.fr) at least one month in advance.

-Reception of a maximum of 600 eggs. Approximative hatching success at the first hatching peak of about 450 eggs (75%). In winter, ask the Inrae team to put the eggs in a specialized box and to add some heaters to prevent excessive heat loss.

Preparation of the reception of the eggs

-Switch on the climate cabinet at the end of the day before reception (Snijders Scientific B.V., Economic Delux, Model ECD10E, ISO9001).

-Switch to the circadian cycle of the machine (light on 8 a.m.-9 p.m. / light off 9 a.m.-8 p.m.). Switch on all the light racks.

-Depending on the desired egg hatching time, set the temperature between 23°C and 28°C. The best temperature is 26°C (hatching eight days after fertilization).

- **Note: The temperature should be set according to the SSP1 (day) and SSP2 (night) programs of the climate chamber. Set the same temperature for the day and night. Otherwise, adapt it to your liking.**

-To maintain a consistent humidity level, add a container with tap water at the bottom of the climate chamber without obstructing the central hole.

-Install an orbital shaker on a grid, leaving another grid available.

-At least 4 days before egg reception, prepare the ”ERS 1X medium” with a pH between 7 and 7.6 using ERS 100X.

- Recipe of the ERS (Egg rearing Solution) 1X medium:
    - In 800 mL of deionized water add:
    - 10 ml of ”ERS 100X”
    - 4g of NaCl (dissolve it completely)
    - Fill the volume with 190 ml of deionized water
    - Autoclave the solution at 120°
    - Indicate the dat
    - Store at 4°C

-Store one bottle in the climate chamber.

-Turn on the air conditioning in the room at 19°C-20°C, especially in summer.

Reception of the eggs

-Most of the time, the eggs arrive at 11 am. Manage the eggs in the 4 hours following the reception. Transfer could be done directly if they are at room temperature (between 18°C and

26°C). If they are outside the temperature range, write it and wait for them to be at room temperature.

-Switch on the orbital shaker at 32 rpm.

-Transfer the eggs to 70 mm sterile glass Petri dishes.

- Method:
    - Discard most of the methylene blue medium. Keep only 5 mL(1/4).
    - Transfer the egg and the rest of the methylene blue medium to the petri dish.
    - Rinse the falcon with 5mL of ERS 1X medium.
    - Add 10 mL of ERS 1X medium in the Petri dishes

-Observe the eggs under a binocular magnifier. If they are dead (mostly the Chorion is blue), extract the egg using a plastic pipette cut at the end and throw it in the bin falcon.

-Transfer the eggs that appear uncertain in another new petri dish called “to verify”.

-Write the remaining number of eggs on each petri dish cover.

-Write in your laboratory notebook the daily number of eggs for each petri dish.

-Place the each petri dish in the orbital shaker in the climate chamber.

Daily management

-Every 24 hours, check every Petri dish with the binocular magnifier.

-Extract the dead eggs and write on the petri dish cover and in your lab notebook the new number of eggs remaining.

-Replace 1/2 of the old medium with fresh ERS 1X medium.

-Repeat this process for each Petri dish and every 24 hours until the hatching peak.

Preparation of the larval exposure

-Each initial condition to analyze should be replicated 3 times. For each replicate, a minimum of 16 larvae should be studied for a total of n = 48 per condition.

-Each condition must be named with a letter from ”g” to ”n” (use ”c” for the control). Add numbers from 1 to 3 for the replicates.

-Fill in a table of that type:

Table 1: Table of leachates samples for Behavior and Heart Rate experiment. Malfo = Malformation. Swim = swimming

Conditions	Replicates	ID	Mortality 24 h	Swim incapacity 24 h	Malfo 24 h	Mortality 48 h	Swim incapacity 48 h	Malfo 48 h
Condition 1	1	g1	...	...	...	...	...	...
Condition 1	2	g2	...	...	...	...	...	...
Condition 1	3	g3	...	...	...	...	...	...
Condition 2	1	h1	...	...	...	...	...	...
Condition 2	2	h2	...	...	...	...	...	...
Condition 2	3	h3	...	...	...	...	...	...

-Prepare a scheme for 48 well-plate with the distribution of the ID conditions (Figure 1).

- In each well plate, all IDs must appear at least 1 time. This should be repeated in several 48-well plates, allowing 16 larvae per ID.
- Do not forget that one condition must be a control, genetic control is better if it comes only from one parent couple (24 larvae) and another positive control if needed (24 larvae).

-Remember to identify the 48 well plates and indicate in which Daniovision machine it will go to.

-Print a scheme with the exact dimension of the well plate to be used when the larvae are placed in (Figure 2).

-According to the sample preparation, set the number of larvae you need by replicates and add, if possible, four as a precaution if there is mortality or malformed ones (20 by replicate, minimum 18).

Opérateur : Date : Plaque : 1
Cycle : T° : Série : 2
Début acclimation : Machine n° : Début comportement :
Fin acclimation : Fin comportement :

	1	2	3	4	5	6	7	8
A	Dmso	Dmso	Dmso	Dmso	Benzo	Benzo	Benzo	Benzo
B	g1	g2	g3	h1	h2	h3	i1	i2
C	i3	j1	j2	j3	k1	k2	k3	l1
D	l2	l3	m1	m2	m3	n1	n2	n3
E	g1	g2	g3	h1	h2	h3	i1	i2
F	i3	j1	j2	j3	k1	k2	k3	l1

Opérateur : Date : Plaque : 2
Cycle : T° : Série : 2
Début acclimation : Machine n° : Début comportement :
Fin acclimation : Fin comportement :

	1	2	3	4	5	6	7	8
A	Dmso	Dmso	Dmso	Dmso	Benzo	Benzo	Benzo	Benzo
B	l2	l3	m1	m2	m3	n1	n2	n3
C	g1	g2	g3	h1	h2	h3	i1	i2
D	i3	j1	j2	j3	k1	k2	k3	l1
E	l2	l3	m1	m2	m3	n1	n2	n3
F	g1	g2	g3	h1	h2	h3	i1	i2

Opérateur : Date : Plaque : 3
Cycle : T° : Série : 2
Début acclimation : Machine n° : Début comportement :
Fin acclimation : Fin comportement :

	1	2	3	4	5	6	7	8
A	Dmso	Dmso	Dmso	Dmso	Benzo	Benzo	Benzo	Benzo
B	i3	j1	j2	j3	k1	k2	k3	l1
C	l2	l3	m1	m2	m3	n1	n2	n3
D	g1	g2	g3	h1	h2	h3	i1	i2
E	i3	j1	j2	j3	k1	k2	k3	l1
F	l2	l3	m1	m2	m3	n1	n2	n3

Opérateur : Date : Plaque : 4
Cycle : T° : Série : 2
Début acclimation : Machine n° : Début comportement :
Fin acclimation : Fin comportement :

	1	2	3	4	5	6	7	8
A	Dmso	Dmso	Dmso	Dmso	Benzo	Benzo	Benzo	Benzo
B	g1	g2	g3	h1	h2	h3	i1	i2
C	i3	j1	j2	j3	k1	k2	k3	l1
D	l2	l3	m1	m2	m3	n1	n2	n3
E	g1	g2	g3	h1	h2	h3	i1	i2
F	i3	j1	j2	j3	k1	k2	k3	l1

Figure 1: Scheme of the 48 well-plates with good dimensions.

Experimentation 09/12/2022 DMSO FN new larves medaka
Plaque 1 - 4

1	1	2	3	4	5	6	7	8
A	Dmso	Dmso	Dmso	Dmso	Benzo	Benzo	Benzo	Benzo
B	g1	g2	g3	h1	h2	h3	i1	i2
C	i3	j1	j2	j3	k1	k2	k3	l1
D	l2	l3	m1	m2	m3	n1	n2	n3
E	g1	g2	g3	h1	h2	h3	i1	i2
F	i3	j1	j2	j3	k1	k2	k3	l1

2	1	2	3	4	5	6	7	8
A	Dmso	Dmso	Dmso	Dmso	Benzo	Benzo	Benzo	Benzo
B	i3	j1	j2	j3	k1	k2	k3	l1
C	l2	l3	m1	m2	m3	n1	n2	n3
D	g1	g2	g3	h1	h2	h3	i1	i2
E	i3	j1	j2	j3	k1	k2	k3	l1
F	l2	l3	m1	m2	m3	n1	n2	n3

Figure 2: Scheme of the 48 well-plates with the distribution of the ID conditions.

Exposure of the larvae (48 h)

-If the exposition medium must be diluted. One day before the hatching peak, prepare 2.5L of mixed water medium: 1/3 tap water/ 2/3 deionized water. The pH should be between 7 and 7.6.

-Put the mixture in the climate chamber and put a clean air pump with a bubble system to oxygenate the medium and evaporate the Chlorine.

-On the morning of the exposure, set the temperature room at the desired temperature.

-After the hatching peak (at least 3/4 of the eggs), the 48-hour exposure can start.

-Prepare one clean glass beaker for each replicate.

-Prepare the exposure medium for each replicate.

- In the context of the Plasfito Research project:
  - 100% DMSO extracts (concentration plastic eq. 0.33 g/mL) are diluted 1000 times.
  - Water leachates (mesoplastic equivalent concentration 10 mg/mL) are tested at 100%.

-Add a minimum of 16 healthy larvae (avoid the malformed and moribund ones) to each beaker using a plastic pipette cut at the end. Add a minimum possible of ERS 1X medium in the beaker (larvae often leave the pipette by themselves when they are not too high in the pipette). When all beakers have 16 larvae, start adding supplementary larvae.

-When a beaker is filled, put it back in the orbital shaker's plate (32 rpm) in the climate chamber to avoid long-term environmental change.

-When all the beakers are filled, note the exposure start time.

-After 24 h of exposure, control each beaker, count and extract dead larvae (Table 1). Replace 1/2 of the medium with the prepared exposure medium with the correct dilutions (if needed) made the same morning.

-After 48 h hours, control each beaker, count and extract the dead larvae. Count the larvae with swimming incapacity and the malformed ones (Table 1).

Fish behavior

Preparation of the Ethovision XT software

-Two days before the fish behavior experiment starts, configure the Ethovision XT software and verify that the Daniovision and the Noldus Cooker are working.

-To configure the software, follow the next steps:

- Start the Daniovision 15 minutes before opening the software.
- 15 minutes later, open the Pylon Viewer software.
- Check that the computer recognizes the camera well and that the ethernet connection works well.
- If everything is working well, continue
- If there is a problem:
    - Check if the Ethernet cable is well connected and if there is light at the level of the computer Ethernet cable. If not, it means that the cable is not well connected, the cable is broken, or the IP configuration is not working. In that case, contact Edgar Dusacre or Christelle Clerendeau.
    - If the light is on, open the Pylon IP configurator. Change the camera's IP to get the same connection as ""Ethernet2". For the subnet, enter the one proposed by the software (bottom right).

- When the camera is detected, close the pylon viewer software and open the Ethovision XT one.
- Create a new experiment using a template from the Plasfito research project.
- Open "Experimental Settings", select "Live Tracking", and select the Basler camera. Set the resolution to 1280x820. Set the frame rate at 30.
- Verify the Arena setting, but with the Plasfito template, you should have a good configuration for 48 well plates.

- Open “Trial Control Settings”. The Plasfito trial comprises 20 minutes of acclimatization in the dark, 51 tapping spaced 1 second apart (force 8/8), 10 minute rest in the dark, and 10 minutes of light (100%), and 10-minute rest in the dark. If you want to adjust it for your project, everything is possible. This routine follows the work of Bedrossiantz et al. (2023). This routine allows for calculating the vibrational startle response (VSR) and habituation, visual motor response (VMR), and basal locomotor activity (BLA) (plus other variables if needed).
- Open “Detection settings.” Fill a 48-well plate with deionized water and install it in the Daniovision chamber. Open the “Advanced” panel, grab a background image (Method A), and save the configuration.
- Open the “Trial List.” And follow the instructions:
    - For each machine, set a good number of trials and try to get the number equal to the well plate number scheme you prepared before.
    - For “Trial,” “Trial” and “Detection settings” select the configuration you just did.
    - Create a variable “Conditions” and add each condition you have.
    - Create a variable “ID” and add each ID corresponding to the condition.
    - Create a variable “Bino” to write any malformation that you could observe during the well plate preparation. Create the levels: “Onbelly”, “Malfo”, “Nomove”, “Small”, and “NA”. Add as many variables as you want.

- Open “Data Profiles” and you will see all the parameters calculated in the context of the Plasfito research project. It allows a quick and tidy data export after the acquisition.
- Open “Analysis profiles”. The three existing ones are sufficient to calculate all the parameters needed. Change the analysis profile according to the used “Data profile”.

Fish behavior experiment

-The evening before the Fish behavior experiment, turn on the air conditioning of the Daniovision room at 26°C if the DanioCoockers are not working.

-After 48 hours of exposure, prepare the well plate for the behavior of the fish using the Noldus’ DanioVision Observation Chamber (DVOC-0041) equipped with a Basler acA1300-60g camera and Ethovision XT software (version 17.0.1630).

-The morning of the fish behavior experiment:

- Set the room temperature to your incubation temperature.

-Place the well plate in the prepared schemes.

-Use the scheme and the ID to fill the well plates.

-Fill two well-plates at the same time.

- Use a 1 mL pipette with cones cut at the end.
- Take each larva with 500 µL of medium and place in the well with the name of its treatment.

- If you have a benzocaïne positive control:
    - 5 minutes after starting to fill the plate, run a 15-minute benzocaine bath (30 mg /L) for the number of control positive larvae you need for the plates.
    - After 15 minutes, rinse the larvae quickly in mixed water medium (1 minute).
    - Add the larvae to the well plates.

-When the well-plates are filled, cover them, put back the beakers in the climate chamber, and bring the well-plates to the Daniovision room.

-Put each well plate in the good Daniovision Chamber.

-Start the acquisition of the two Daniovision Chambers at exactly the same time to avoid a double noise effect of tapping because of a time lag between the two machines.

-30 minutes before the routine ends, prepare the next two well plates.

-When acquisition is made, remove the well-plates, replace them with the new one, and launch a new acquisition.

-Put the well-plates back in the climate chamber for the Heart rate.

-Repeat this process for all the well-plates that you have to analyze.

Fish behavior data analysis

Using video files and data, verify any “outlier” individual that may need to be withdrawn from the data due to its very different behavior compared to other individuals of the same replicate.

-Using the “Data Profiles” and the “Analysis profiles” export the data in a good format and import them into the software you want to use.

-In the Plasfito Research project context, a semi-automatic RStudio (version 2022.12.0+353) script was created if needed. https://github.com/EdgarDusacre/Plasfito_public/tree/c56a2eece7b5a3dc9c1d9b37196a12f411aec0ec/Protocols/Bioassays/Fish%20larvae

Heart rate

Methylcellulose preparation

-For the heart rate experiment, 12 well-plates are used. Each replicate will use two wells.

- Therefore, to know how many well plates to fill with two drops of methylcellulose (MCE) (3%) do: (nb_condition x3 x2)/12.

-To make 240 ml of MCE:

- Put 240 mL of phosphate-buffered saline (PBS) in the microwave for 2 minutes (max power).
- Add 7.2g of MCE.
- Stir for 5 minutes with a magnetic stirrer.

- Divide the mix into 80 mL aliquots into three 100 mL glass beakers.
- Store them for 30 minutes at 4°C.
- Repeat the stirring and cooling process three times.
- Store the Glass beakers for 48 hours.
- Divide each beaker into two 50 mL falcon tubes.
- Centrifuge for 10 minutes at 3G and room temperature to reduce the number of air bubbles.

-Fill the 12 well plates according to the number of well plates you need for the analysis.

-Put the other in the fridge.

-Divide the remaining MCE (3%) into 15 mL falcon tubes.

-Store 15 mL falcon tubes in the freezer at minus 20°C.

Tricaïne preparation

-To decrease the movement of the larvae, without affecting the cardiac rhythm, the larvae should undergo a tricaïne bath before being placed in the MCE.

-To prepare the solution Tricaine at 170 mg/L:

- Prepare a stock solution of 4 g/L using tricaine powder.
- Adjust the pH to 7.2 using NaOH. Be careful because pH jumps are sensitive.
- Sample 2.125 mL of the stock solution and add 50 mL of mixed water medium.
- Store it at 4°C to use in the following days.
- Store the rest at -20°C.

Heart rate analysis

-One day before the heart rate experiment, it must be controlled that the computer, the OBS studio software, and the Danioscope software work well and that the camera (Basler acA1300-60gm) of the stereomicroscope (Olympus SZX2-TR30) is well recognized.zed.

-The morning of the experiment: open *Pylon Viewer*

-Check if the camera is recognized and take a picture to verify.

-If everything works without an error message, skip the next step.

-If no:

- Click right, *pylon IP configurator*.
- Click right on the *camera line*. Click "*Assign temporary IP"*
- Enter the end of the IP suggested on the bottom left of this page;
- Click *Refresh*.
- Close this window.
- Try again to take a picture.

- If it works, close the Pylon viewer. If not, try the last few steps again or contact Edgar Dusacre

-Open the *OBS studio* software.

-Look in the *Sources* panel. You will see a line with "*Basler_bino"*

-Click right on it and open the settings.

-Normally, if the stereomicroscope is ON, you can see the image. If not, make sure you have turned on the light, pulled the bar to allow camera vision, and opened the diaphragm.

-Click on "*Fichier, Paramètres, Sortie"*, look at the panel "*Enregistrement",* click "*Parcourir"* and choose where you want to save your video files that you will capture during the experiment.

-Configure your capture time (45 seconds or 60 seconds...):

- Click on *"Outils"* (top left)
- Click *"Minuterie des sorties"*
- Set the desired video time on the line "*Arrêter l'enregistrement après:*".
    - Note: Set the time in the "seconds" area and maintain the other at 0.
- Click "*Fermer"*

-Do not close the software until your experiment.

-Between 18 and 24 individuals should be used to study heart rate. The objective is to get a final number of individuals equal to 15 by condition. Remember that some individuals will not be treated during the data analysis.

-In the case of the Plasfito Research project, eight individuals were analyzed by replicate (Ntot = 24).

-Before starting the analysis, label the 12 well plates. Each plate must be numbered. Each well must be named on the cover. The names correspond to the ID of each condition and replicate. In the context of the Plasfito research project, two replicate wells with four larvae were used.

-Prepare as much as 5 mL glass beaker needed if conditions are replicated and label them with the ID.

-Fill each beaker with 3 mL of tricaine (170 mg/mL).

-One hour after the first behavior analysis is finished, the heart rate analysis can start.

-Make one condition by one condition (if you are trained and feel comfortable, you can do it two by two or three by three if you are fast):

- In the 48 well plates, take the needed number of larvae using a plastic pipette with the cut end and place them in the corresponding 5 mL glass beaker with tricaine.
- Wait between 2:00 and 2:30 minutes.
- *If you want to do two or three different conditions at the same time: At the time you put the first four larvae in one condition, start a new condition. It is recommended to leave at least 30 seconds between the two conditions to have the time to put the larvae of the first condition in the MCE before the second condition time ends.*

- After 2:00 min, put four larvae by the well in the micro-plate.
- Remove the tricaine supernatant using a p200 micropipette. To do this, take a volume from the surface of the MCE. Do not hesitate to discard the cone frequently, as it becomes blocked by the viscosity of the MCE. Remove volume from several places. Repeat this at least three times. Sometimes, more pipetting is required if a lot of volume was added when the larvae were deposited.
    - **Note: the more volume stays, the poorer the quality of the videos.**
- Continue until all wells you want to analyze simultaneously are filled. A half plate simultaneously (i.e., six wells) is recommended.

-Bring the plate to the Danioscope room **(the light of the room must be ON).**

-Acclimate for at least 5 minutes the plate under strong white light.

-Do not use the grey or black filter of the stereomicroscope. Set the light level of the stereomicroscope to a medium intensity (1 or 2).

-Using a 100 µL cone, manage to place the larvae on their back or side (to see the heart), close to each other, in the same layer in the MCE (for a good focus), and centered in the middle of the well.

-Do a good focus looking on the computer screen. All larvae must be seen at the same time.

-Start a video capture: click *"Démarrer l'enregistrement"* (bottom right).

- **Note: sometimes there is a little issue and the first click only starts a try of 1 second. Click a new time on "*Démarrer l'enregistrement*" and it will work.**

-Have a look at your folder. You will see two files. One of the trial (1s) and your video. Delete the trial (1s video) and rename your video.

-Repeat this process until you get all the video you need.

-If a plate is being treated while another one is ready, put it under high-intensity white light in the same room.

-Close the *OBS Studio* Software when your experiment is finished.

Video conversion

-Convert your video file to be able to analyze them in the Danioscope software:

- Download the *Any Video Converter* software
- Open the *Any Video Converter* software
- Click the wheel settings icon (top right)
- Select the folder where you want to save your converted videos. Click *Appliquer.*
- Add all the videos you want to convert: by sliding them from the folder where they are or by clicking *Ajouter une video*.
- Click on the the *FPS* drop-down menu.
- Select *"***25***"*
- Click *Options Audio*, select *Désactiver le son*.

- Click *Commencer*

-When finished, close the software.

Heart rate data analysis

-To analyze the converted video registered using the *OBS studio* software, use the *Noldus Danioscope* software (version 1.0).

-Import the video into the software.

-In the Experiment setting select only *"Cardiology".*

-In the *Independent Variable* create three variables:

- Conditions (add all your conditions)
- ID (add all your ID)
- Hole (1, 2)

-Open the panel *cardiology.*

-Import all the videos.

-Select the *first video*.

-Click on *Heart rate*, and *zoom* at 150%.

-*Draw a small area* on the right or left heart valve (try to draw the area on the same valve every time for more reproducibility).

-For each individual, draw three areas at three different times. If you have a 60s video or two times if you have a 45s video:

- Area one: from 0.004 to 20 s
- Area two: from 21 to 40s
- Area three: from 22 to 59s

-If the larvae move too much or if there are image jumps in the video, reduce the video time to a minimum of 15 s.

-Click on *Acquire All*. If the heart rates are not homogeneous or display 0.01 or 0.03 and do not correspond to what you see on the individual, manage *the area* and *the time range* to get better results. If not possible or if the fish died, just remove this individual.

-Repeat this process for all the videos.

-In *statistics* you can see a data frame with all the results. Export the statistical data frame (not the Raw one). In this data frame, you will have the mean value of the two or three measurements you did for each individual. You don't need to consider the standard deviation of this value. You will use the one you will calculate when you do the mean of the replicates.

-Export the data. In the Plasfito Research project context, a semi-automatic RStudio (version 2022.12.0+353) script has been created if needed.

https://github.com/EdgarDusacre/Plasfito_public/tree/c56a2eece7b5a3dc9c1d9b37196a12f411aec0ec/Protocols/Bioassays/Fish%20larvae

References

Bedrossiantz, J., Faria, M., Prats, E., Barata, C., Cachot, J., & Raldúa, D. 2023. "Heart Rate and Behavioral Responses in Three Phylogenetically Distant Aquatic Model Organisms Exposed to Environmental Concentrations of Carbaryl and Fenitrothion." Science of the Total Environment 865(161268). https://doi.org/10.1016/j.scitotenv.2022.161268.

Annex B. UV chamber description

UV-accelerated weathering chamber for low-temperature material degradation: technical specifications

Edgar Dusacre[1,2], Christian Portier[1] and Yannick Geerebaert[1].

[1]Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805, F-33600 Pessac, France
[2] CBET Research Group, Dept. Zoology and Animal Cell Biology; Faculty of Science and Technology and Research Centre for Experimental Marine Biology and Biotechnology PiE, University of the Basque Country UPV/EHU, Basque Country, Spain

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October 18, 2024

Contents

1 Introduction 3
2 Operating instructions 3
3 Physical and electrical characteristics 3
4 LED panels 3
5 Power density 4
6 Materials 5
7 Electrical diagram 6
8 References 6

Version 03





Figure 1: Overall view of the UV chamber.

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Version 03

1 Introduction

This UV chamber is designed to irradiate materials to simulate their accelerated degradation using high surface power light (several hundred W/m^2) at low temperatures (around 20°C). A study conducted by a team from the University of Le Mans (IMMM Mixed research Unite, Le Mans, France) using an incandescent UV lamp served as the basis for defining its characteristics.

2 Operating instructions

The typical use of this chamber is at half-power continuously for 10 to 60 days.

Ultraviolet radiation is invisible to the eye but not without danger, so it is recommended to wear dedicated protective glasses. The surface of the chamber can reach a relatively high temperature, so be careful of the risk of burn.

A safety feature prevents UV lamps from operating with the door open. To turn on the UV lamps, close the door and press the "ON" button. In the event of a momentary power outage, the chamber does not restart automatically; "ON" button must be pressed again.

The fans and electrical outlets inside the chamber operate without the door safety feature.

The chamber is relatively heavy; to move it, do not carry it by the electrical panel !

3 Physical and electrical characteristics

- Dimensions (LxPxH) : ~ $630 \times 530 \times 445$mm
- Usable dimensions (LxPxH) : ~ $430 \times 400 \times 240$mm
- Weight : ~ 21kg
- Main power supply: $100 - 300V_{AC}$, $47 - 63$Hz, 1A
- Fuse: 5x20mm, 4 A time-delay

4 LED panels

The LED panels were custom-made by Agrotek with the following characteristics:

- Mix U.V. : ratio UVA:UVB = 79 : 21%
- UV-A, SMD 3030, 0.8W, quantity 100, relative spectral distribution : 365nm [360nm-370nm], manufacturer LEDESTAR, reference : LDR-3030TTAV365-V0V A0H0
- UV-B, SMD 3535, 1.2W, quantity 25, relative spectral distribution : 310nm [290nm-350nm], manufacturer LEDESTAR, reference : LDR-3535CNZV310-M0
- Driver with power dimming in 20% increments, MeanWell XLG-150-H-AB

Yannick Geerebaert - Univ. Bordeaux, CNRS, Bordeaux INP, EPOC, UMR 5805 yannick.geerebaert@u-bordeaux.fr

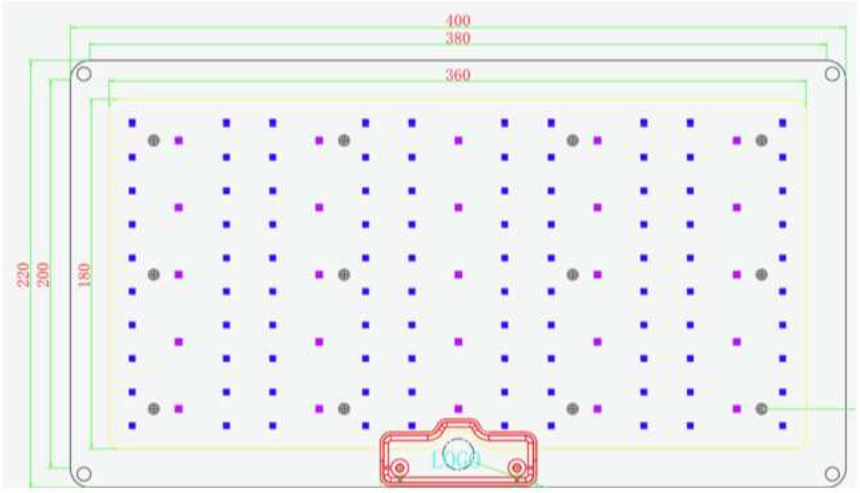

Figure 2: Distribution of UV-A and UV-B LEDs on the panels. Blue squares represent the UV-A LEDs, while purple squares represent the UV-B LEDs.

Measurements carried out on a driver panel assembly at ambient temperature *:

Pos.	R_{add}(kΩ)	P_{line}(W)	V_{led}(V)	I_{led}(A)	P_{led}(W)	η_{elec}(%)	T_{in}(°C)	T_{rise}(°C)
0	0	0	4,1	0,000	0	-	20,8	0
20	47	13,4	37,6	0,325	12,22	91,19	23,9	3,1
40	138	45,0	39,1	1,060	41,45	92,10	30,2	9,4
60	220	74,4	39,9	1,713	68,35	91,87	35,3	14,5
80	280	96,1	40,4	2,197	88,76	92,36	38,6	17,8
100	infini	122,0	40,8	2,764	112,775	92,44	42,9	22,1

** : Electrical values were measured for one LED panel coupled with one driver. For the power corresponding to the chamber, multiply the values by two. Temperatures were measured while both panels operate simultaneously.*

5 Power density

The average power density (PD) have been measured for the two most frequently used positions 20% and 40% that won't damage LED panels over long periods of use. The device used to make measurements on the chamber platform was the PM400 optical power and energy meter console (Thorlabs) equipped with the thermal power sensor head S401C (surface absorber, 0.19 - 20 µm, 10 µW - 1 W, 10 mm).

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Version 03

Pos.	$PD_{UV-A}(W/m^2)$		$PD_{UV-B}(W/m^2)$	
	Average	CI 95%	Average	CI 95%
20	309	[252 - 366]	91.17	[47.4 - 136]
40	894	[748 - 1040]	281	[199 - 363]

6 Materials

The chamber is primarily made of plywood panels painted on the exterior and covered with aluminum sheets on the interior. The electrical enclosure is made of polycarbonate.

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Version 03

7 Electrical diagram

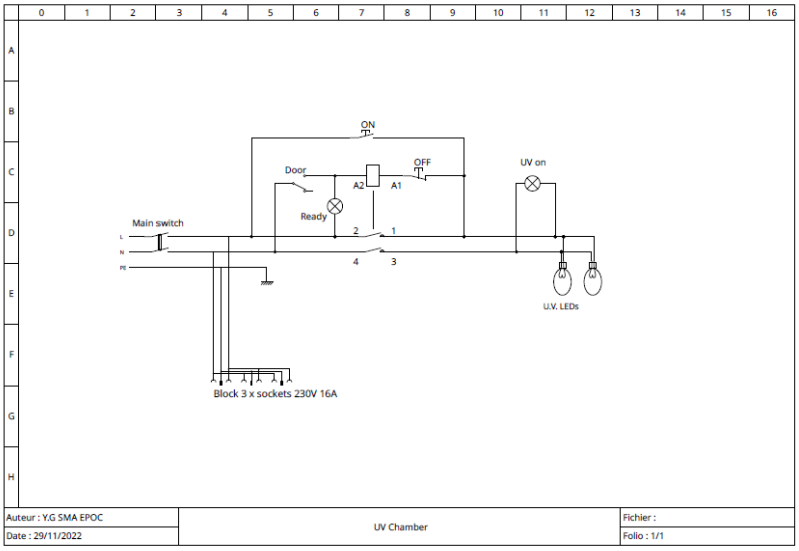

Figure 3: Electrical diagram of the UV chamber.

8 References

This up-to-date notice can be downloaded at the following address:
https://github.com/EdgarDusacre/Plasfito_public/blob/main/UV_chamber/Dusacre_et_al_2024.pdf

Agrotek SAS
25 rue General Ferrié
38100 Grenoble, FRANCE
https://www.agrotek.fr

Product Family Data Sheet Rev:2.0 21.08.05

Middle Power LED
3030

302G UVA Series
Purple



Features & Benefits

- 1.0 W class middle power LED
- Mold resin for high reliability
- Standard form factor for design flexibility (3.0 × 3.0 mm)
- Radiant Efficiency @300mA: typ.31.1%



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1. Characteristics

a) Absolute Maximum Rating

Item	Symbol	Rating	Unit	Condition
Ambient / Operating Temperature	T_a	-40 ~ +105	°C	-
Storage Temperature	T_{stg}	-40 ~ +105	°C	-
LED Junction Temperature	T_j	125	°C	-
Forward Current	I_F	300	mA	-
Pulse Forward Current	I_{FP}	600	mA	Duty 1/10, pulse width 10ms
Assembly Process Temperature	-	260 <10	°C s	-
ESD (HBM)	-	0.5	kv	-

b) Electro-optical Characteristics (I_F = 300 mA, Ts = 25°C)

Item	Unit	Rank	Min.	Typ.	Max.
Forward Voltage (V_f)	V	V0	3.2	3.4	3.6
Reverse Current(I_R) (@VR=5V)	uA		-	-	1
Peak wavelength (λp)	nm	VA0	365	-	375
Radiant Power	mW	H0	280	300	350
Electrical thermal resistance junction/ solderpoint with efficiency ($R_{ths\,elec.}$)η_L= 31.1%	°C/W		-	13	-
Beam Angle	°		-	120	-

Note:

Ledstar maintains measurement tolerance of: Radiant Power = ±7 %, forward voltage = ±0.1 V, Wavelength = ±2 nm

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2. Product Code Information

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
L	D	R	–	3	0	3	0	T	T	A	V	3	6	5	–	V	0	V	A	0	H	0

Digit	PKG Information	Code	Specification
1 2 3	Ledstar Package Middle Power	LDR	
4 5 6 7	Package Model and Size	3030	3.0 x 3.0 x 0.65mm
8	Product Category	T	Top View
9	Bractek Type	T	PCT & Cu
10	Version	A	
11	Color	V	Violet
12 13 14	Wavelength Typical (nm)	365	365~375
15 16	Forward Voltage (V)	V0	3.2~3.6 Bin Code: H0 3.2~3.4 H1 3.2~3.3 H2 3.3~3.4 J0 3.4~3.6 J1 3.4~3.5 J2 3.5~3.6
17 18 19	Peak Wavelength (nm)	VA0 VA1 VA2	VA1 VA2 365~370 370~375
20 21	Radiant Power (mW)	H0 HY HZ	HY HZ 280~300 300~350

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a) **Voltage Bins (I_F = 300 mA, T_s = 25 °C)**

Product Code	Voltage Rank	Voltage Bin		Voltage Range (V)
LDR-3030TTAV365-V0VA0H0	V0	H0	H1	3.2 ~ 3.3
			H2	3.3 ~ 3.4
		J0	J1	3.4 ~ 3.5
			J2	3.5 ~ 3.6

b) **Wavelength Bins (I_F = 300 mA, T_s = 25 °C)**

Product Code	Wavelength Rank	Wavelength Bin	Wavelength Range (nm)
LDR-3030TTAV365-V0VA0H0	VA0	VA1	365 ~ 370
		VA2	370 ~ 375

c) **Radiant Power Bins (I_F = 300 mA, T_s = 25 °C)**

Product Code	Power Rank	Power Bin	Power Range (mW)
LDR-3030TTAV365-V0VA0H0	H0	HY	280 ~ 300
		HZ	300 ~ 350

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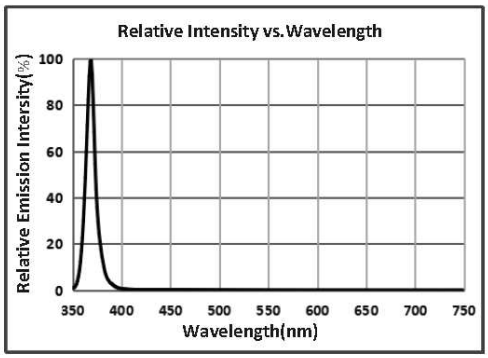
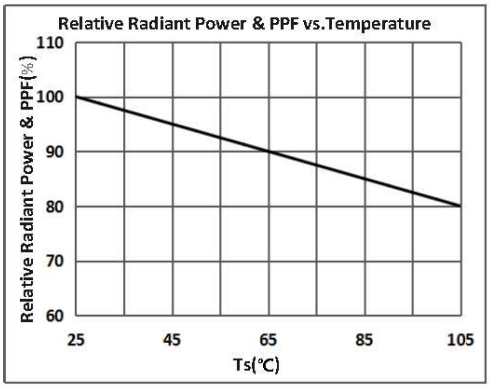
07

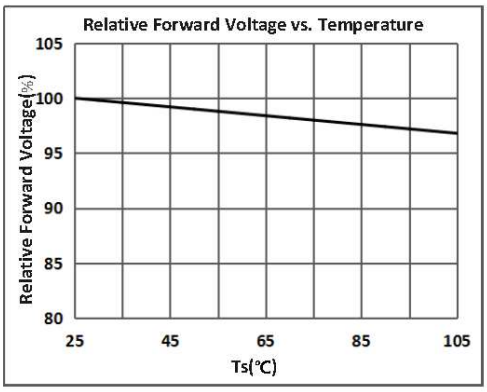
3. Typical Characteristics Graphs

a) Spectrum Distribution (I_F = 300 mA, T_s = 25℃)

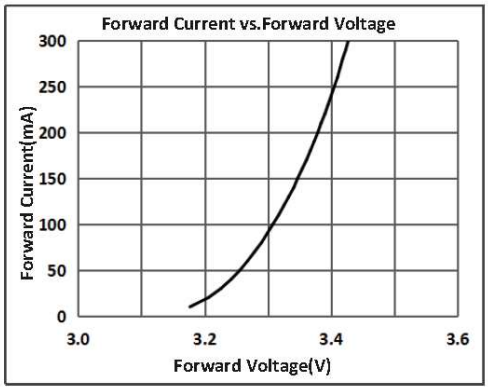


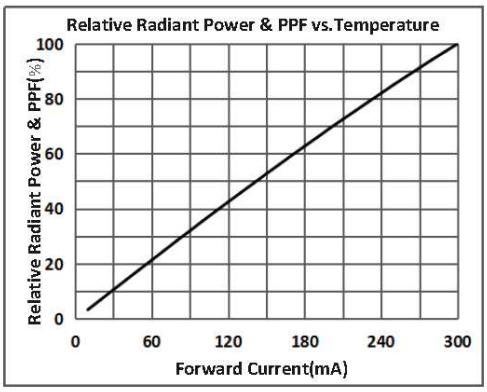
b) Temperature Characteristics (I_F = 300 mA)





c) Forward Current Characteristics (T_s = 25℃)





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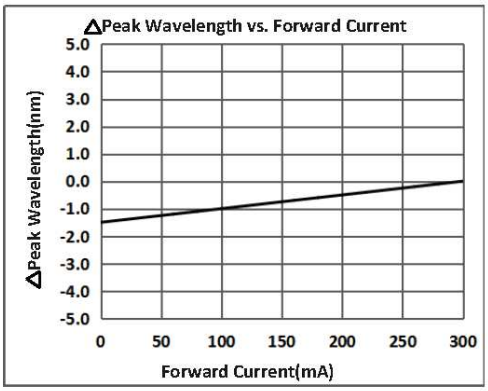
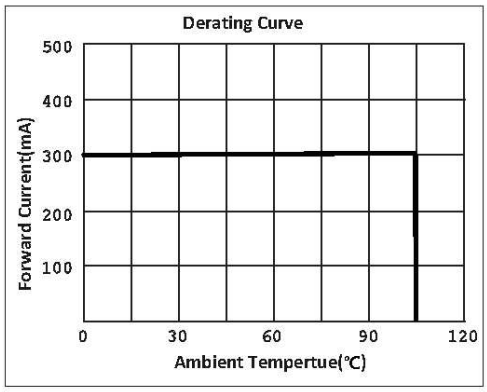
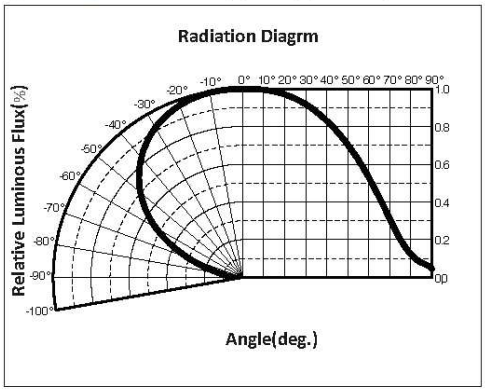
d) Color Shift Characteristics, T_s = 25℃



e) Derating Curve



f) Beam Angle Characteristics (T_s = 25℃, I_F = 300 mA)



Product Family Data Sheet Rev.1.0 21.09.01

Middle Power LED
3535

351W UVB Series Ultraviolet

For Disinfect Lighting



Features & Benefits

- 0.8 W class middle power LED
- Mold resin for high reliability
- Standard form factor for design flexibility (3.5 × 3.5 mm)

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1. Characteristics

a) Absolute Maximum Rating

Item	Symbol	Rating	Unit	Condition
Ambient / Operating Temperature	T_a	-40 ~ +60	°C	-
Storage Temperature	T_{stg}	-40 ~ +60	°C	-
LED Junction Temperature	T_j	85	°C	-
Forward Current	I_F	100	mA	-
Pulse Forward Current	I_{FP}	200	mA	Duty 1/10, pulse width 10ms
Assembly Process Temperature	-	220 <10	°C s	-
ESD (HBM)	-	6	kV	-

b) Electro-optical Characteristics (I_F = 40 mA, Ts = 25 °C)

Item	Unit	Rank	Min.	Typ.	Max.
Forward Voltage (V_F)	V	M0	5.0	-	7.0
Peak wavelength (λ_P)	nm	VB0	305	-	315
Radiant Power (Φ_e)	mW	L0	3	-	5
Thermal Resistance (junction to solder point)	°C/W		-	5	-
Beam Angle	°		-	120	-

Note:

Ledstar maintains measurement tolerance of: **forward voltage = ±0.1 V, Radiant Power = ±5 %,** Wavelength = ±2 nm

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04

2. Product Code Information

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
L	D	R	–	3	5	3	5	C	N	Z	V	3	1	0	–	M	0	V	B	0	L	0

Digit	PKG Information	Code	Specification
1 2 3	Ledstar Package Middle Power	LDR	
4 5 6 7	Package Model and Size	3535	3.5 x3.5 x1.47mm
8	Product Category	C	Ceramics
9	Bractek Type	N	AlN
10	Version	Z	With Zener
11	Color	V	Violet
12 13 14	Wavelength Typical (nm)	310	305~315
15 16	Forward Voltage (V)	M0	5.0~7.0
17 18 19	Peak Wavelength (nm)	VC0 VB1 VB2	VB1 VB2 305~310 310~315
20 21	Radiant Power (mW)	LA LC LD	LC LD 3.0~4.0 4.0~5.0

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a) Voltage Bins (I_F = 40 mA, T_S = 25 °C)

Product Code	Voltage Rank	Voltage Range (V)
LDR-3535CNZV310-M0VB0L0	M0	5.0 ~ 5.5
		5.5 ~ 6.0
		6.0 ~ 6.5
		6.5 ~ 7.0

b) Wavelength Bins (I_F = 40 mA, T_S = 25 °C)

Product Code	Wavelength Rank	Wavelength Bin	Wavelength Range (nm)
LDR-3535CNZV310-M0VB0L0	VB0	VB1	305 ~ 310
		VB2	310 ~ 315

c) Radiant Power Bins (I_F = 40 mA, T_S = 25 °C)

Product Code	Power Rank	Power Bin	Power Range (mW)
LDR-3535CNZV310-M0VB0L0	L0	LC	3 ~ 4
		LD	4 ~ 5

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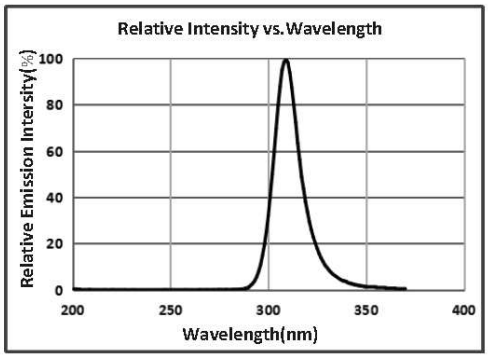
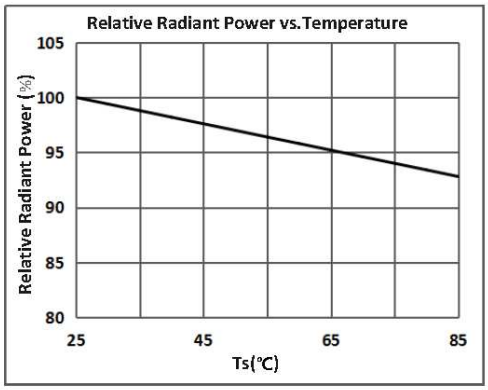
3. Typical Characteristics Graphs

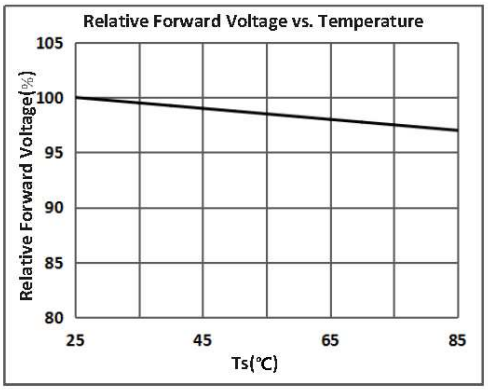
a) Spectrum Distribution (I_F = 40 mA, T_s = 25℃)

WLP305-315nm

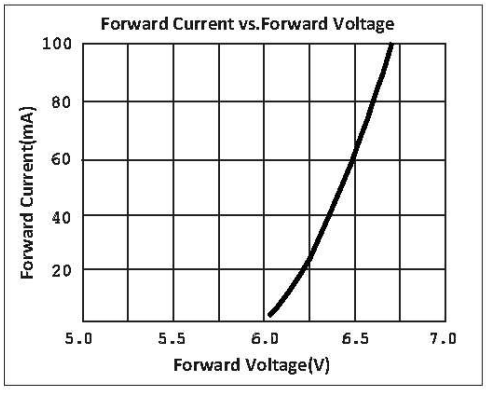


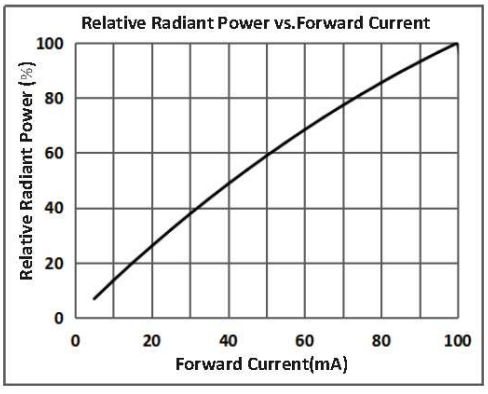
b) Temperature Characteristics (I_F = 40 mA)





c) Forward Current Characteristics (T_s = 25℃)





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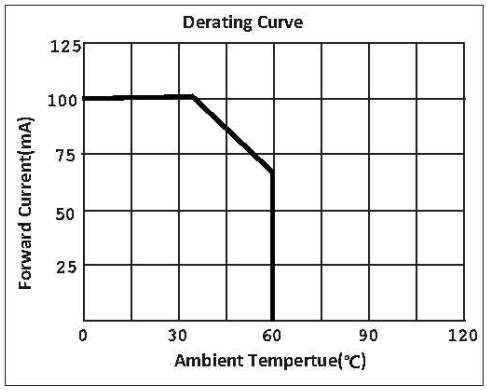
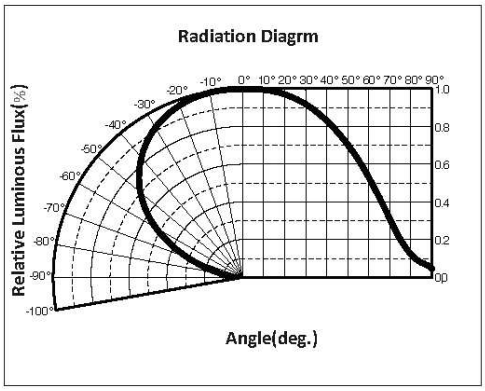
d) Derating Curve



e) Beam Angle Characteristics (T_s = 25℃, I_F = 40 mA)



Annex C. Identification key of lost fishing gear

The accompanying manual for the identification key employed in 0 can be accessed in the provided GitHub repository below. It includes a comprehensive overview of the various fishing *métiers* and gear. Furthermore, this manual explains the utilization of the identification key and how to do the distinctions between different fishing gear. Additionally, this repository contains the files necessary for sampling and data analysis.

https://github.com/EdgarDusacre/FG_Identification_Key.git

Annex D. Data and R scripts repository

All the data and Rmardown scripts that were used to produce the results presented in this Ph.D. thesis are available on the following GitHub repository.

https://github.com/EdgarDusacre/Plasfito_public.git

Annex E. Publications, congress and scientific outreach

E.5 Publications

Lefebvre, C., Le Bihanic, F., Jalón-Rojas, I., **Dusacre, E.,** Chassaigne–Viscaïno, L., Bichon, J., Clérandeau, C., Morin, B., Lecomte, S., & Cachot, J. 2023. "Spatial Distribution of Anthropogenic Particles and Microplastics in a Meso-Tidal Lagoon (Arcachon Bay, France): A Multi-Compartment Approach." *Science of the Total Environment* 898. https://doi.org/10.1016/j.scitotenv.2023.165460.

Ghiglione, J.-F., Barbe, V., Bruzaud, S., Burgaud, G., Cachot, J., Eyheraguibel, B., Lartaud, F., Ludwig, W., Meistertzheim, A.-L., Paul-Pont, I., Pesant, S., ter Halle, A., Thiebeauld, O., Ghiglione, J. F., Philip, L., Odobel, C., Pandin, C., Pujo-Pay, M., Conan, P., Luckas, N., Barbe, V., Wincker, P., Bruzaud, S., Kedzierski, M., Palazot, M., Soccalingame, L., Burgaud, G., Philippe, A., Cachot, J., Morin, B., **Dusacre, E.**, Clérandeau, C., Lefebvre, C., Eyheraguibel, B., Lartaud, F., Ludwig, W., de Madron, X. D., Weiss, L., Meistertzheim, A. L., Calves, I., Lebaron, K., Lavergne, E., Paul-Pont, I., Huvet, A., Dubreuil, C., Pesant, S., ter Halle, A., Albignac, M., Thiebeauld, O., Crenn, K., Gassane, T., Merakeb, L., Bauvois, C., Galgani, F., Gerigny, O., Pedrotti, M. L., Gorsky, G., Lombard, F., Alligant, S., Lacroix, C., Navarro, L., Sperandio, B., Diémé, B., Bowler, C., Troublé, R., Hentinger, R., Abreu, A., Thomas, M., Bourdreux, M., Schramm, J., Moulin, C., Bernollin, E., Hertau, M., Audrain, S., Bin, N., Tournon, Y., Boulon, L., Aurat, F., Blijdorp, L., Pire, C., Bin, S., Gicquel, C., Oriot, M., & the Mission Tara Microplastics consortium. 2023. "Mission Tara Microplastics: A Holistic Set of Protocols and Data Resources for the Field Investigation of Plastic Pollution along the Land-Sea Continuum in Europe." *Environmental Science and Pollution Research* 32(16):10032–49. https://doi.org/10.1007/s11356-023-26883-9.

Dusacre, E., Le Picard, C., Hausard, V., Rigolet, C., Ekoja, F., Jean, M., Clérandeau, C., Villette, S., Lagarde, F., Lecomte, S., Morin, B., Cachot, J., & Cajaraville, M. P. 2025. "Distinct Toxicity Profiles of Conventional and Biodegradable Fishing Nets' Leachates after Artificial Aging." *Journal of Hazardous Materials* 489:137609. https://doi.org/10.1016/j.jhazmat.2025.137609.

Dusacre, E., González-Soto, N., Bihanic, F. L., Galib, A. H., Boura, C., Fuertes, I., Ballesteros, M., Moreno, C., Lecomte, S., Cajaraville, M. P., & Cachot, J. 2025. "From Sea to Shore: Estimating Used, Lost and Stranded Fishing Gear in the Southeastern Bay of Biscay through Models and a Novel Gear Identification Key." *Marine Pollution Bulletin* 224(119090). 10.1016/j.marpolbul.2025.119090.

E.6 Participation in scientific conferences

Platform presentations

Dusacre, E., Ekoja, F., Gonthier, C., Llech, C., Lecomte, S., Clérandeau, C., Morin, B., Lagarde, F., Cajaraville, M. P., & Cachot, J. 2022. "Degradation and Ecotoxicity of New and Used Fishing Nets. First Results of a Research Project in the South- Eastern Bay of Biscay.". MICRO, Online. https://micro2022.sciencesconf.org/427248/document.

Dusacre, E., González-Soto, N., Bihanic, F. L., Hil, G. A., Boura, C., Fuertes, I., Moreno, C., Lecomte, S., Cajaraville, M. P., & Cachot, J. 2025. "From Sea to Shore: Estimating Fishing Gear Loss in the Southeastern Bay of Biscay through Models and a Novel Gear Identification Key.". GDR Plastiques, Environnement, Santé, Bordeaux.

Dusacre, E., González-Soto, N., Clérandeau, C., Lecomte, S., Lagarde, F., Cajaraville, M. P., & Cachot, J. 2024. "Toxicity Patterns of New and Used Fishing Net Extracts: A Comparative Study of Conventional and Biodegradable Polymers.". MICRO, Lanzarote.

Dusacre, E., Le Picard, C., Clérandeau, C., Lecomte, S., Morin, B., Lagarde, F., Cajaraville, M. P., & Cachot, J. 2023. "Écotoxicité du mélange de microplastiques, de nanoplastiques et d'additifs issus de la dégradation des filets de pêche.". GDR Polymères et Océans, Rennes.

Dusacre, E., Lefebvre, C., Clérandeau, C., Villette, S., Lagarde, F., Lecomte, S., Morin, B., Cajaraville, M. P., & Cachot, J. 2025. "Cinétique de dégradation et toxicité induite lors du vieillissement des filets de pêche en plastique.". ECOBIM, Sousse.

Dusacre, E., Picard, C. L., Hausard, V., Rigolet, C., Ekoja, F., Clerandeau, C., Lagarde, F., Lecomte, S., Morin, B., & Cachot, J. 2024. "Distinct Toxicity Profiles Caused by Conventional and Biodegradable Fishing Nets' Leachates on the Marine Bacteria *Aliivibrio Fischeri* and the Fish *Oryzias Latipes*.". PRIMO 22, Nantes.

Dusacre, E., Picard, C. L., Hausard, V., Rigolet, C., Ekoja, F., Clerandeau, C., Lagarde, F., Lecomte, S., Morin, B., Cajaraville, M. P., & Cachot, J. 2024. "Profils de toxicité distincts de filets de pêche conventionnels ou biodégradables après vieillissement artificiel.". GDR Plastiques, Environnement, Santé, Marseille.

Lefebvre, C., **Dusacre, E.**, González-Soto, N., Morin, B., Clerandeau, C., Lecomte, S., Cajaraville, M. P., & Cachot, J. 2025. "Ecotoxicity of Fishing Nets Leachates after up to One Year of in Situ Weathering in Two Harbors of the Bay of Biscay.". One Ocean Congress, Nice. https://doi.org/10.5194/oos2025-1188.

Lefebvre, C., **Dusacre, E.**, Soto, N. G., Morin, B., Clerandeau, C., Lecomte, S., Cajaraville, M. P., & Cachot, J. 2024. "Ecotoxicity of Fishing Nets Leachates after up to One Year of in Situ Weathering in Two Harbors of the Biscay Bay.". MICRO, Lanzarote.

Morin, B., Rakwe, M. E., Amédéo, F., Thomas, L., **Dusacre, E.**, Burger, V., Mazeas, F., Dreanno, C., Maes, C., Lecomte, S., Cachot, J., & Prado, E. 2025. "Naviguer pour la science : une étude collaborative pour évaluer la pollution microplastique dans l'océan Atlantique.". ECOBIM, Sousse.

Paul-Pont, I., Reynaud, S., George, M., Fabre, P., Duflos, G., Dignac, M.-F., Gasperi, J., Mercier-Bonin, M., Ghiglione, J.-F., Ter Halle, A., Huvet, A., Lagarde, F., Kedzierski, M., Akoueson, F., **Dusacre, E.**, & Cousin, X. 2025. "How Scientific Networks Can Help Advancing Both Scientific Knowledge and Public Policies: The Case Study of the 'Plastics, Environment and Health' Network.". One Ocean Congress, Nice. https://doi.org/10.5194/oos2025-775.

Poster presentations

Dusacre, E., Clérandeau, C., Lecomte, S., Lagarde, F., Morin, B., Cajaraville, M., & Cachot, J. 2023. "Ecotoxicity of the Mixture of Microplastics, Nanoplastics, and Additives Produced from the Degradation of Fishing Nets.". SETAC Europe 33rd Annual Meeting, Dublin. https://doi.org/10.13140/RG.2.2.12720.88323.

Dusacre, E., Ekoja-Smah, F. O., Grillard, E., Lagarde, F., Lecomte, S., Le Bihanic, F., Cajaraville, M., & Cachot, J. 2022. "Devenir et Effets Des Microplastiques, Nanoplastiques et Additifs Issus de La Dégradation Des Engins de Pêche Au Cours de Leur Cycle de Vie. Étude Dans Le Golfe de Gascogne.". GDR Polymères et Océan, Brest. https://doi.org/10.13140/RG.2.2.17325.56809.

Dusacre, E., Gonzalez Soto, N., Galib, A., Boura, C., Le Bihanic, F., Fuertes, I., Ballesteros, M., Moreno, C., Lecomte, S., Cajaraville, M., & Cachot, J. 2024. "Novel Identification Approach for Quantifying and Characterizing Abandoned, Lost, or Otherwise Discarded Fishing Gear.". MICRO, Lanzarote. https://micro2024.sciencesconf.org/559754/document.

Franco, J., Jalón-Rojas, I., Ruiz-Gonzalez, V., **Dusacre, E.**, Cajaraville, M. P., & Cachot, J. 2024. "Numerical Modelling of Fishery Litter Pathways in Southern Bay of Biscay.". ISOBAY 18, La Rochelle. https://apps.univ-lr.fr/actes/ged/colloques/docWeb/2405311602.0/Poster_Abstract_Book.pdf.

Gonzalez Soto, N., **Dusacre, E.**, Fuertes, I., Ballesteros, M., Moreno, C., Bilbao, E., Orbea, A., Lecomte, S., Cachot, J., & Cajaraville, M. P. 2024. "Characterization of Plastics Obtained in the OSPAR Monitoring for Marine Litter in 5 Beaches of the Bay of Biscay.". SETAC Europe 34rd Annual Meeting. https://www.setac.org/resource/seville-2024-abstract-book-pdf.html.

Jalon-Rojas, I., Franco, J., Ruiz-González, V., **Dusacre, E.**, Cajaraville, M. P., & Cachot, J. 2025. "The Journey of Fishing Gear in the Southern Bay of Biscay: From Source to Sink and Vice Versa.". One Ocean Congress, Nice. https://doi.org/10.5194/oos2025-741.

Morin, B., Rakwe, M. E., Amédéo, F., Thomas, L., **Dusacre, E.**, Burger, V., Dreanno, C., Benoit, A., Maes, C., Lecomte, S., Cachot, J., & Prado, E. 2025. "Evaluation de la pollution microplastique dans l'océan Atlantique basée sur une collaboration Science et Voile.". GDR Plastiques, Environnement, Bordeaux.

Veclin, C., Le Picard, C., Bergue, V., Bellier, B., Akoueson, F., Darragon, A., Dusacre, E., Lefebvre, C., Perez, J., & **Dusacre, E.** 2025. "Evaluation des effets des campagnes de communication en faveur du recyclage sur la gestion des dechets plastiques et la pollution plastique.". GDR Plastique, Environnement et Santé, Bordeaux https://doi.org/sciencesconf.org:gdrpes2025:638662.

E.7 List of workshops and scientific outreach

Workshops

Dusacre, E., Cachot J., Cajaraville M.P., 2023. "Ecotoxicity of the mixture of micro, nanoplastics, and additives produced from the degradation of fishing nets.". JDED annual Workshop. Université de Bordeaux.

Dusacre, E., Cachot J., Cajaraville M.P., 2023. "Dusacre, E., Cachot J., Cajaraville M.P., 2025. "Toxicity patterns of new and used fishing net extracts: a comparative study of conventional and biodegradable polymers.". CTA/ECT annual Workshop. Universidad de Pais Vasco/Euskal Herriko Unibertsitatea.

Dusacre, E., Le Picard, C., Lagarde, F., Morin, B., Lecomte, S., Cajaraville, M. P., Cachot, J., 2024. "Ecotoxicity of the mixture of microplastics, nanoplastics, and additives produced from the degradation of fishing nets.". LTC SAREUS Workshop. Fundazioa Euskampus

Dusacre, E., González-Soto, N., Clérandeau, C., Lecomte, S., Lagarde, F., Cajaraville, M. P., & Cachot, J. 2024. "Dusacre, E., Cachot J., Cajaraville M.P., 2024. "Fate and effects of microplastics, nanoplastics and additives from the degradation of fishing nets in the Bay of Biscay.". CTA/ECT annual Workshop. Universidad de Pais Vasco/Euskal Herriko Unibertsitatea.

Dusacre, E., Cachot J., Cajaraville M.P., 2025. "Fate and effects of microplastics, nanoplastics and additives from the degradation of fishing nets in the Bay of Biscay.". IX Jornadas de investigacion de la Facultad de Cienca y Tecnologia. Universidad de Pais Vasco/Euskal Herriko Unibertsitatea

Scientific outreach

Dusacre, E., 2021. "Euskampus Eguna Donostia: Devenir et effets des microplastiques, nanoplastiques et additifs issus de la degradation des filets de pêche.". Fundazioa Euskampus, Université de Bordeaux, Universidad de Pais Vasco/Euskal Herriko Unibertsitatea.

30 young French citizens, working or involved in the ecology and waste reduction sector, 2021. "World café – Génération Zéro Déchet. Comment mieux réduire nos déchets en Europe ?". European Comission, Académie du Climat. https://france.representation.ec.europa.eu/events/world-cafe-generation-zero-dechet-comment-mieux-reduire-nos-dechets-en-europe-2021-11-25_fr

Dusacre, E., Bilbao, E., 2021. "Zientzia Astea 2021 Ciencia ciudadana: pesca de residuos marinos". MATER Museao, Universidad de Pais Vasco/Euskal Herriko Unibertsitatea.

Dusacre, E., 2022. "Fate and effect of microplastics, nanoplastics and additives coming from fishing net degradation.". Ocean Plastic Webinars. https://www.youtube.com/watch?v=tTy6a-k6IM0

Dusacre, E., 2022. "Ma thèse en 180 secondes, finale régionale : Devenir et effets des microplastiques, nanoplastiques et additifs issus de la degradation des filets de pêche.".

Université de Bordeaux, France Universités, CNRS. https://www.youtube.com/watch?v=7j52eWplufI

Dusacre, E., 2022. “La pêche : source de microplastiques et de nanoplastiques dans les océans ? Qu’en savons nous ? Etat des lieux et impacts.”. Société Scientifique d’Arcachon.

Amédeo F., **Dusacre, E.**, Cachot, J., Lecomte, S., 2022. “Rencard du savoir - Attention marins, plastiques à babord. ». CNRS, Université de Bordeaux, Fondation Bordeaux Université, Maison de l’Europe Bordeaux. https://soundcloud.com/universitedebordeaux/attention-marin-plastiques-a-babord?in=universitedebordeaux/sets/les-rencards-du-savoir-saison

Dusacre, E., Cachot J., 2022. “Village des Sciences : Pollution Plastique, reconnaître les engins de pêche échoués sur les plages ou perdu en mer.”. CAP SCIENCES, Bordeaux.

Gontard, N., Moisan D., Derot, C., Guillard Bounsaythip, F., **Dusacre E.**, Nardi, J., Thiand B., Irigaray, M., 2022. “TIC du PLASTOC. Table ronde: Civilisation plastique, quel avenir ?”. Harmonie Mutuelle.

Dusacre, E., Cajaraville M.P., 2023. “Pitch Euskampus Misiones 1.0: Destino y efectos de los microplásticos, nanoplásticos y aditivos derivados de la degradación de redes de pesca durante su ciclo de vida, un estudio en el Golfo de Bizkaia.”. Fundazioa Euskampus.

Gontard, N., **Dusacre E.**, Vanhove I., Niveaux F., 2023. “TIC du PLASTOC. Table ronde: Civilisation plastique, quel avenir ?”. Harmonie Mutuelle. https://jagiscollectif.harmonie-mutuelle.fr/agora-mutualiste/civilisation-plastique-quel-avenir

Dusacre, E., Bilbao, E., 2024. “Zientzia Astea 2024 Ciencia ciudadana: pesca de residuos marinos”. MATER Museao, Universidad de Pais Vasco/Euskal Herriko Unibertsitatea.

Gontard, N., **Dusacre E.**, Gayssot C., 2024. “TIC du PLASTOC. Table ronde: Civilisation plastique, quel avenir ?”. Harmonie Mutuelle. https://jagiscollectif.harmonie-mutuelle.fr/agora-mutualiste/civilisation-plastiquequel-avenir

Academic members of GDR PES, **Scientifc board of GDR PES**, Capucine Dupuy, Bobika, Break Free From Plastic, 2025, “A look back at the Plastics, Environment and Health GDR days 2024”. CNRS, ANSES. https://gdr-po.cnrs.fr/docs/GDR_2024_en_image_English.pdf

Cachot, J., Reyboulet, C., Charbonnier, M., Bougrat S., Coma, V., **Dusacre, E.**, 2025. Animateur de la soirée grand public et de la conférence-débat : Les défis de la pollution plastique.". Ville de Bordeaux, Science avec et pour la société, Université de Bordeaux. https://www.u-bordeaux.fr/evenements/les-defis-de-la-pollution-plastique

E.8 List of interventions or articles in the media

Capdevielle, S., 2022. “Edgar Dusacre - le cauchemar du plastique”. Terre et Toits, Dealers de Sciences, Université de Bordeaux, Université de Bordeaux Montaigne. https://www.terre-et-toits.dealersdescience.com/edgar-dusacre/

Menet, S., 2022. “Arcachon : une conférence sur les origines et les impacts des microplastiques dans le bassin”. Sud Ouest. https://www.sudouest.fr/gironde/arcachon/arcachon-une-conference-sur-les-origines-et-les-impacts-des-microplastiques-dans-le-bassin-8342593.php

TheMetaNews, 2023. “SA VIE, SON PHD : Edgar Dusacre, activiste scientifique.” TheMetaNews, Les Deeptech. https://themeta.news/phdnumero8/

Challandes, J., Morin, M., 2023. “Documentaire : De l'assiette à l'océan”. Blutopia. https://blutopia.org/tournage-assiette-ocean-journal-7/

E.9 Involvement in research group or science-related NGOs

Représentant des étudiants-chercheurs et chercheurs en contrats non permanents au sein du conseil scientifique du Groupe de Recherche Plastique, Environnement, Santé. CNRS, ANSES. Septembre 2023 – Avril 2025. https://gdr-po.cnrs.fr/

Fondateur et président de l’ONG scientifique 4P Scienseas. 2019 – 2025. https://4pscienseas.org/