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# THÈSE DE DOCTORAT

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## Temperature impacts across biological scales: from physiology to the functioning of aquatic ecosystems

**Discipline**
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# Affidavit

Je soussignée,  Julie MORLA, déclare par la présente que le travail présenté dans ce manuscrit est mon propre travail, réalisé sous la direction scientifique de Martin DAUFRESNE et Arnaud SENTIS, dans le respect des principes d'honnêteté, d'intégrité et de responsabilité inhérents à la mission de recherche. Les travaux de recherche et la rédaction de ce manuscrit ont été réalisés dans le respect à la fois de la charte nationale de déontologie des métiers de la recherche et de la charte d'Aix-Marseille Université relative à la lutte contre le plagiat.

Ce travail n'a pas été précédemment soumis en France ou à l'étranger dans une version identique ou similaire à un organisme examinateur.

Fait à Aix-en-Provence, le 20 avril 2024



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# Liste des publications et participations aux conférences

## 1) Liste des publications dans le cadre du projet de thèse :

1. *Multigenerational exposure to temperature influences mitochondrial oxygen fluxes in the Medaka fish (Orizias Latipes)*. **Julie. Morla[1]**, Karine. Salin[2], Rémy. Lassus[1], Julie. Favre-Marinet[1], Arnaud. Sentis[1], and Martin. Daufresne[1]. *(Statut: Under Review with minor comments status, Acta-physiologica).*

2. *Multigenerational effects of temperature exposure on thermal tolerance ($CT_{max}$) and mitochondrial functioning in Medaka (Orizias Latipes) fish brain.* **Julie. Morla[1]**, Mélissa. Richard[1], Rémy. Lassus[1], Nicolas. Pichaud[3], Martin. Daufresne[1] & Arnaud. Sentis[1]. *(Statut: Submitted, Proceedings of the Royal Society B).*

3. *Impact of temperature and body size reduction on ecosystem functioning.* **Julie. Morla[1]**, Simon. Bazin[1], Marina. Campana[1], Thalia. Zambeaux[1], Virginie. Diouloufet[1], Ange. Molina[1], Julie. Favre-Marinet[1], Gaetan. Fremont[1], Stéphan. Jacquet[4]; Serena. Rasconi[4], Nathan. Armaing[1], Dorian. Milesi[1], Camille. Leclerc[1], Remy. Lassus[1], Tiphain. Peroux[1], Fanny. Colas[5], Martin. Daufresne[1], Arnaud. Sentis[1]. *(Statut: In. prep. Draft).*

4. *Artificial warming decreases heterotroph densities and predator-prey biomass ratio in aquatic systems.* Simon. Bazin[1]; Virginie. Diouloufet[1]; Ange. Molina[1]; Tiphaine. Peroux[1]; Rémy. Lassus[1]; Jose. Montoya[6]; Simon. Blanchet[6]; Eric. Edeline[7]; Stéphan. Jacquet[4]; Serena. Rasconi[4]; Stéphanie. Fayolle[8]; Marina. Campana[1]; Thalia. Zambeaux[1'8]; Camille. Leclerc[1]; **Julie. Morla[1]**; Martin. Daufresne[1]; Arnaud. Sentis[1]. *(Statut: Submitted, Oikos).*

## 2) Participation aux conférences nationales et internationales au cours de la thèse :

**1** **Colloque d'Ecophysiologie Animale, 5eme édition (CEPA-5)**. 02-05 Novembre 2021. Campus Triolet de l'Université de Montpellier, Pl. Eugène Bataillon, 34090 Montpellier. « *Influence of thermal multi-generational exposition on mitochondrial functioning in the medaka fish (Oryzias Latipes)"*. Julie. Morla[1], Karine. Salin[2], Rémy. Lassus[1], Julie. Favre-Marinet[1], Arnaud. Sentis[1], and Martin. Daufresne[1]. **(Présentation orale).**

**2** **Groupe De Recherche (GDR) Théorie et modélisation de la Biodiversité (Theomodiv).** 22-24 Novembre 2021. Bâtiment du FORUM, Technopôle de l'Environnement Arbois-Méditerranée Domaine du petit Arbois Avenue Louis Philibert 13545 Aix-en-Provence. *« Ecological consequences of temperature-induced body size shifts on aquatic ecosystem* ». Julie. Morla[1], Simon. Bazin[1], Marina. Campana[1], Thalia. Zambeaux[1], Virginie. Diouloufet[1], Ange. Molina[1], Julie. Favre-Marinet[1], Gaetan. Fremont[1], Stéphan. Jacquet[4]; Serena. Rasconi[4], Nathan.

Armaing[1], Dorian. Milesi[1], Camille. Leclerc[1], Remy. Lassus[1], Tiphain. Peroux[1], Fanny. Colas[5], Martin. Daufresne[1], Arnaud. Sentis[1]. **(Présentation orale)**.

3 **Congres Ecole Doctorale Science de l'Environnement EDSE.** 5-6 Mai 2022. M.I.O (Institut Méditerranéen d'Océanologie) Bât. Méditerranée, Campus de Luminy-Océanomed, 13009 Marseille. *« Impact du changement climatique sur les organismes, la structure des réseaux trophiques et le fonctionnement des écosystèmes aquatiques ».* Julie. Morla[1], Simon. Bazin[1], Marina. Campana[1], Thalia. Zambeaux[1], Virginie. Diouloufet[1], Ange. Molina[1], Julie. Favre-Marinet[1], Gaetan. Fremont[1], Stéphan. Jacquet[4]; Serena. Rasconi[4], Nathan. Armaing[1], Dorian. Milesi[1], Camille. Leclerc[1], Remy. Lassus[1], Tiphain. Peroux[1], Fanny. Colas[5], Martin. Daufresne[1], Arnaud. Sentis[1]. **(Poster)**.

4 **Journée des doctorants de l'INRAE.** 27 juin 2022. Site INRAE du Tholonet 3275 route de Cézanne, 13182 Aix-en-Provence. *« Impact du changement climatique sur les organismes, la structure des réseaux trophiques et le fonctionnement des écosystèmes aquatiques ».* Julie. Morla[1], Simon. Bazin[1], Marina. Campana[1], Thalia. Zambeaux[1], Virginie. Diouloufet[1], Ange. Molina[1], Julie. Favre-Marinet[1], Gaetan. Fremont[1], Stéphan. Jacquet[4]; Serena. Rasconi[4], Nathan. Armaing[1], Dorian. Milesi[1], Camille. Leclerc[1], Remy. Lassus[1], Tiphain. Peroux[1], Fanny. Colas[5], Martin. Daufresne[1], Arnaud. Sentis[1]. **(Poster)**.

5 **International Congres on the Biology of Fish (ICBF).** 28 june - 01-jully-2022. Corum de Montperllier, Place Charles de Gaulle, 34000 Montpellier, France. *« Multigenerational exposition to warming reduces mitochondrial oxygen flux in the medaka fish (Orizias Latipes)* ». Julie. Morla[1], Karine. Salin[2], Rémy. Lassus[1], Julie. Favre-Marinet[1], Arnaud. Sentis[1], and Martin. Daufresne[1]. **(Présentation orale)**.

6 **SFEE-GFO Congres, Conférence Internationale « Ecology and Evolution: New perspectives and societal challenges »** (Société Française d'Écologie et d'Évolution SFE[2] – The Ecological Society of Germany GfÖ –European Ecological Federation EEF), Metz France, 21-25 November 2022. Parvis de l'amphithéâtre - 100 rue aux Arènes 57000 - Metz, France. *« Ecological consequences of temperature-induced body size shifts on aquatic ecosystem ».* Julie. Morla[1], Simon. Bazin[1], Marina. Campana[1], Thalia. Zambeaux[1], Virginie. Diouloufet[1], Ange. Molina[1], Julie. Favre-Marinet[1], Gaetan. Fremont[1], Stéphan. Jacquet[4]; Serena. Rasconi[4], Nathan. Armaing[1], Dorian. Milesi[1], Camille. Leclerc[1], Remy. Lassus[1], Tiphain. Peroux[1], Fanny. Colas[5], Martin. Daufresne[1], Arnaud. Sentis[1]. **(Poster)**.

7 **Séminaire annuel du réseau INRAE « EcoSyst ».** Octobre 2023. Campus Supagro, 2, place Viala, Montpellier, France. *« A mesocosm experiment to study how changes in community affect the ecosystem functioning ».* Julie. Morla[1], Simon. Bazin[1], Marina. Campana[1], Thalia. Zambeaux[1], Virginie. Diouloufet[1], Ange. Molina[1], Julie. Favre-Marinet[1], Gaetan. Fremont[1], Stéphan. Jacquet[4]; Serena. Rasconi[4], Nathan. Armaing[1], Dorian. Milesi[1], Camille. Leclerc[1], Remy. Lassus[1], Tiphain. Peroux[1], Fanny. Colas[5], Martin. Daufresne[1], Arnaud. Sentis[1]. **(Présentation orale)**.

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## Diffusion de la culture scientifique scolaire et grand public

1. **Nuit Européenne des Chercheur.e.s grand public** (24 septembre, 2021). Aix-en-Provence. Cellule de culture scientifique et technique de la Direction de la Recherche et de la Valorisation d'Aix-Marseille Université. Intervention auprès du public sous la forme d'escales scientifiques : discussion avec le grand public à partir d'un objet représentant mon travail de recherche. 4h.

2. **Nuit Européenne des Chercheur.e.s scolaire** (30 septembre, 2021). Marseille. Cellule de culture scientifique et technique de la Direction de la Recherche et de la Valorisation d'Aix-Marseille Université. Intervention auprès de scolaires (3 classes de 3eme) sous la forme d'une mini conférence autour de mon sujet de recherche « *Impact du changement climatique sur le fonctionnement des systèmes aquatiques* ». 5h.

3. **ASTEP (Partenaire Scientifique Pour la Classe)** (03 janvier au 15 juillet 2022). Ecole élémentaire de Gréasque (3 classes de CM2). Intervention autour de séances en classe sur le travail de chercheur et sur le changement climatique. Présentation du travail de chercheur, présentation des principaux risques climatiques et humains. Mise en place d'animations et d'ateliers : experience d'acidification ; création « jeu du réseau trophique », « jeux de dégradation des déchets », organisation d'exposés et restitutions. 30h.

4. **Fête de la Science : Journée de création en médiation scientifique** (29 avril 2022). Maison du Développement Industrielle, 38 rue Joliot Curie, 13013 Marseille. Journée de création de supports de médiation autour de mon sujet de recherche à l'aide du grand-public. Création prototype de jeu de société autour de l'impact de la temperature sur les organismes aquatiques. Organisée par la coordination régionale de la Fête de la Science (Petits Débrouillards, DRARI, Région Sud). 8h.

5. **Journée de l'Europe** (14 mai 2022). Marseille parc Longchamp. Animation atelier autour des invertébrés aquatiques *« Bébêtes aquatiques face au changement climatique : Impact du changement climatique sur le fonctionnement des systèmes aquatiques »* et des expériences scientifiques que je mène à l'inrae d'Aix-en-Provence (création d'un mini atelier autours de l'échantillonnage des organismes aquatiques). 10h.

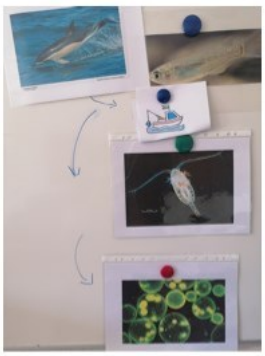
6. **Intervention scolaire en primaire pour la fête de la science 2022** (15 Novembre 2022). Ecole élémentaire d'Orgon, 13660 (classe de CE2 ; Enseignant : Mr Sagnimote David). Présentation du travail de chercheur(se), illustration de mon travail en quelques photos *« impact du changement climatique sur les organismes aquatiques »*, premières approches de sensibilisation à l'impact du changement climatique, les bons gestes à adopter et organisation d'ateliers autours du climat (mise en place d'experience d'acidification ; jeu du réseau trophique, jeu de la dégradation des déchets). 3h.

7. **Intervention scolaire en primaire pour la fête de la science 2022 (**8 Décembre 2022). École élémentaire Marceau Ginoux de Salon de Provence, 13300 (classe de CE2 ; Enseignante: Mme Milani Aurèlie). Présentation du travail de chercheur(se), illustration de mon travail en quelques photos *« impact du changement climatique sur les organismes aquatiques »*, premières approches de sensibilisation à l'impact du changement climatique, les bons gestes à adopter et organisation d'ateliers autours du climat (mise en place d'experience d'acidification ; jeu du réseau trophique, jeu de la dégradation des déchets). 2h30.

8. **Intervention scolaire en Lycée pour la fête de la science 2022** (12 Décembre 2022). Lycée Cézanne d'Aix-en-Provence, 13100 (classe de 1ere). Illustration de mon travail de recherches *« impact du changement climatique sur les organismes aquatiques »*, initiation à une démarche scientifique, présentation simplifiée de mes résultats de recherche et des principaux enjeux, Présentation de la crise climatique problématique de la disponibilité en eau, de la surpêche, des modes de consommation en relation avec le programme de 1ere. 4h.

9. **Intervention scolaire en primaire pour la fête de la science 2022** (6 Janvier 2023). École des Fabrette de Marseille, 13015 (classe de CM2; Enseignante: Mme Truong Aurelie). Présentation du travail de chercheur(se), illustration de mon travail en quelques photos *« impact du changement climatique sur les organismes aquatiques »*, premières approches de sensibilisation à l'impact du changement climatique, les bons gestes à adopter et organisation d'ateliers autours du climat (mise en place d'experience d'acidification ; jeu du réseau trophique, jeu de la dégradation des déchets). 2h30.






# Encadrement

**Co-encadrement stagiaire Master 2.** Janvier - juillet 2021. *Impacts de la température sur la croissance d'un prédateur, Oryzias Latipes, et ses conséquences sur l'abondance et la taille d'une communauté zooplanctonique*, INRAE, site d'Aix en Provence - Le Tholonet UMR RECOVER - Équipe FRESHCO par. **Campana Marina.**

**Co-encadrement stagiaire IUT.** Avril - Juin 2021. *Impact d'une acclimatation à la température sur le fonctionnement mitochondrial chez Oryzias Latipes*, INRAE, site d'Aix en Provence - Le Tholonet UMR RECOVER - Équipe FRESHCO par. **Favre-Marinet Julie.**

**Co-encadrement stagiaire Master 1.** Avril - juin 2023. *Tolérances thermiques et respiration mitochondriale du poisson médaka dans un contexte de changement climatique*, INRAE, site d'Aix en Provence - Le Tholonet UMR RECOVER - Équipe FRESHCO par. **Richard Melissa.**

# Résumé

Les écosystèmes d'eau douce sont parmi les écosystèmes les plus vulnérables au réchauffement climatique. La température régule une grande partie des processus physiologiques et écologiques, avec des effets en cascade sur la biodiversité et le fonctionnement des écosystèmes. Plusieurs auteurs ont avancé l'idée que l'écologie des organismes et des communautés découle du métabolisme. Plus généralement, la taille, la température et le métabolisme semblent jouer un rôle majeur dans la structure des réseaux trophiques et le fonctionnement des écosystèmes. La réponse à l'exposition à la température peut également dépendre de la durée de l'exposition, les réponses à court terme pouvant être modifiées à long terme par la plasticité et l'évolution. Pour mieux anticiper la réponse des organismes au changement climatique, il est important de prendre en compte les conséquences de l'exposition à la température aux différentes échelles du vivant en fonction de la durée d'exposition.

L'objectif principal de cette thèse est d'améliorer notre compréhension de l'effet d'une exposition multigénérationnelle à la température sur les ectothermes d'eau douce en tenant compte de différentes échelles écologiques. Les processus influencés par la température sont abordés à l'aide de différentes approches expérimentales. Tout d'abord, j'ai utilisé une approche physiologique, axée sur la respiration cellulaire à des températures élevées, en me concentrant sur les mitochondries, jouant un rôle central dans le contrôle du métabolisme et des caractéristiques de l'histoire de la vie. Ensuite j'ai combiné respiration cellulaire et mesure de limites thermiques pour déterminer si une exposition multigénérationnelle peut moduler ces deux traits. Finalement j'ai utilisé une approche en mésocosmes visant à étudier les processus de l'écosystème sous pression thermique en présence de poissons d'origines thermiques contrastées.

De manière générale, les résultats de cette thèse indiquent que l'exposition multigénérationnelle a une température élevée conduit à (i) des flux respiratoires mitochondriaux plus faibles pour une même température de test et des flux respiratoires mitochondriaux très proches entre les poissons du froid et ceux du chaud testés à leur température d'élevage, pouvant refléter l'existence d'un flux respiratoire optimal, et (ii) une augmentation significative du CTmax des poissons. Nos résultats suggèrent également un lien entre CTmax de l'organisme entier et performance mitochondriale cérébrale. Enfin, l'étude en mésocosmes met en évidence (iii) la complexité de l'impact de la température sur les fonctions de l'écosystème par le biais d'effets directs et indirects. Même si la présence et l'origine des poissons ont des effets plus faibles sur les fonctions de l'écosystème que les effets de température, notre étude démontre l'importance des relations trophiques et des effets antagonistes complexes sur la réponse des écosystèmes à la température.

Dans l'ensemble, cette thèse contribue à la connaissance de la façon dont la température modifie la physiologie et la tolérance thermique d'un poisson d'eau douce et fournit des informations précieuses sur la façon dont le fonctionnement des écosystèmes aquatiques peut être affecté par le réchauffement climatique.



**Mots clés :** Augmentation des températures, Mitochondrie, écosystèmes d'eau douce, Ectothermes, Multi-Échelle biologiques, Exposition multigénérationnelle.

# Abstract

Freshwater ecosystems are among the most vulnerable to global warming. Temperature regulates a large part of physiological and ecological processes, with cascading effects on biodiversity and ecosystem functioning. Several authors have put forward the idea that the ecology of organisms and communities derives from metabolism. More generally, size, temperature and metabolism appear to play a major role in the structure of food webs and the functioning of ecosystems. The response to temperature variations may also depend on the duration of exposure, short-term responses being potentially modified over the longer term by plasticity and evolution. To better anticipate the response of organisms to climate change, it is important to take into account the consequences of exposure to temperature at the different ecological scales, depending on the duration of exposure.

The main objective of this thesis is to improve our understanding of the effect of a multigenerational exposure to temperature on freshwater ectotherms, taking into account different ecological scales. The biological processes influenced by temperature are addressed using different experimental approaches. Firstly, a physiological approach, focusing on cellular respiration at elevated temperatures, through a focus on mitochondria, which play a central role in controlling metabolism and life-history characteristics. Secondly, an approach studying ecosystem processes under thermal pressure in the presence of fish from two thermally contrasting lineages, through a mesocosm experiment.

Generally, this study indicates that multigenerational exposure to a high temperature leads to (i) lower respiratory fluxes for fish test at the same temperature and very similar respiratory fluxes between cold and warm fish tested at their rearing temperature, which may reflect the existence of an optimal respiratory, and (ii) a significant increase in the CTmax of the fish. Our results also suggest a link between whole organism CTmax and cerebral mitochondrial performance. Finally, the mesocosm study highlights (iii) the complexity of the impact of temperature on ecosystem functions through direct and indirect effects. Although the presence and size/metabolism differences of fish have smaller effects on ecosystem functions than temperature effects, our study demonstrates the importance of trophic relationships and complex antagonistic effects on ecosystems response to temperature.

Overall, this thesis contributes to the knowledge on how temperature modifies the physiology and thermal tolerance of a freshwater fish and provides valuable information on how the functioning of aquatic ecosystems can be affected by global warming.



**Keywords:** Temperature increase, Mitochondria, Freshwater ecosystems, ectotherms, Biological multi-scale, Multi-generational exposure.

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"Tu n'es plus là où tu étais, mais tu es partout là où je suis. "

Victor. Hugo

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# Foreword

Temperature has an essential role for living organisms as it regulates a large part of their physiological and biological processes. In the current context of global warming, a better understanding of the impact of temperature on living organisms is crucial. According to the AR6 IPCC (2023) latest report (Calvin et al., 2023) global temperatures will rise by 1.5°C in 2030, ten years earlier than the IPCC's previous forecast (AR5, IPCC 2014: Pachauri et al., 2014). According to the IPCC (2023), "*global warming is now a reality, with consequences irreversible for centuries or millennia*". Freshwaters are particularly vulnerable to climate change as they are climate-dependent shallow habitat with poor migration and dispersion possibilities and are particularly exposed to anthropogenic stressors (Dudgeon et al., 2006; Woodward et al., 2010). Rising temperatures particularly affect freshwater ectotherms whose body temperature depends on environmental temperature (Falfushynska et al., 2016; Woodward et al., 2010).

Many studies focused on the individual or population scales rather than higher levels of organization (i.e. communities, ecosystems). The complexity of the impact of temperature lies in the multi-scale ranging from the metabolism of individuals (Arrhenius, 1896; Fry and Hart, 1948) through the biology of organisms to the functioning of ecosystems (Vasseur et al., 2014; Jeppesen et al., 2014; Jeppensen et al., 2010; Pereira et al., 2010) (Fig.1). Moreover, there is intraspecific and interspecific variation in ecotherms organisms responses to temperature exposure (Fangue et al., 2006; Klok, J, & Chown, 2003; Seebacher et al., 2010). Quantitative theories such as the Metabolic Ecological theory have been focusing on the multiscale aspects of ecological systems since the 2000s. However, to better anticipate the response of organisms to current and future climate change, and to propose tools to protect ecosystems and aquatic biodiversity, it is necessary to take into account empirically the different temporal and biological scales at which temperature acts on ecological systems.

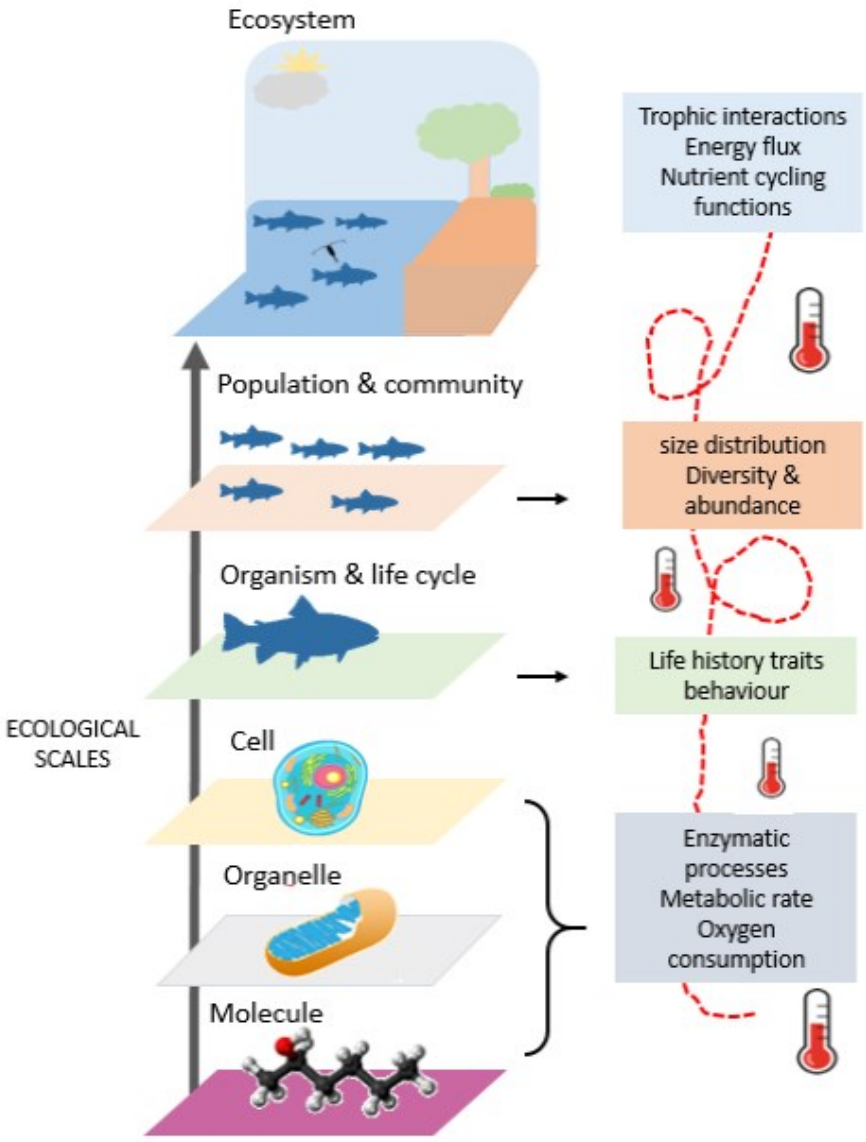


***Figure 1:*** *Conceptual diagram of the nested ecological scales and their links to temperature.*

# General Introduction

*« Je suis de ceux qui pensent que la science est d'une grande beauté.*
*Un scientifique dans son laboratoire est non seulement un technicien:*
*Il est aussi un enfant placé devant des phénomènes*
*naturels qui l'impressionnent comme des contes de fées. »*

**Marie Curie**
*Physicienne, Scientifique (1867 - 1934)*
*Madame Curie, ed. Gallimard, Eve Curie*

# 1. Climate change and rising temperatures

## 1.1 Current climate context and global changes

Arrhenius was among the first in 1896 to suggest a link between the burning of fossil resources at the beginning of the industrial era and an increase in global atmospheric temperature, proposing a relationship between temperature and the concentration of carbon dioxide in the atmosphere (Arrhenius, 1896). However, we had to wait until 1972 for the emergence of the first United Nations conference on the environment and 1990 for the first assessment report of the Intergovernmental Panel on Climate Change (IPCC) recognizing the responsibility of Human in global warming. For several years, the influence of Human activities on the climate system has been clearly established. Anthropogenic emissions of greenhouse gases, mainly carbon dioxide and methane are the highest ever observed, reinforcing the natural greenhouse effect and inducing unprecedented warming on a global scale (Fig.2ab; IPCC, 2014; IPCC 2023). In addition, climate change is accelerating and concerns are growing around the impacts of future climatic crisis. In September 2023, the United Nations Secretary General António Guterres, announced that the *'climate breakdown has begun'* after record summer heat and he stressed that it is time to "*turn up the heat now for climate solutions*."

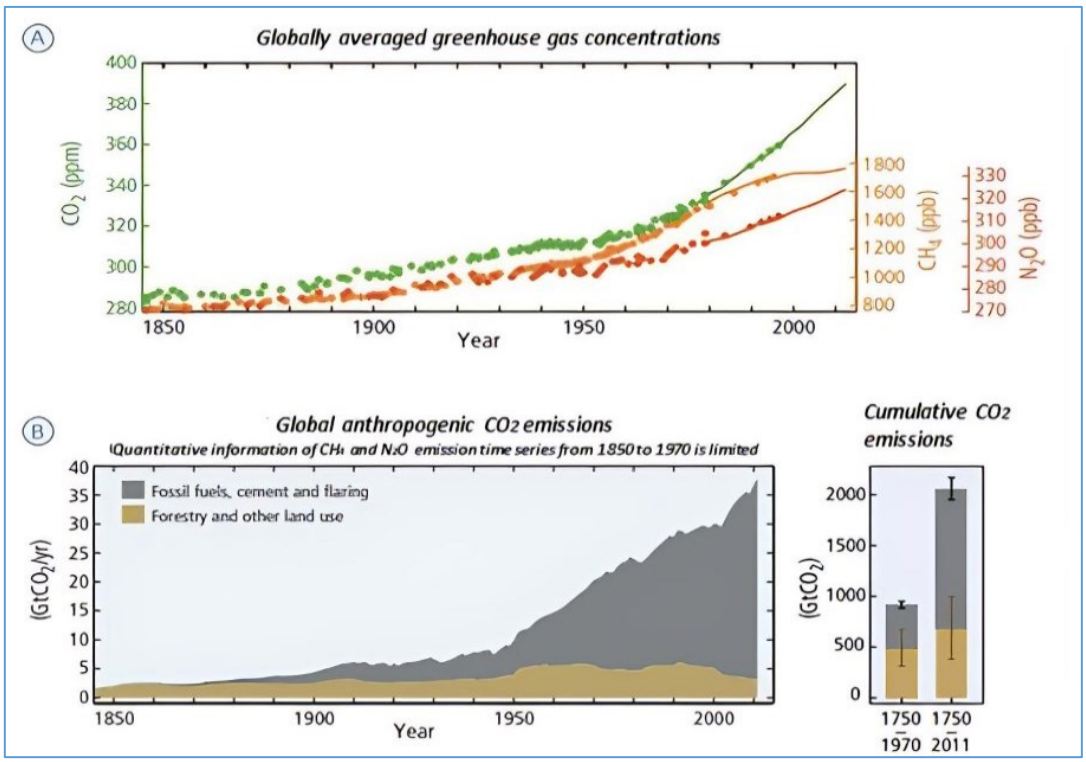


***Figure 2**: (A) Atmospheric concentrations of greenhouse gases carbon dioxide ($CO_2$, green), methane ($CH_4$, orange) and nitrous oxide ($N_2O$, red) determined from ice core data (dots) and from direct atmospheric measurements (lines). (From IPCC 2014). (B) Global anthropogenic $CO_2$ emissions from forestry and other land use as well as from burning of fossil fuel, cement production and flaring. Cumulative emissions of $CO_2$ from these sources and their uncertainties are shown as bars and whiskers, respectively, on the right hand side. (From IPCC 2014)*

Of all climatic variables, the increase in temperatures is one of the most important factors of climate change and the subject of numerous predictions. The observation that the average temperature of the planet increased by around 0.85°C over the period 1880-2012 and that temperature has already risen by 1.1°C over the period 2011-2020 compared to 1850-1900 alarms us about future risks. Human activities, principally through emissions of greenhouse gases, have unequivocally caused global warming. IPCC (2014) predicted an increase by 4°C in 2100 (RCP. 8.5) (Fig.3ab; Fig.4a; IPCC, 2014) and latest IPCC predictions based on Earth System Model projections (AR6, IPCC, 2023) suggest a general increase across the globe up to 1.5°C by 2030. However, 2023 is already the hottest year on record, almost 1.5°C above pre-industrial levels (ERA5 dataset).

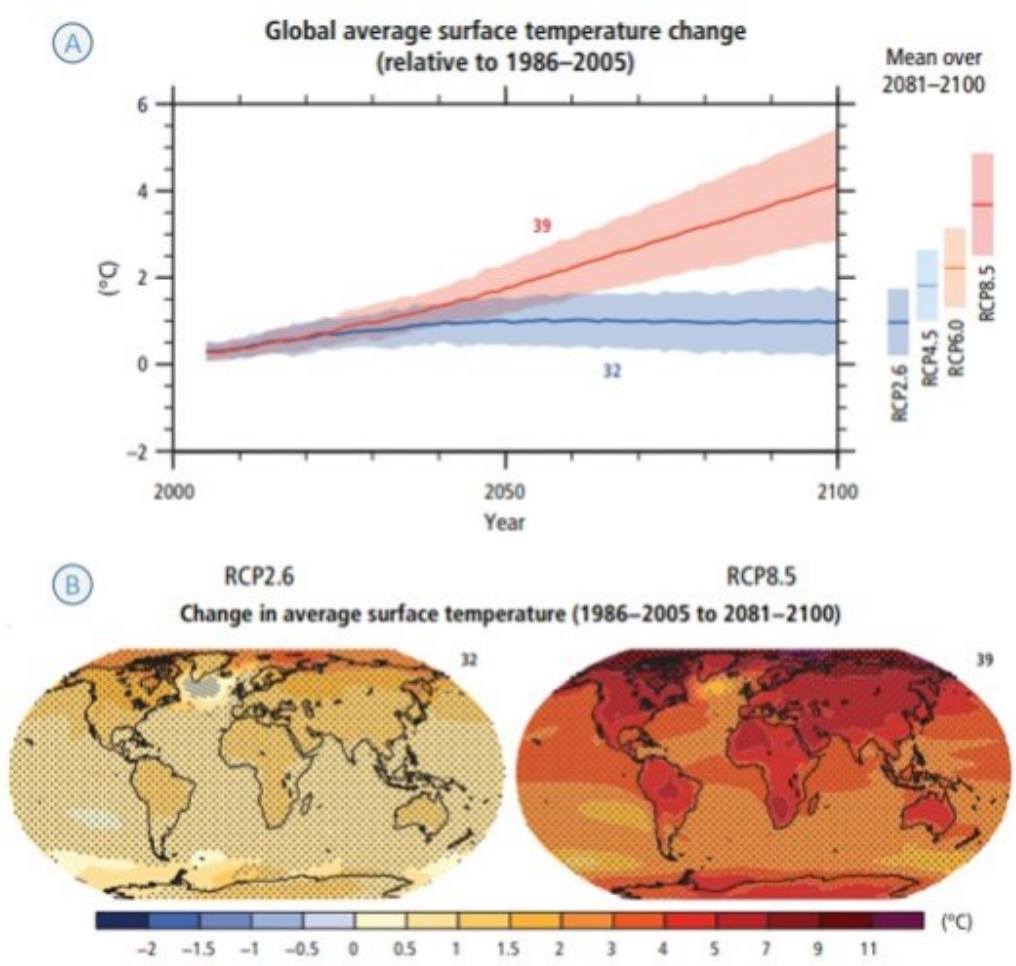


***Figure 3**: (A) Global average surface temperature change from 2006 to 2100 as determined by multi-model simulations. All changes are relative to 1986–2005. Time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (blue) (optimistic scenario) and RCP8.5 (red) (pessimistic scenario). The mean and associated uncertainties averaged over 2081–2100 are given for all RCP (i.e. Representative Concentration Pathway) scenarios as coloured vertical bars at the right hand side of each panel. (B) Change in average surface temperature based on multi-model mean projections for 2081–2100 relative to 1986–2005 under the RCP2.6 (left: scenario the most optimistic corresponding to a radiative forcing of +2.6 W/m2) and RCP8.5 (right scenario, the least optimistic corresponding to a radiative forcing of +8.5 W/m2) (from IPCC 2014).*

Besides temperature, other environmental changes will occur, linked or not to the increase in temperature, with important ecological impacts. Climate change, due to the main impacts on the water cycle, result globally in a modification of the physical characteristics of environments through an intensification of extreme climatic events since 1950 (IPCC, 2014). These events are associated with changes in precipitation patterns (heavy rain, hail, monsoons), an increase in natural extreme events (such as hurricanes and cyclones), and an increase in periods of aridity and drought having repercussions on terrestrial and aquatic environments (e.g. increasing ocean salinity due to higher evaporation rates) (IPCC, 2014; 2023). Global warming also leads to a dilatation of sea water which, coupled with the intensification of the melting of glaciers under thermal pressure (Fig. 4b), has resulted in an increase of the average ocean level by about 20 cm between 1901 and 2018 (IPCC 2023; IPCC 2014; Fig. 4c). Then, the increase in atmospheric $CO_2$ concentrations leads to an increase in $CO_2$ trapped in the oceans (around a third of atmospheric $CO_2$). The presence of $CO_2$ trapped in large quantities is causing a chemical reaction, resulting in ocean acidification and in a decrease in water pH due to high $HCO_3$ concentrations. Overall, IPPC Earth System Model projections indicate a decline in surface ocean pH toward the end of the 21st century. The most pessimistic scenarios (RCP8.5) suggests a decrease in pH ranging from 0.30 to 0.32 (increase in acidity from 100 to 109%) by 2100 (IPCC 2014; Fig. 4d) resulting in the destruction of the calcareous structures of organisms such as corals, shelled molluscs or planktonic exoskeletons. Finally, increased temperatures, disturbances of currents and eutrophication phenomena (i.e. concentration of nutrients from agriculture and wastewater discharge) can lead to hypoxia (i.e. oxygen concentration is insufficient to persist) (Qian et al., 2018). The IPCC has defined that some of these modifications can take hundreds or even thousands years to revert even if greenhouse gas emissions are reduced.

Thus, environmental factors such as global warming and the expected significant increase in temperature will impose strong constraints on organisms, populations, communities and ecosystems (IPCC 2023). The increase in temperature will have an impact on the survival and distribution of organisms by bringing organisms closer to their thermal tolerance range and by disrupting their life cycle (Barbarossa et al., 2021; Poloczanska et al., 2013). Only few options are available to organisms to cope with such pressure.

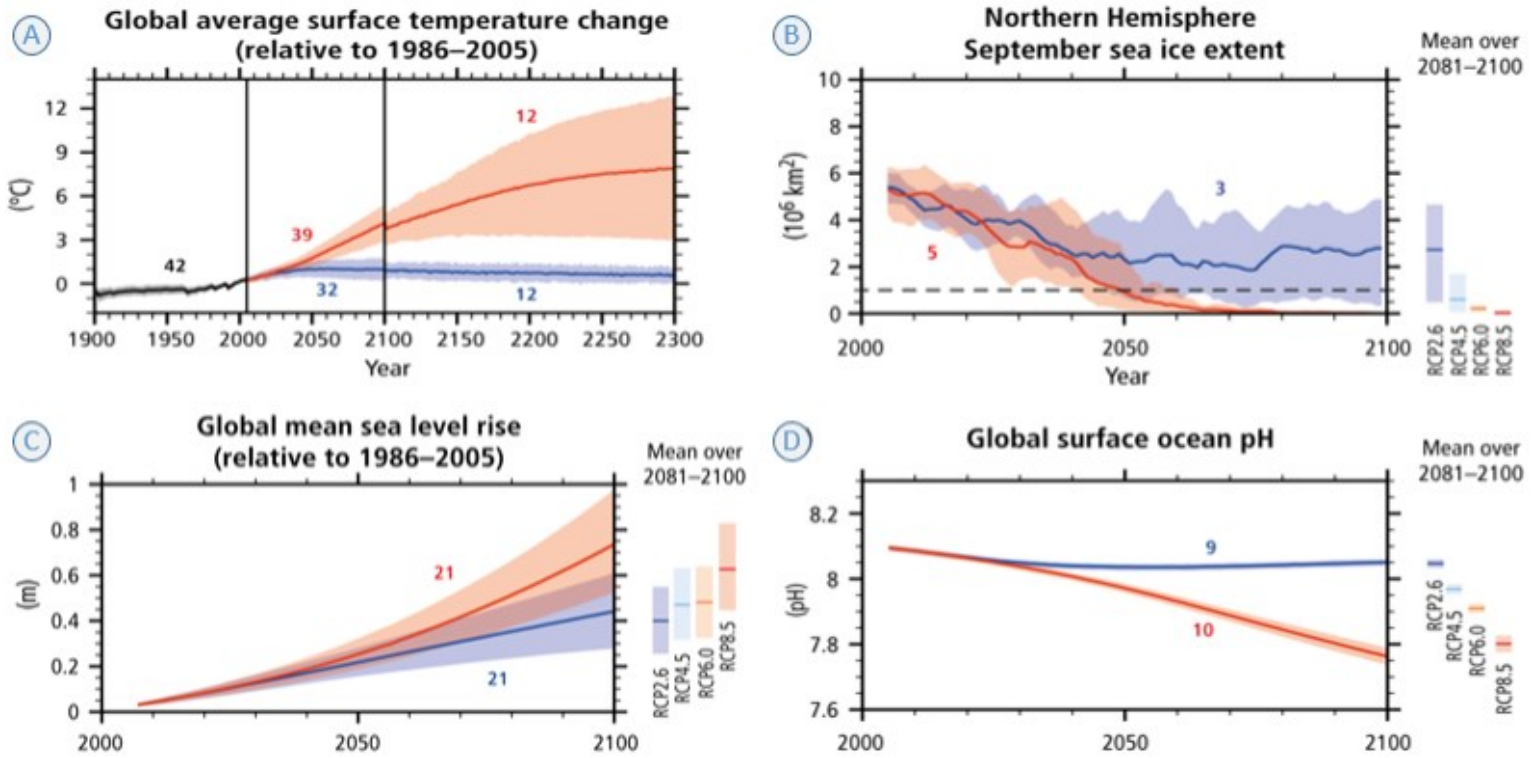


***Figure 4:*** *(A) Time series of global annual change in mean surface temperature for the 1900–2300 period (relative to 1986–2005) from Coupled Model Inter-comparison Project Phase 5 (CMIP5) concentration-driven experiments. Projections are shown for the multi-model mean (solid lines) and the 5% to 95% range across the distribution of individual models (shading). Grey lines and shading represent the CMIP5 historical simulations. (B) Change in Northern Hemisphere September sea-ice extent (5 year running mean). The dashed line represents nearly ice-free conditions (i.e., when September sea-ice extent is less than 106 km² for at least five consecutive years). (C) Change in global mean sea level. (D) Change in ocean surface pH. For all panels, changes are relative to the 1986–2005 period; time series of projections and a measure of uncertainty (shading) are shown for scenarios RCP2.6 (blue) and RCP8.5 (red). (From IPCC, 2014).*

## 1.2 How organisms can cope with temperature increase?

The complexity of the impact of environmental factors such as temperature on living organisms lies in the fact that their influence depends on the duration of exposure, the degree of exposure and the nature of organisms and ecosystems.

Temperature effect over a short period of time relative to an individual's lifespan, mainly results in plastic responses (i.e. capacity for expression of several phenotypes from the same genotype under different environmental constraints; Pigliucci, 2001; Pigliucci et al., 2006) following acclimation (i.e. coordinated phenotypic response developed by the animal to a specific stressor in the environment; Fregly et al., 1996). Thus an organism with strong phenotypic plasticity for a particular trait will be able to produce different versions of this trait depending on the thermal regime to which it will be exposed (Fig. 5a). Acclimation through phenotypic plasticity can allow organisms to deal with environmental changes and provide populations time to adapt genetically in the long-term particularly for organisms with a relatively long generation time such as fish (Crozier and Hutchings, 2014). Three general forms of plasticity are recognized (Angilletta., 2009): (i) reversible plasticity which occurs over a few days/months, in response to environmental changes, (ii) developmental plasticity, where exposure to an environmental condition in early life influences performance in that environment later in life, and (iii) transgenerational plasticity, where environmental conditions experienced by parents have a significant effect on offspring performance in that environment (Donelson et al., 2012). Animals can improve their performance in a new environment (i.e., acclimated) through one or more of these forms of plasticity (Fig. 5b). Acclimation through developmental and transgenerational plasticity is particularly important in the context of climate change (Boukal et al., 2019; Loisel et al., 2019). Temperature can also have a long-term effect corresponding to an exposition over several generations, which may result from local genetic adaptation over generations (Geerts et al., 2015). Thus, species response to new thermal conditions may be the result of natural selection. When selection is involved, a new form of the phenotypic trait is favoured, this will correspond to an adaptive response, and the fitness of the individual will be maintained. Finally, epigenetic mechanisms that reversibly, transmissibly and adaptively modify gene expression without changing the nucleotide sequence (i.e. any mitotically or meiotically heritable contribution to the phenotype that does not involve a change in DNA sequence) can also affect the response of organisms (Sentis et al., 2018; Shen et al., 2023).

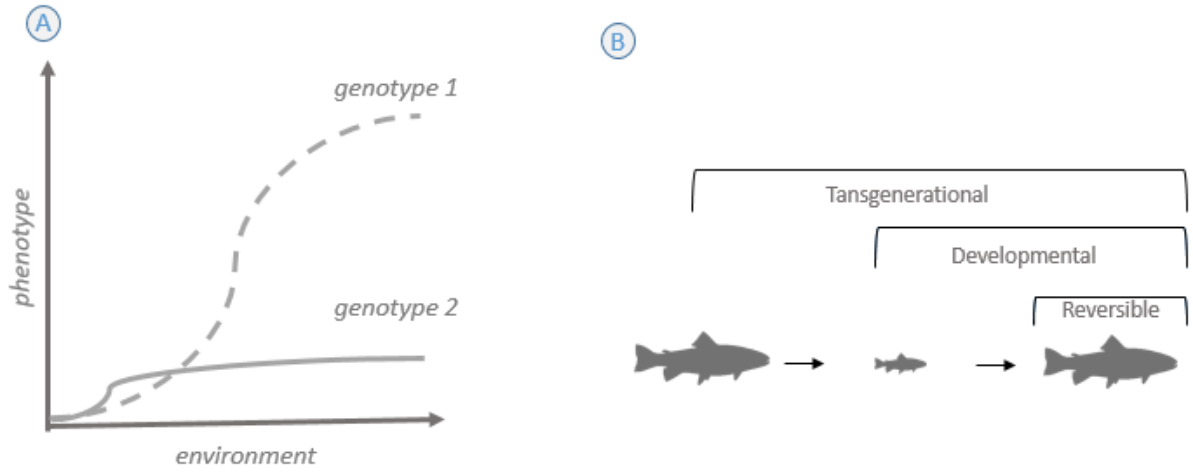


***Figure 5**: (A) ability to express several phenotypes from the same genotype according to different environmental constraints for two different genotypes. (B) Three general forms of phenotypic plasticity: (i) reversible plasticity that occurs over days to months, (ii) developmental plasticity, where exposure to an environmental condition in early life influences performance in that environment later in life, and (iii) transgenerational plasticity, where the environmental conditions experienced by parents have a significant effect on the performance of the offspring in that environment. (adapted from Rummer and Munday, 2017).*

The short and long-term effects of temperature influence the biology (growth, reproduction, senescence) and physiology of organisms (cellular, enzymatic/protein functioning), the density and diversity of populations, the structure of communities, trophic networks and the functioning of ecosystems (productivity, resource management) and can even determine the survival of species and their geographic distribution (Abele et al., 2002; Angilletta., 2009; Bideault et al., 2019; Butchart et al., 2010; Clotfelter et al., 2013; Daufresne et al., 2009; Jeppesen et al., 2014; Ohlberger, 2013; Sentis et al., 2012; IUCN, 2022).

Throughout the scientific literature, three major universal responses to global warming are most commonly observed: (i) Changes in phenology (i.e. chronological variations in the life cycle of organisms determined by thermal conditions and seasonality like hatching, emergence or reproduction) which can lead to temporal mismatches between consumers and resource dynamics (Fig. 6a; Cushing, 1974; Dembski et al., 2006; Jeppesen et al., 2014; Visser et al., 2006). (ii) Rapid changes in distribution areas through shifts in species ranges to higher altitudes and latitudes, which can lead to the arrival of new species in an existing environment and the extinction of native ones, potentially altering the ecosystem ecological processes. This phenomenon seems to occur more quickly in aquatic than in terrestrial species (Fig. 6b; Lenoir et al., 2020; Parmesan and Yohe, 2003; Root et al., 2003; Sunday et al., 2012). Finally, (iii) a decrease in the body size of organisms and populations which can alter ecosystem interactions and food-web structure (Fig. 6c; (Atkinson, 1994; Daufresne et al., 2009; Edeline et al., 2013; Gardner et al., 2011; Horne et al., 2017, 2015; Ohlberger, 2013; Sheridan and Bickford, 2011).

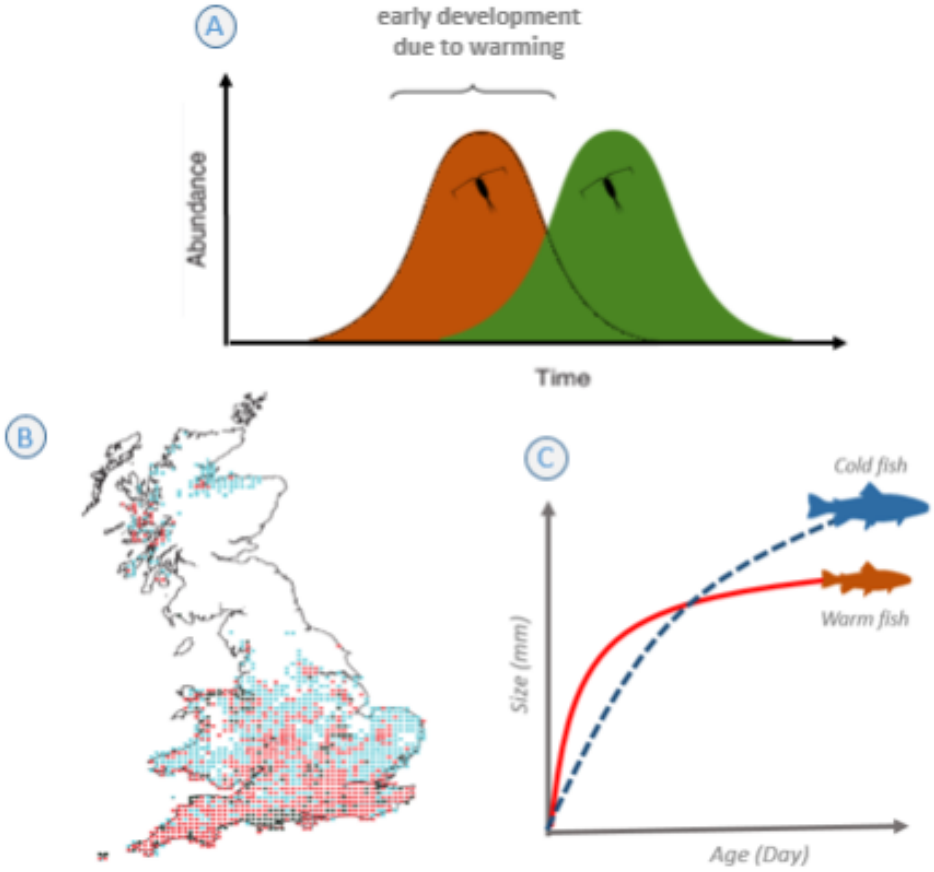


***Figure 6**: (A) the Cushing's match-mismatch hypothesis (adapted from Cushing, 1974). (B) Poleward shifts of species' ranges associated with regional warming. The cells indicate the presence of at least one tircis population recorded during national monitoring between 1915 and 1939 (dark green), between 1940 and 1969 (red) and between 1970 and 1977 (blue), showing a northward extension of the range of Pararge aegeria in Great Britain (from Parmesan et al. 1999). (C) TSR pattern (adapted from Atkinson 1994).*

More specifically, the climatic dependence of thermal and hydrological regimes, the high heat capacity of the water and the limited dispersal capacity of many freshwater organisms (Comte and Grenouillet, 2013; Domisch et al., 2013) make freshwater ecosystems particularly vulnerable to climate change despite their crucial importance for living organisms and humans.

## 1.3 Vulnerability of freshwater ecosystems to climate change

Freshwater ecosystems represents ~3% of the terrestrial surface (Downing et al., 2006), including lakes, ponds, rivers, streams, springs, peat bogs and wetlands. Freshwater ecosystems are particularly sensitive to increases in water temperature and changes in the hydrological cycle (Capon et al., 2021; Livingstone and Dokulil, 2001; Magnuson et al., 1997; Schindler, 1990). In addition, they are warming-up faster than many terrestrial or marine habitats and are among the most disturbed and vulnerable ecosystems (Dudgeon

et al., 2006; Falfushynska et al., 2016; Haase et al., 2023; Woodward et al., 2010). Finally, the strength of the temperature-size response is greater in aquatic than terrestrial species (Forster et al., 2012) and aquatic food webs are generally more structured by size than terrestrial ecosystems (Potapov et al., 2019; Shurin et al., 2012, 2006).

The consequences of climate change are more significant in spatially constrained environments such as ponds or lakes. Indeed, their small surface area and their low mixing favour acidification and evaporation and also eutrophication, ultimately leading to hypoxia (Adrian et al., 2009; Crozier and Hutchings, 2014; Jeppesen et al., 2010; Woodward et al., 2010). Oxygen is a key factor in the interactions between metabolism, temperature and the biological functions of organisms. However, the distribution of oxygen is not always homogeneous, the diffusivity of oxygen is lower in aquatic environments than in aerial environment and, the solubility of oxygen decreases when temperature increases (Forster et al., 2012; Horne et al., 2015; Verberk et al., 2011). Therefore, a rapid increase in temperature, particularly in aquatic environments and shallow areas sensitive to air temperature fluctuations, is a critical stressor for organisms with cascading impacts on biodiversity and ecosystems functioning (Crozier and Hutchings, 2014; Horne et al., 2015; Woodward, 2009). Together, these characteristics make freshwater ecosystems some of the most threatened ecosystems on the globe (Dudgeon et al., 2006).

In this thesis, I focus on freshwater ecosystems and the key role of temperature on ectothermic aquatic organisms at several scales, from physiology through life-history traits to ecosystem processes. A large part of the thesis focuses on cellular metabolism, that is particularly sensitive to temperature and plays a determining role in most of biological and ecological processes of organisms and ecosystems (Brown et al., 2004; Gillooly, 2001).

# 2. Increase in temperatures & physiology of organisms

## 2.1 Temperature & metabolism

According to Brown et al (2004), "*Metabolism provides a basis for using first principles of physics, chemistry, and biology to link the biology of individual organisms to the ecology of populations, communities, and ecosystems*". The metabolism of organisms corresponds to all the biochemical transformation processes (i.e. chemical reactions of molecules synthesis and degradation necessary for the functioning of a cell) occurring in the tissues of living organisms. These biochemical processes allow taking advantage of environmental resources (i.e. oxygen, nutrients) by converting them into energy resources necessary for biological processes (i.e. growth, reproduction, survival) (Brown et al., 2004; Harman, 1956; Kirkwood and Holliday, 1979).

Arrhenius in 1889 early established the thermal dependence of the speed of chemical reactions (Arrhenius, 1889). Gillooly et al. (2001) proposed later a formulation derived

from the Kleiber equation (1932) and the Arrhenius law (1889) (BOX. 1) to describe the dependence of individual metabolism ($I$) on mass ($M$) and temperature ($T$, in Kelvin):

$$Eq.1: I=I_0 M^b \exp^{(-E/kT)}$$

Where the metabolic rate ($I$) increases with body temperature ($T$, expressed in degrees Kelvin) (Brown et al., 2004) and depends on the mass $M$ of the organism, $b$ being the allometric coefficient, $k$ the Boltzmann constant, $E$ the activation energy, and $I_0$ a constant independent of mass and temperature (Gillooly, 2001).

**BOX 1: COMPONANTE OF THE METABOLISM EQUATION (I)**

TEMPERATURE

At increased temperatures, chemical reactions proceed faster. This relationship is described by the Boltzmann factor (1872) or the Van't Hoff-Arrhenius relation (1889), where $E$ is activation energy in electronvolts or joules, $T$ is the absolute temperature in kelvins, and $k$ is the Boltzmann constant in $eV/K$ or $J/K$:

$$Eq.2: e^{-E/kT}$$

SIZE:

Huxley (1932) highlighted that most size-related variation can be described by so-called allometric equations, which are power functions of the form:

$$Eq.3: Y = Y_0 M^b$$

$Y$, such as metabolic rate, a body mass, $M$, and two coefficients, a normalization constant, $Y_0$, and an allometric exponent, $b$.

According to Kleiber's law (1932), metabolic rate scales with the mass of an organism of a given species, where $B$ is whole organism metabolic rate, $M$ is organism mass and $B_0$ is a mass-independent normalization constant:

$$Eq.4: B = B_0 . M^{3/4}$$

Thus, exposure to heat leads to changes in the kinetic of a multitude of chemical and enzymatic reactions underpinning physiological responses. Some of these physiological responses occur into mitochondria, organelles that produce energy for cells and are therefore particularly interesting for understanding how temperature influences organisms.

## 2. 2 Temperature & mitochondria

Mitochondria are organelles often defined as "*the Powerhouse of the Cell*" because they determine the amount of energy available to maintain the essential functions of the body.

The mitochondria (Fig.7) is found in the cytoplasm of most cell, measuring few µm in diameter. They contain the enzymatic systems required to convert the energy of nutrients into cellular energy by producing ATP (Adenosine Triphosphate) within the respiratory chain constituted of 4 protein complexes and an ATP synthase pump (Fig. 8, BOX.2). This conversion process is called oxidative phosphorylation (OXPHOS), corresponding to the coupling between the oxidation of substrates and the phosphorylation of ADP (Adenosine Diphosphate) into ATP using the energy released by the oxidation of electron donors in the respiratory chain. In more detail, protein complexes I and II of the respiratory chain oxidize substrates resulting from the products of the Krebs cycle and glycolysis, releasing electrons, which are transported along the respiratory chain by two mobile transporters to the oxygen (known as the terminal electron acceptor). Each electron transfer causes the expulsion of protons ($H^+$) from the matrix towards the intermembrane space. This processes leads to a proton gradient (i.e. proton-motive force, BOX.2) that is used by the mitochondria to synthesize ATP from ADP thanks to the passage of protons through the ATP synthase pump (Fig. 8) (Mitchell, 1966).

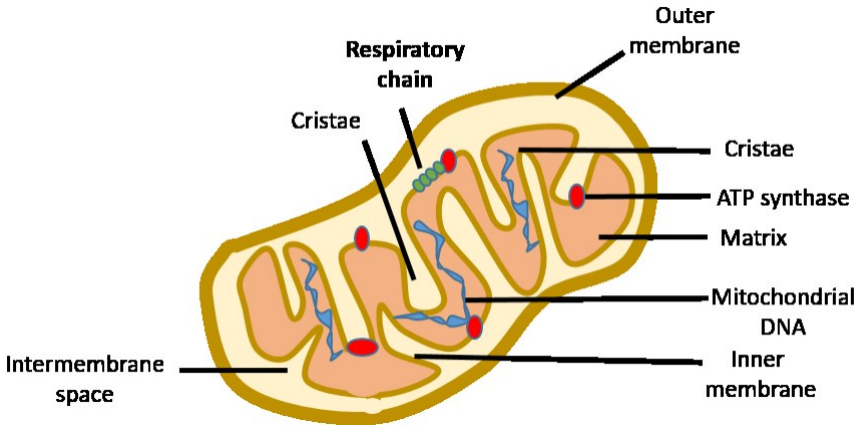


***Figure 7** : An illustration of a mitochondria*

In the majority of cases, the electrons released by the oxidation of substrates are transported along the respiratory chain. However, some electrons can spontaneously reduce oxygen, leading to the production of Reactive oxygen species (i.e. ROS), molecules containing at least one oxygen atom and one or more unpaired electrons including oxygen free radicals (e.g. superoxide anion $O_2^-$) (Harman, 1972). Harman (1956) postulated that the use of oxygen at the cellular level during mitochondrial respiration causes tissue damage due to the production of these free radicals (Harman, 1956). During the life of the organism, the accumulation of these cellular wastes could accelerate senescence (i.e. the deterioration of biological functions of individuals with age) and death (Rubner, 1908). However, the involvement of oxidative damage in the senescence remains complex (Finkel and Holbrook, 2000; Sohal and Weindruch, 1996). Protons can also return to the matrix independently of ATP synthase, a process known as proton leak. In this case, protons energy is not transformed into chemical energy (ATP) but into heat. This processes is referred to as mitochondrial uncoupling (i.e. any decrease in the proton motive force not linked to ATP synthesis which uncouples the oxidation of substrates

from phosphorylation). This leak of protons is possible either by phospholipids of the internal membrane or by proteins such as UCPs (i.e. Uncoupling Proteins) (Blanc et al., 2003; Nègre-Salvayre et al., 1997; Ricquier and Bouillaud, 2000). According to some authors, strong uncoupling would thus limit the production of free radicals at the cost of lower efficiency in terms of ATP production per molecule of oxygen consumed (Brand, 2000; Skulachev, 1998). This could thus lead to a trade off between energy production and ROS production, needed to be confirmed by future studies.

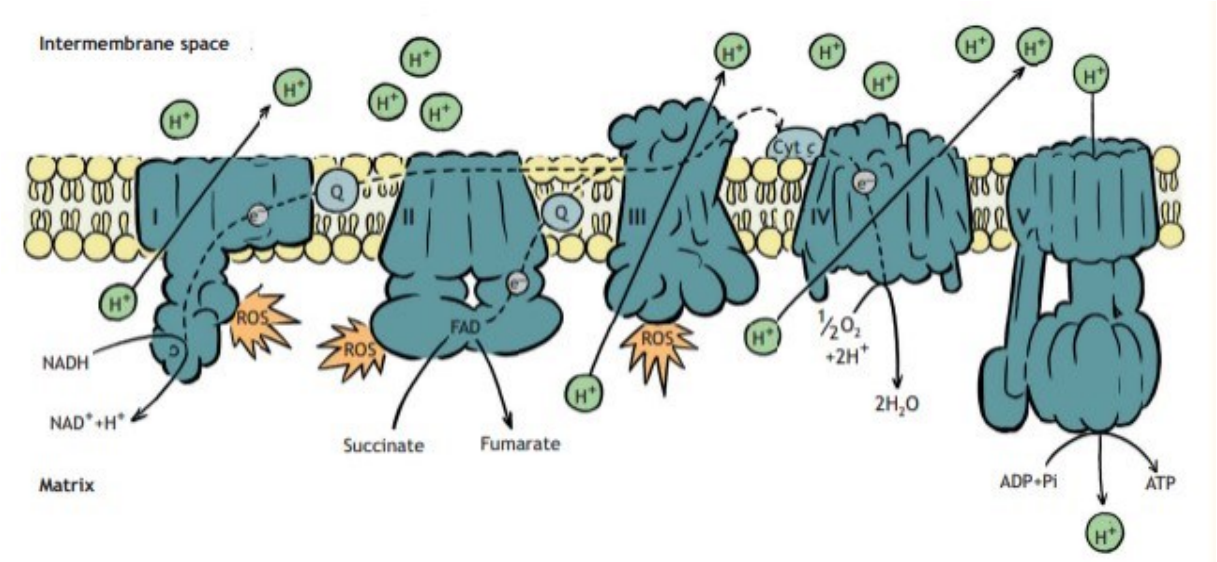


***Figure 8**: mitochondrial respiratory chain (adapted from Chung and Schulte, 2020): the electron transport system (ETS) accepts electrons from reducing equivalents and generates a proton gradient across the inner mitochondrial membrane (IMM) that is used to synthesize ATP (Mitchell, 1961). ETS function generates free radicals (e.g. reactive oxygen species; ROS) as a by-product. These become damaging as their production exceeds mitochondrial and cellular scavenging capacity (Munro and Treberg, 2017; Murphy, 2009). The generation of free radicals is primarily associated with complexes I and III (Brand, 2010), and, to a lesser degree, complex II (Quinlan et al., 2012).*

**BOX 2: RESPIRATORY CHAIN COMPLEXES**

**Proton motive force:** An electrochemical gradient composed of a pH gradient and an electrical potential component (Ψm) driving proton movement across the inner mitochondrial membrane, used to synthesise ATP from ADP.

**Complex I** (NADH ubiquinone oxidoreductase) oxidises NADH, releasing electrons transported along the respiratory chain to oxygen, the final acceptor. Each electron transfer causes the expulsion of protons ($H^+$) from the matrix into the intermembrane space.

**Complex II** (succinate dehydrogenase) oxidises $FADH_2$, releasing electrons transported along the respiratory chain to oxygen. Unlike Complex I, no protons are transported to the intermembrane space in this pathway.

**Complex III** ($CoQH_2$-cytochrome c reductase) transports 4 protons from the mitochondrial matrix to the intermembrane space.

**Complex IV** (cytochrome c oxidase) catalyzes the dioxygen reduction reaction (i.e. the transfer of electrons to an $O_2$ molecule, while pumping protons across the inner mitochondrial membrane). Two $H_2O$ molecules are formed per reduced oxygen molecule, while four protons are expelled towards the mitochondrial intermembrane space.

**ATP synthase** is sometimes described as Complex V of the electron transport chain. Allow the phosphorylation of ADP into ATP thanks to the proton motive force.

Many mitochondrial reactions are impacted by an acute increase in temperatures (Abele et al., 2002, 1998; Chung and Schulte, 2015; Roussel and Voituron, 2020). According to previous studies, the exposure of mitochondria to high temperatures results in a multitude of biochemical, enzymatic, kinetic and protein changes. The main reported affects are:

(1) An increase in the rate of cellular respiration, which results in an exponential increase in the rate of oxygen molecule consumed (Abele, 2011; Abele et al., 2002, 1998; Chung and Schulte, 2015; Hilton et al., 2010; Keller et al., 2004; Roussel and Voituron, 2020; Somero et al., 2017).

(2) An increase in cellular damages (i.e. production of free radicals) linked to the acceleration of cellular respiration (Abele et al., 2002; Clotfelter et al., 2013; Keller et al., 2004; Lushchak, 2011; Roussel and Voituron, 2020; Salin et al., 2019).

(3) A modification/alteration of the activities and functionalities of several mitochondrial enzymes and enzyme complexes, membrane proteins, and an increase in the fluidity of mitochondrial membranes (Abele et al., 1998; Clotfelter et al., 2013; Dahlhoff et al., 1991; Somero, 2010; Somero et al., 2017).

(4) A high level of uncoupling (Blanc et al., 2003; Brand, 2000; Nègre-Salvayre et al., 1997; Ricquier and Bouillaud, 2000, 1998).

In parallel, acclimation can induce a modification of mitochondrial structure and function (Guderley and St-Pierre, 2002; Seebacher et al., 2010). Mitochondrial density is also known to be modified by long-term temperature exposure resulting in lower mitochondrial density following exposure to warming (Dhillon and Schulte, 2011; Guderley, 2004; Keller et al., 2004; Kraffe et al., 2007; Schulte, 2015; Tyler and Sidell, 1984). Warm acclimation may results in decreased mitochondrial metabolism at high temperatures, due to decreased mitochondrial respiration rates, decreased mitochondrial density, or changes in mitochondrial membrane composition (Chung et al., 2017; Ekström et al., 2016; Schulte, 2015; Yan and Xie, 2015). Finally, thermally induced mitochondrial changes, such as adjustments in mitochondrial densities or loss of the ability to generate ATP, can contribute to setting limits of whole-organism thermal tolerance (Harada et al., 2019; Healy and Burton, 2023; Iftikar et al., 2014; Iftikar and Hickey, 2013; Pörtner, 2001).

## 2.3 Temperature and thermal tolerances of organisms

Temperature directly influences the kinetics of biochemical reactions underlying the cellular activity of organisms (Hochachka and Somero, 2002). This thermal dependence therefore affects the performance of individuals. However, the thermal sensitivity of metabolism differs between organisms. Indeed, we can briefly differentiate organisms into two groups, ectotherms and endotherms.

Endotherms can control their body temperature through their internal metabolism by producing thermal energy (i.e. thermoregulation). However, the internal temperature in ectotherms is directly influenced by the environmental temperature (i.e. no or very weak thermoregulation) (Fig. 9). They mainly use physiological and behavioural means to adjust their body temperature (Beitinger and Fitzpatrick, 1979; Brattstrom, 1979; Lagerspetz and Vainio, 2006; Heinrich 1977; Danks 2004). An increase in the environmental temperature will quickly accelerate their metabolism. However, beyond the organisms' optimum temperatures, a decline in physiological and biological processes can be observed (Martínez et al., 2016).

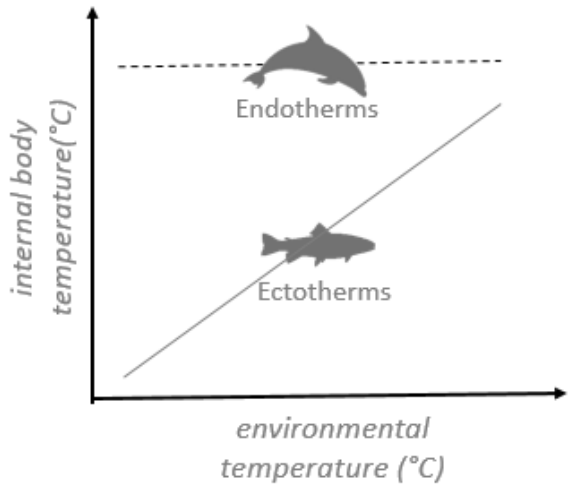


***Figure 9**: Relationship between internal and external temperatures in endotherms (grey solid line) and ectotherms (black dotted line).*

There are several ways to measure the thermal limits of an organism. Firstly, the temperature-performance relationship can be modelled by a "thermal performance curve" (BOX.3) (Schulte et al., 2011). Such curves are mainly composed of: (1) an ascending phase as temperature increases; (2) a “plateau” phase that encompasses the thermal optimum (Topt) and (3) a steep declining phase at higher temperatures. Thus, for each species we can define specific tolerated temperature ranges in which optimum temperature values represent maximum performance (T°Opt) as well as temperatures at which performance is negatively affected and where the species survival is jeopardized, defined as upper thermal limit (Tmax) and Lower thermal limit (Tmin) (Fig. 10) (Angilletta., 2009; Dell et al., 2011; Huey and Stevenson, 1979; Krenek et al., 2012; Martínez et al., 2016).

Thermal limits calculated from the shape of the thermal performance curve (TPC) are generally used for a particular trait and defined as either the temperature that causes a performance characteristic to fall to a predefined level below its optimal value or to zero. Most studies describing thermal performance curve expose groups of organisms to a range of temperatures and measure their performance at each temperature. Given that estimating the shape and position of thermal performance curve requires multiple measurements at several temperatures for one trait, specific methods have been developed to a point estimate of critical thermal limits.

The two main methods are (1) the static or lethal-temperature (LT) method, developed primarily by F.E.J. Fry and colleagues (1942) involving holding duration constant while temperature is varied, with assessments based on survival of a given proportion of the exposed individuals. The resultant lethal temperature (LT50) is the temperature at which 50% of the individuals survive (i.e. the temperature lethal to 50% of individuals) (Brett 1944; Fry et al. 1942; Fry 1958) and (2) the dynamic method, which involves increasing temperature until a non-lethal end point (point of physiological failure) is reached (i.e. minimal and maximal critical thermal limits, CTmin and CTmax). Endpoint can either be defined as the loss of the righting reflex, loss of equilibrium, or the onset of muscle spasms at which the organism locomotion movements become disordered and no longer allow it to escape conditions that can lead to death (Beitinger et al., 2000; Ziegeweid et al., 2008; Cowles & Bogert, 1944). As mentioned in Schulte & chung 2020 the terms CTmax and Tmax are sometimes used interchangeably, but they derive from different tests and are not necessarily the same (Chung and Schulte, 2020; Ern et al., 2016; Jutfelt et al., 2019).

Performances of ectotherms organisms decreases after their thermal optimum ($T_{opt}$), with increasing temperature (Fig. 10), (Payne et al., 2021; Sunday et al., 2019). However, thermal tolerance varies with prior thermal exposure and can be improved after a period of acclimation to elevated temperature (Angilletta, 2001; Bilyk and DeVries, 2011). The upper thermal limits of organisms in the environment also depend on oxygen availability (Andreassen et al., 2022). This is of concern for aquatic environments where warming decreases oxygen solubility (Truesdale et al., 1955; Verberk et al., 2011) inducing a mismatch between demand in response to increased metabolic activity and the amount of oxygen available in the water (Atkinson and Sibly, 1996; Pörtner, 2002).

Recent studies suggest that, for many species, there can be a link between mitochondrial failure and whole-organism thermal limits (Chung and Schulte, 2020; Ekström et al., 2016; Fangue et al., 2009; Harada et al., 2019; Hraoui et al., 2020; Iftikar et al., 2014; Iftikar and Hickey, 2013; Martínez et al., 2016). Mitochondrial dysfunction (i.e. in terms of oxygen consumption or ATP production) is a possible mechanism underlying neuronal failure highlighting the link between mitochondrial failure (i.e. whether in terms of oxygen consumption or ATP production) and the 'failure' of the entire organism (Chung et al., 2017; Willis et al., 2021) that may constrain the thermal limits of the whole organism (Ern et al., 2015; Somero and DeVries, 1967). Nevertheless, the link between mitochondrial failure and thermal tolerance of the organism remains poorly understood (Clark et al., 2013; Ern et al., 2015; Ørsted et al., 2022). Moreover, conventional measures of mitochondrial function suggest that mitochondria can function at temperatures higher than those limiting organismal function (Chung and Schulte, 2020; Pörtner, 2002; Somero,

2002). Finally, the physiological thermal dependence of ectotherms and their thermal limits are determining in ecological studies because they strongly regulate their associated biological functions representing their life history traits (Angilletta 2004; Clotfelter et al. 2013; Sunday et al., 2019).

**BOX 3: THERMAL PERFORMANCE CURVE**

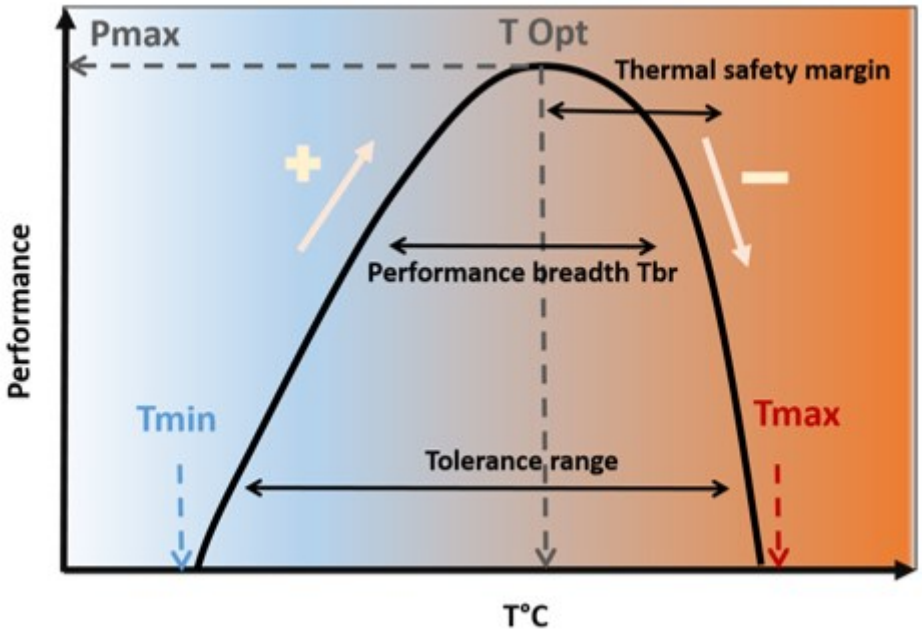


***Figure 10**: (A) General shape of a thermal performance curve describing the relationship between organismal performance and temperature. (Black line: follows a Gaussian, inverted 'U'-shaped curve), modified from Huey and Kingsolver (1989).*

**The optimum temperature** (Topt) – temperature at maximum performance.

**Tmax** – Upper thermal limit of performance.

**Tmin** – Lower thermal limit of performance.

**Thermal tolerance range** – range of temperatures tolerated by the organism delimited by Tmin and Tmax

**Performance breadth** is the range of temperatures over which a species maintained at least 80% of the peak curve.

**Thermal safety margin** – various definitions are in use, but TSM is generally inversely proportional to the risk of an animal experiencing temperatures above Tmax. Here, we define it as the difference between Topt and Tmax.

# 3. Response of life-history traits to elevated temperatures

## 3.1 Effects on development, survival and fertility

All phenotypic traits directly related to fitness of an organism are called life history traits. The main life history traits are fecundity (number of eggs, egg quality), size (size at hatching, growth rate and size at sexual maturity) and survival (survival at birth, lifespan) (Stearns, 1992). These traits are subject to natural selection, which can lead to maximizing the organism's selective value (i.e. fitness) and can also be influenced by the environment due to the plastic response of organisms (particularly in ectotherms).

The effect of short-term exposure to high temperature on ectotherms is well documented. Numerous studies have shown that short-term exposure of ectotherms to high temperatures increasing metabolism results in:

• An early sexual maturity; a reduction in egg incubation time, and an early emergence (Atkinson, 1995, 1994; Blaxter, 1992) (Fig. 11).

• Higher juvenile growth rates (Fig. 11); (Atkinson, 1994; Atkinson and Sibly, 1996).

• A reduction in adult body size (Fig. 11); (Atkinson, 1994; Cheung et al., 2013; Daufresne et al., 2009; Gardner et al., 2011; Horne et al., 2017; Munday et al., 2017; Sentis et al., 2017; Sheridan and Bickford, 2011).

• A reduction in lifespan leading to an accelerated life cycle but also to a reduction in the survival rate of adults (Fig. 11); (Dembski et al., 2006). As discussed in section 2.8, the production of cellular damages increases with warming which could explain why organisms die young under warming.

The effect of short-term exposure to high temperature on ectotherms seems to be confirmed after several generations' exposure to specific temperature. For example, a study on an ectothermic marine polychaete (*Ophryotrocha Labronica*) showed that multi-generational exposure (6 generations) to warming caused an increase in the development rate of juveniles, and a decrease in the average body size at maturity (Gibbin et al., 2016) A second study carried out on Medaka fish (*Orizias Latipes*) in 2019 presents a response pattern along the same lines after seven generations of exposure although the response pattern is less strong at the end of the generations compared to the beginning in F0 (Loisel, 2019).

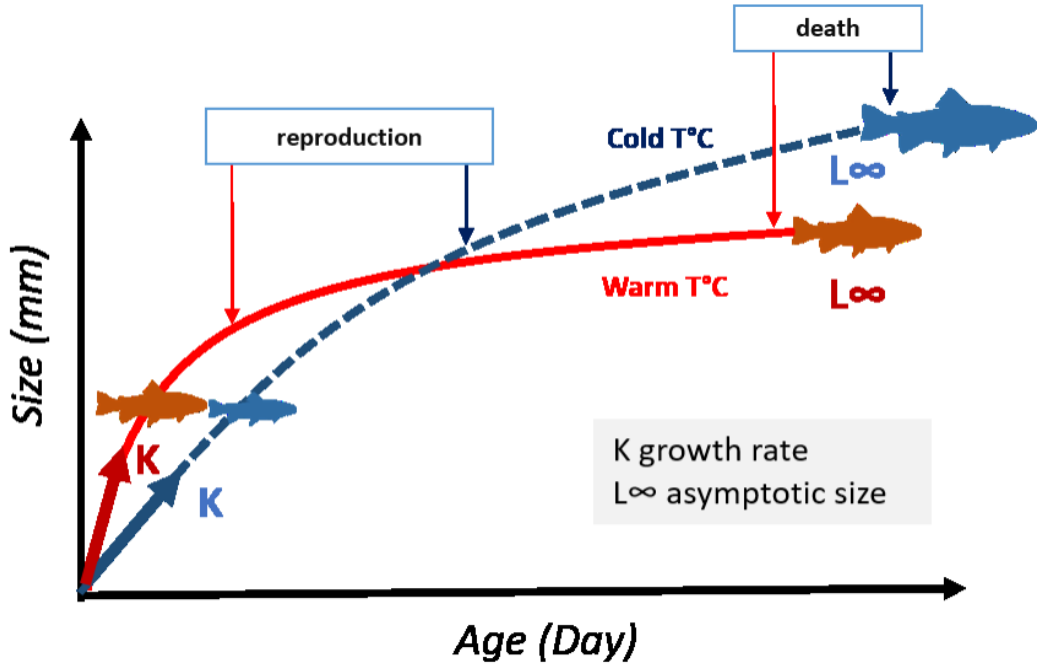


***Figure 11:*** *Effect of exposure to elevated temperature in ectotherms on key life-history traits (size, growth, survival)*

Several studies have attempted to highlight the link between life history traits and mitochondria in terms of maintenance, growth, reproduction and longevity, based on the idea that oxidative stress may underpin life history trade-offs (Costantini et al., 2010; Dowling and Simmons, 2009; Monaghan et al., 2009; Speakman and Garratt, 2014). In humans, mitochondrial dysfunction is thought to contribute to cognitive declines and age-related declines in walking performance (Santanasto et al., 2016). There also appears to be a strong relationship between variation in mitochondrial efficiency and growth performance (Salin et al., 2019). Finally, tissue-specific relationships between mitochondrial properties and whole-organism metabolic features were highlighted. Specifically, SMR and MMR were positively associated with mitochondrial proton leak respiration. These results imply that a high SMR or MMR, rather than signalling a higher capacity for ATP synthesis induced by respiration, could actually reflect greater energy dissipation, caused by proton leak (Salin et al., 2016). However, it would be interesting to deepen the link between individual metabolic rate and mitochondrial functioning in a context of global warming due to their strong dependence on temperature and oxygen.

A particularly interesting impact of exposure to high temperature in ectotherms is a shorter lifespan. In 1908, Rubner measured the metabolic rates of five mammal species with different lifespans. He found that there was a correlation between lifespan and metabolic rate, *"the faster an organism's metabolism, the faster it ages and the shorter its lifespan"* (Beckman and Ames, 1998; Rubner, 1908). Fifty years later, Harman's radical theory of aging (1956) which was later integrated into life history compromises, highlights the link between accumulation of cellular damages and senescence of organisms (Beckman and Ames, 1998; Selman et al., 2012). By focusing on the link

between organism metabolism and life characteristics, it would later inspire the principle of resource allocation (Stearns, 1992) as an explanatory parameter for life strategies and "the rate of living" hypothesis (Pearl, 1928) which establishes the foundations of the link between oxygen consumption and life expectancy. Today, different studies used the metabolic theory of aging predicting that life expectancy is inversely correlated with metabolism (Orr et al., 1992; Sohal and Orr, 1992; Beckman and Ames, 1998). This association between metabolism and life expectancy also seems to be correlated with size, small organisms tending to adopt "live fast" strategies with short longevity (Kleiber, 1932; Speakman et al., 2002; Speakman and Król, 2010). Size and body mass thus appear to be key life history parameters to predict life history strategy (Bergmann 1847; Peters 1983; Calder 1984; Brown et al. 2004).

## 3.2 Temperature & body size reduction

Size is an essential life history trait in ecology because it drives many biological and ecological functions of organisms, communities and ecosystems (Brown et al., 2004; Peters and Wassenberg, 1983; Peters 1983; Calder 1984).

In 1847 Carl Bergmann, a German biologist, was one of the first to report a link between temperature and the size of organisms. He stated that, within a taxonomic group, the species living at the highest latitudes are larger than those living in temperate zones closer to the tropics or the equator. He thus observed a positive correlation between latitude and the average size of species belonging to the same genus, mainly in mammals and birds (Bergmann, 1847). His rule posited that selection favoured larger species at colder temperatures because surface-to-volume ratio decrease with body size, which, in turn, limits total heat loss (Fig. 12a). Even if this rule was initially expounded for endotherms (the heat loss argument is difficult to apply to ectotherms because their internal body temperature fluctuate with the environmental temperature) it has been observed that ectotherms can also exhibit larger body sizes in colder environments (Blackburn et al., 1999; Olalla-Tárraga et al., 2006; Timofeev, 2001). However, several factors are covarying with latitude and temperature may not be the only explanation for this reduction in size.

More than a century later, Atkinson (1994, 1995) proposed the "Temperature-Size Rule" (TSR) (Fig. 12b) based on the observation that an ectotherm living in a hot (non-stressful) environment have an higher initial growth rate ($k$) but a lower adult size compared to the same organism from a cold environment (Atkinson., 1994; Atkinson and Sibly, 1997). He observed this rule on 91% of the studied ectothermic species. This phenomenon would occur because the developmental rate (speed at which an organism grows in age) increases faster with temperature than its individual growth rate (rate at which an organism grows in size). Thus, at higher temperatures, organisms develop faster than they grow and therefore reach a smaller adult size (Zuo et al., 2012). In addition, rapid senescence induced by increased metabolism would explain the high prevalence of TSR in organisms exposed to high temperatures (Kindlmann et al. 2001). Indeed, Harman's theory of aging (1956) highlights the link between accumulation of cellular damage and

senescence of organisms (Beckman and Ames, 1998; Selman et al., 2012). If we link the observations of Max Rubner who postulates that organisms of smaller size or body mass (with a short lifespan) have a faster metabolism (Rübner 1908; Pearl 1928; Kleiber 1932, 1947; Speakman & Król 2010), the reduction in body size would be a response to a high level of cellular stress. Thus, organisms with a short life but rapid development will be able to maximize their selective value (capacity to reproduce) leading to a "live fast die young strategy".

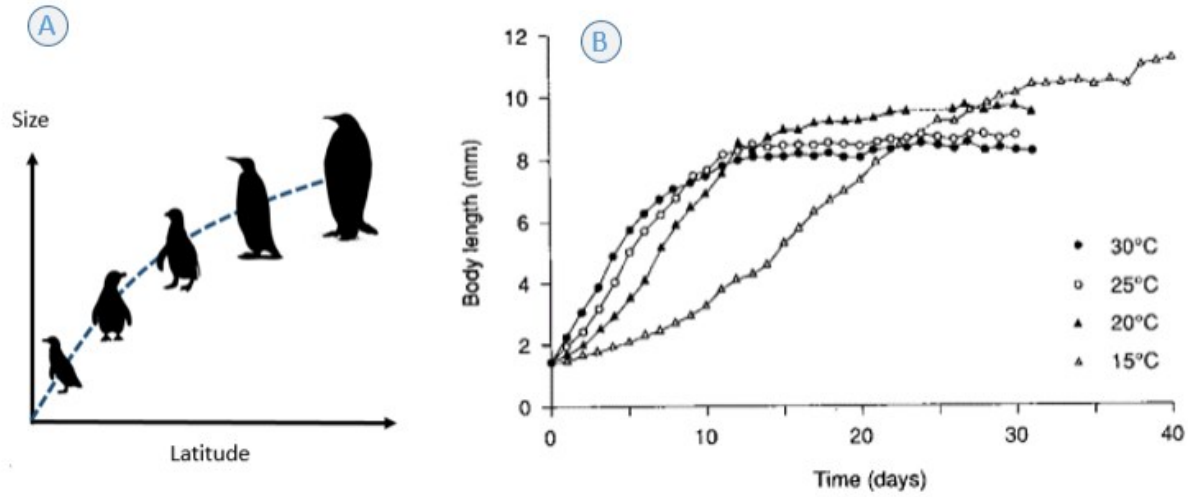


***Figure 12:*** (A) illustration of Carl Bergmann's Eco-geographic rule - the link between temperature (latitude for southern hemisphere) and the size of endothermic organisms: species living at the highest latitudes are larger than those living in temperate zones (regions closer to the tropics or the equator). (B) Growth in mean body length of larvae of the midge *Chaoborus* at different temperatures (from Atkinson, 1994 after Hanazato and Yasuno, 1989).

Thus, body size and its thermal dependence are increasingly taken into account in understanding the functioning of aquatic ecosystems. Indeed, body size strongly structures aquatic ecosystems through the regulation of trophic and competitive interactions, consumer-resource size relationships, community structure and the energy efficiency of individuals. (Cuthbert et al., 2020; Rall et al., 2010; Sentis et al., 2017; Vagnon et al., 2021; Vucic-Pestic et al., 2010; Woodward et al., 2005; Yodzis and Innes, 1992). These studies are therefore essential for a better understanding of the impact of climate change on the scale of entire ecosystems.

# 4. Global warming & ecosystems functioning

## 4.1 Size - temperature - Metabolism: Metabolic theory of ecology

The metabolic theory of ecology (hereafter, MTE; Brown et al. In 2004; BOX. 4; Fig. 13) offers a conceptual framework to evaluate quantitatively the relationships between body size, temperature and ecological variables through the scope of metabolism (Eq.5, Eq.6, Gillooly et al., 2001; West et al., 2002). The use of the MTE seems to be particularly helpful to describe the functioning of aquatic ecosystems (Allen et al., 2005; Delmas et al., 2019; Laigle et al., 2018).

**BOX 4: METABOLIC THEORY OF ECOLOGY (MTE) (Brown et al., 2004)**

The MTE is a quantitative theory that explains the link between metabolism, body size and body temperature. Firstly introduced by Arrhenius and Kleiber and synthesised in 2004 by Brown et al. This theory predicts "how metabolic rate, by setting the rates of resource uptake from the environment and resource allocation to survival, growth, and reproduction, controls ecological processes at all levels of organization" (including developmental rate, population interactions, carrying capacity, rates of competition).

INDIVIDUAL PERFORMANCE

The joint effects of body size, mass, M, and temperature, T (in K), on individual metabolic rate, I, can be described by combining Eqs.2 & 4; BOX. 1:

$$\text{Eq.5: } B = b_o M^{3/4}. e^{-EkT}$$

Where $b_o$ is a normalization constant that is independent of body size or temperature: under this equation, large organisms have higher metabolic rates than small organisms, and organisms at high body temperatures have higher metabolic rates than those at low body temperatures.

However, mass-specific metabolic rate (SMR, in watts/kg) is given by

$$\text{Eq.6: } SMR = (B / M) = b_o M^{-1/4} e^{-EkT}$$

Hence large organisms have a lower SMR than small organisms. This negative correlation can be explained by the surface/volume ratio of the organisms.

POPULATIONS

According to the theory, small organisms with a high internal temperature have a higher growth rate (Rmax) than larger organisms with a cooler internal temperature (Slobodkin 1962, Blueweiss et al. 1978)

&

The population will be denser if resources are increased, and if the size of the individuals is small. Carrying capacity is reduced if temperature rises.

ECOSYSTEM PROCESSES

SMR equation provides the basis for predicting how organism size and temperature determine flux rates within and between compartments for primary producers, herbivores, predators and detritivores and patterns of trophic dynamics (Gauzens et al., 2019)

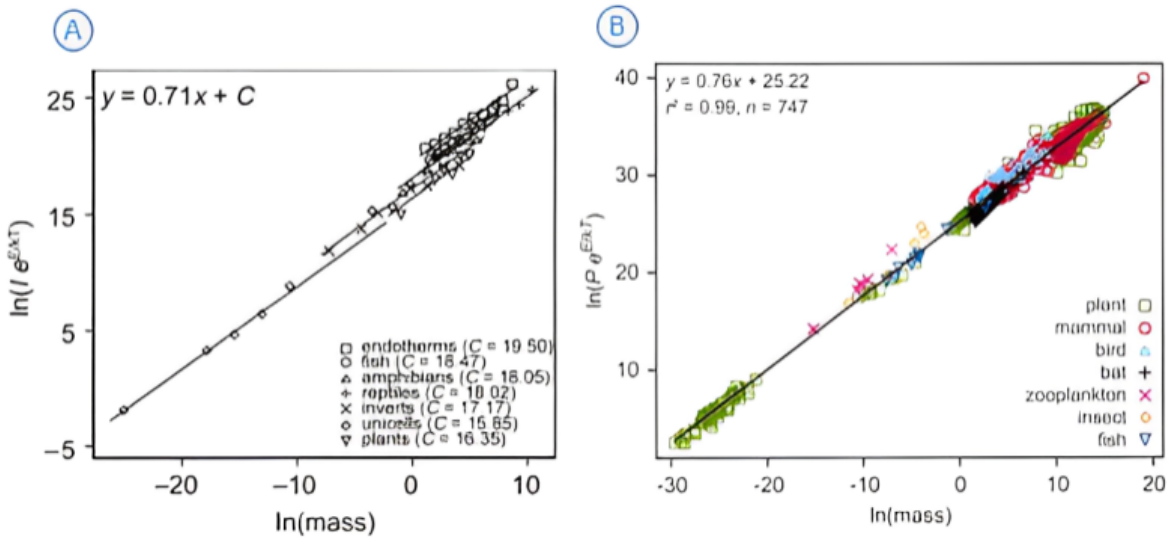


***Figure 13**: (A) Universal relationship between log mass and metabolism (calculated for a given temperature). (B) Metabolic allometry linking metabolism, temperature and size; body mass of the organism. Figure adapted from Brown et al., 2004.*

Thus, using the MTE as a basis Allen, Gillooly and Brown in 2005 used models providing a synthetic and mechanistic framework to link global biogeochemical cycles to cellular, individual and community processes. By explicitly linking photosynthesis and respiration rates to the effects of body size and temperature on plant, animal and microbial metabolism. Models takes into account numerous variables such as the size dependence of photosynthetic rates, the temperature dependence of ATP synthesis in the respiratory complexes and the differences between the temperature dependence of short and long term (The model assumes that flows remain at a steady state over periods of a year or more, but that short-term imbalances between photosynthesis production and consumption can occur as a result of diurnal and seasonal fluctuations of temperature) of $CO_2$ flow throughout the ecosystem confirming the importance of multi-scale studies.

## 4.2 Complex effects of temperature and body size reduction on ecosystems functioning

In the context of climate change, ecosystems functions are often used as indicators of the environmental pressure on ecosystems (Gessner and Chauvet, 2002; Gücker et al., 2006; Wallace et al., 1996; Woodward and Warren, 2007).

One of the main functions of ecosystems is primary production. Light energy is converted into chemical energy to enable the production of organic molecules from mineral substances ($CO_2$ and mineral salts) by autotrophic plants (Fig.14). An increase in

temperature will affect metabolism and enzymes involved in photosynthesis. Gross primary productivity and respiration can thus increase (exponentially) with temperature limiting by the normal range (non-stressful) of environmental temperatures encountered in environments (i.e. thermal tolerance range, as discussed in section 2.3) (Allen et al., 2005; Brylinsky and Mann, 1973; Davison, 1991; Lee et al., 2007). In addition, as mentioned above (see section 3.2), global warming affects individuals by reducing their body size (Atkinson, 1994; Daufresne et al., 2009; Laurel et al., 2008). These reductions in size will have consequences on the functioning of ecosystems, for instance by modifying prey-predator interactions, mainly structured by size in aquatic ecosystems (Audzijonyte et al., 2013; Cuthbert et al., 2020; Rall et al., 2010; Sentis et al., 2017; Vagnon et al., 2021; Woodward et al., 2005). Thus, primary producer's abundance and size can be modified by the size of consumers and top predators (Forster et al., 2012; Renneville et al., 2016).

The organic matter produced by plants is transferred to upper trophic levels of consumers (heterotrophic organisms) to produce their own organic matter. The efficiency of this transfer ranges between 10 and 20% (Pauly and Christensen, 1995) and the energy loss is released into the environment in the form of heat and faeces. However, warming would reduce the efficiency of energy transfer between resources and consumers by more than an half (Barneche et al., 2021). The use of organic molecules by organisms is based on respiration (i.e. complete degradation of organic molecules in the presence of oxygen). An increase in metabolic rate (thermal dependant, but also scales body size; section 2.1, BOX.1 and section 4.1, BOX.4) leads to greater energy requirements. However, organisms generally increase their energy intake by ingesting nutrients to counteract increasing energy demand, even if metabolism increases faster than nutrient ingestion with temperature (Bideault et al., 2019; Lemoine et al., 2013; Rall et al., 2010; Sentis et al., 2012). As a consequence organisms must consume more prey and oxygen which, in turn, modifies the interactions between consumers and resources.

The non-living organic matter produced by each trophic level (corpses of living beings, excrements) accumulates in the environment and is ultimately available to decomposers (i.e. bacteria, fungi, and insects). Decomposers recycles organic matter into inorganic matter that is then reused by autotrophic and heterotrophic organisms. The decomposition of organic matter has three phases: (I) leaching (i.e. the process of releasing nutrients in the water mainly during the first days of immersion, but can last several weeks) (Gessner et al., 1991), (II) microbial conditioning (i.e. colonization by aquatic fungi in particular), and (III) fragmentation by invertebrates. Fungi are considered the main decomposers, especially at the beginning with the activity of invertebrates while bacteria are more important in the later decomposition stages (Baldy et al., 1995; Hieber and Gessner, 2002; Pascoal and Cássio, 2004). The temperature dependence of organic matter decomposition is of considerable importance in the context of climate change because it determines the amounts of carbon released with warming. The rate of enzymatic degradation of organic matter is closely linked to the kinetic of chemical reactions as for others metabolic processes. Thus, decomposition rates increase with temperature (Davidson and Janssens, 2006; Friedlingstein et al., 2006) which leads to faster litter decomposition during warmer seasons at higher temperatures and for

lower altitudes and latitudes (Englert et al., 2015; Ferreira et al., 2015; Taylor and Chauvet, 2014; Tiegs et al., 2019, Ferreira and Canhoto, 2015). According to Follstad Shah et al., 2017, leaf litter decomposition is expected to increase by up to 20% considering both microbial and invertebrate taxa within a projected 4°C increase in average temperature water (Follstad Shah et al., 2017).

However, in some cases, overall litter decomposition may not differ between cold and warm streams or may have a contrasting impact depending on the energy efficiency of individuals, the nature of the detritivores community, the community size, the thermal adaptation of organisms and litter quality (Boyero et al., 2011; Cornwell et al., 2008; Ferreira and Canhoto, 2015; Follstad Shah et al., 2017; Gonçalves et al., 2013; Griffiths and Tiegs, 2016; Makkonen et al., 2012; Strickland et al., 2015). Furthermore, if warming decreases oxygen solubility, litter decomposition may be inhibited (Medeiros et al., 2009). Indeed, studies have examined the size-temperature relationship on the decomposition function and shown that reducing detritivore size (modulating its energy efficiency) led to a decrease in organic matter decomposition at elevated temperatures (Réveillon et al., 2022). Consequently, the individual size of organisms present in an ecosystem can affect ecosystem functions such as matter degradation kinetics and nutrient cycling. To conclude, the pattern of response to temperature is not very clear and antagonistic effects can inhibit the effect of warming.

The strong thermal dependence of the metabolism and its relationship with organism size will have cascading effects on the entire ecosystem. Thus, temperature will directly alter the carbon sequestration capacity and the greenhouse gas efflux of ecosystems through its effects on the balance between plant growth and decomposition but also through a significant increase in respiration rates (Allen et al., 2005; López-Urrutia et al., 2006; Yvon-Durocher et al., 2010a, 2010b). According to Yvon-Durocher et al., (2010a), who tested theoretical models derived from the metabolic theory of ecology in an ecosystem-level manipulation experiment (mesocosm) over a one-year period, a warming of 4°C decreased $CO_2$ sequestration by 13%, increased the fraction of primary production efflux in the form of methane by 20% and the fraction of ecosystem respiration in the form of methane by 9%.

These results underline that all ecosystem processes are linked through the flow of matter and energy and the carbon cycle within ecosystems (Fig. 14) and that the effects of temperature and size at each trophic level will have repercussions on the other trophic levels and on the overall dynamics of the ecosystem, playing a major role in the contribution of greenhouse gases at global scale.

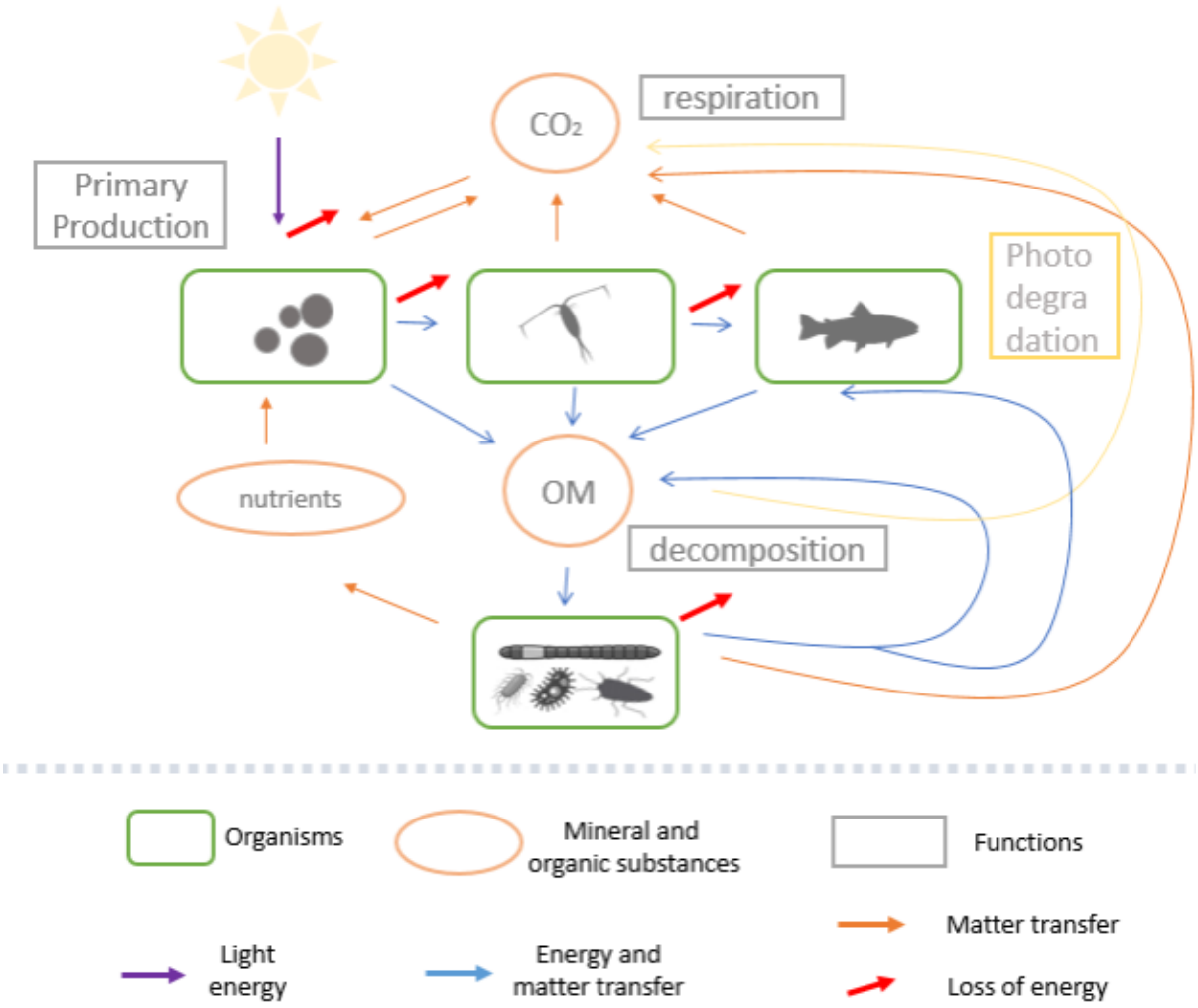


***Figure 14**: matter and energy flows within an ecosystem. Illustrating the main trophic relationships and functions of the ecosystem. Primary producers convert sunlight into energy. Consumers obtain energy by eating. Decomposers break down organic matter, recycling matter and nutrients.*

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To conclude this general introduction, the rise in temperatures and the thermal dependence of body size will impose strong constraints on ectothermic organisms which can in turn have repercussions at all levels of organization up to the scale of ecosystems and biogeochemical cycles. The complexity of the impact of temperature therefore lies in this multiplicity of scales at which it can influence processes. In addition, responses may depend on exposure duration and to the ability of organisms to acclimatize or evolve. To better anticipate the response of organisms and ecosystems to current and future climate change, it will be important to consider different temperature exposure based on the duration and the intensity of exposure through empirical approaches. This general introduction confirms the importance contribution of the different levels of organization of life to the flow of matter an energy and more generally greenhouse gases emissions

# 5. Research questions

## Problem statement

The main objective of this thesis is to improve our understanding of the effect of a multigenerational temperature exposure on freshwater ectotherms organisms taking into account different ecological scales from cellular metabolism and body size reduction, to the functioning of the ecosystem.

My work is a continuation of the work of two previous doctoral students, Claire Hemmer-Brepson (2010-2013) and Ayala Loisel (2015-2019), who studied the ecology of populations of Japanese medaka (*Oryzias Latipes*) exposed experimentally to 20°C and 30°C over several generations. Their work demonstrated a link between oxidative balance under elevated temperature (increased oxidative damage, damage to membranes and proteins) and the life strategies adopted by medaka fish in contrasting thermal environment (TSR patterns). They determined different antioxidant strategies depending on exposure temperatures and sexes. Finally, they demonstrated that thermal phenotypic plasticity persisted for acclimated organisms even after several generations (Hemmer-Brepson, 2013; Hemmer-Brepson et al. 2014; Loisel et al., 2019; Loisel, 2019).

To extend these finding, we conducted a similar evolutionary experiment with lineages of medaka fish (*Oryzias Latipes*) reared at 20°C or 30°C. We then used these fish to determine whether multigenerational exposure to contrasting temperatures can modulate the physiological, biological and ecosystemic effects of temperature on ectotherms.

## Questions

To highlights the multigenerational temperatures exposures effects on ectotherms and their impact at multiple biological and ecological scales I aimed at answering the following questions:

*1 What are the long-term (multigenerational) effects of temperature on the cellular metabolism (mitochondrial respiration) of an ectotherm fish (Orizyas latipes)?*

To answer this question, the first chapter of my thesis focuses on the cellular metabolism of individuals Medaka fish (*Orizyas latipes*) raised for several generations (two years) at 20°C or 30°C. The aim of this chapter is to better understand the response of ectotherms to temperature after a multigenerational exposure and to highlight modulation of the mitochondria response.

*2 What are the long-term (multigenerational) effects of temperature on the critical maximum thermal tolerances (CTmax) of medaka fish and is there a link between dysfunction of fish brain mitochondria and the thermal tolerance of the whole organism?*

The second chapter of my thesis focuses on the CTmax of medaka fish maintained over several generations (five years) to cold (20°C) and warm (30°C) temperatures and

aims to highlight a link between dysfunction of fish brain mitochondria and the thermal tolerance of the whole organism (CTmax).

*3 What are the temperature-induced effects of size reduction (TSR) and metabolisms modification of ectotherms individuals (medaka fish) on the functioning of the ecosystem?*

A final chapter revolves around a three-month experiment in mesocosms where we manipulated the temperature (+4°C; IPCC, 2023) and the thermal origin of the medaka fish. The aim is to determine the temperature-induced effects of body size reduction and metabolism on ecosystem functions (primary production, ecosystem respiration, greenhouse gaz production).

## Biological model

The Japanese medaka fish (*Oryzias Latipes)* is a small (20-40 mm) amphidromous freshwater fish from Southeast Asia. This species is eurythermic with a thermal range from zero to 40 °C (Dhillon and Fox, 2007; Hirshfield, 1980; Shima and Mitani, 2004; Yamamoto, 1975) and a thermal optimum of 25°C (Dhillon and Fox, 2007; Hirshfield, 1980; Kirchen et al., 1976; Leaf et al., 2011; Yamamoto, 1975; Egami and Etoh 1969; Dhillon and Fox 2004). In its native range, the medaka generally lives in small bodies of water such as rice paddies, in the upper part of the water column or in shallow habitats areas that are often sensitive to important temperature variations, with temperatures ranging from 5 to 35°C (Kirchen & West 1976; Hirshfield 1980). Under experimental conditions and at shallow depths in mesocosms medaka can support up to 43°C (Sentis. A, personal observation). It is a good biological model for multigenerational thermal experimentation as it has a short generation time of 3 months and can reach sexual maturity in about 10 weeks (Hirshfield, 1980) (The age at maturity observed in our facilities varied between 60-70 days after hatching at 30°C and up to 160 days at 20°C). Its genome is sequenced and published. Males have a more developed dorsal fin than females and generally have a more slender appearance allowing to be visually differentiated without manipulation (Fig. 15). Reproduction takes place continuously. It has an average lifespan of 1 year in the wild and up to 5 years in captivity (Shima & Mitani, 2004). The medaka produces eggs prolifically and oviparously (Leaf et al., 2011; Fig. 15). It is an omnivorous species that feeds primarily zooplankton (rather in their juvenile stage) but also on diatoms, algae, and aquatic insects (larvae and adults on the surface) (Edeline et al., 2016; Furutani-Seiki and Wittbrodt, 2004; Kinoshita et al., 2009; Mano and Tanaka, 2012; Shima and Mitani, 2004).

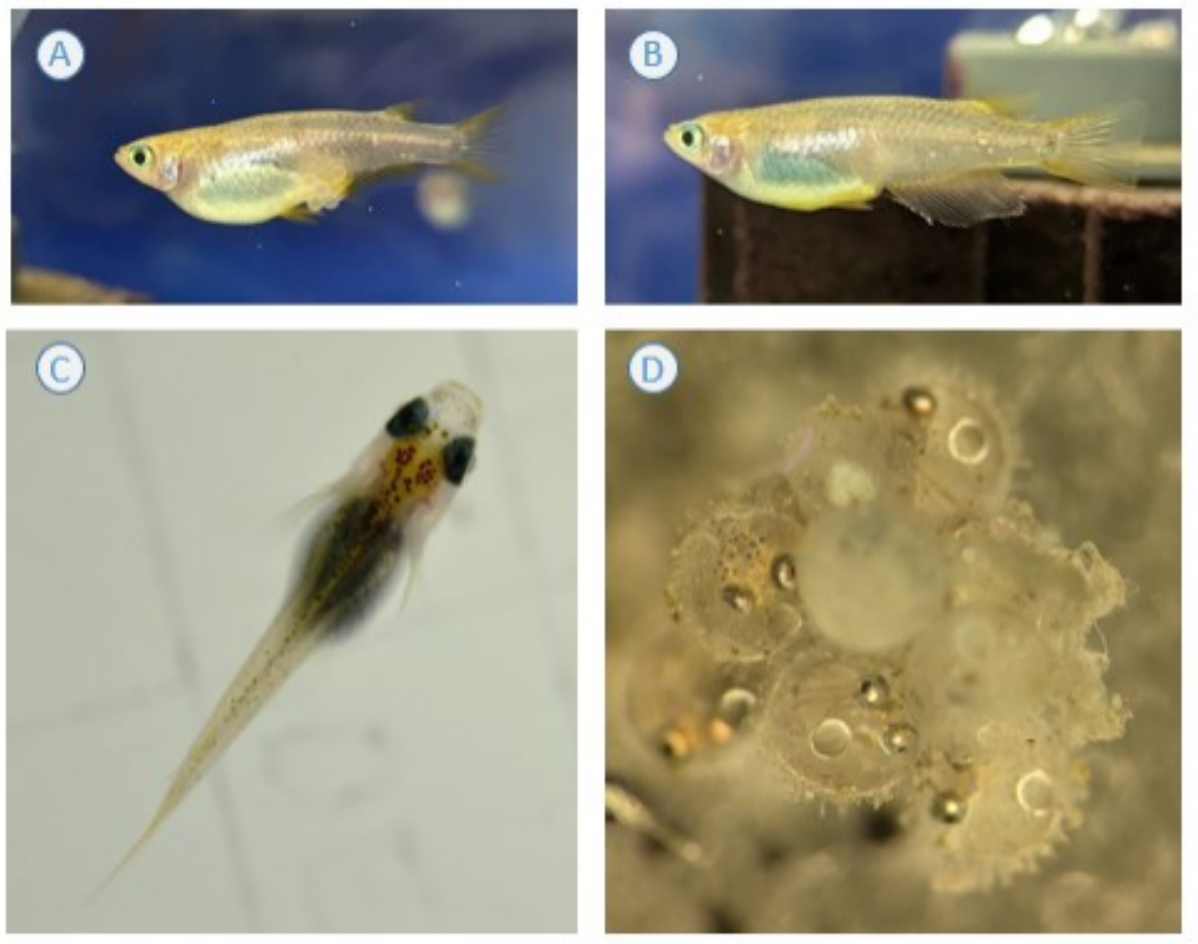


***Figure 15**: (A) Female medaka with eggs showing a triangular anal fin. (B) Male medaka showing a flag-shaped anal fin. (C) Juvenile medaka (~ 40 days). (D) Developing medaka egg cluster showing characteristic medaka spikes on the egg outline. (©Remy. Lassus).*

## Rearing conditions

Medaka used in this thesis were reared at 20 °C (COLD fish) or 30 °C (WARM fish) (non-stressful temperatures) over several generations under controlled experimental conditions in climatic chambers.

Climatic chambers consists of two air-conditioned rooms at approximately 20 °C (COLD fish) and 30 °C (Warm fish), delimited via an airlock (Fig. 16). Each air-conditioned room contains 15 aquariums (40 x 25 x 20 cm; 20 L) arranged on height-adjustable aluminium cabinets in rows of 5 aquariums (Fig.16). Aquariums are annotated from 1 to 30. There is also a 64 L (80x40x20) egg incubation aquarium. The airlock between the two rooms ensures no direct contact with the rearing aquariums. Hygrometry is controlled and adjusted to around 50%, by means of the air conditioning system, combined with a portable dehumidifier. Each room has its own air-conditioning system. The temperature is easily adjustable, from a few °C to 40°C. The room temperature is maintained at 2 degrees below the desired temperature and, in each aquarium, heaters (Juwel aqua 100) are used to bring all the aquariums to the same temperature (i.e. aquariums at 20°C or 30°C). Temperatures in all aquariums are measured daily to an accuracy of 0.1°C. Each room is equipped with 2 "FLUMEN LTPF701560NW" 50W, 4000 K, 6000 Lm LED neon

lights. Timers regulate switch-on and switch-off on a daily 12-hour schedule. The photoperiod can be adjusted (according to the phenology of the species and the experiment underway) to mimic seasonal rhythms and stimulate reproduction.

Aquariums have three walls covered with an opaque blue film to limit the stress of the fish. Water is derived from the river *Cause*, and is successively filtered by a 25µm mechanical filter and a second mechanical 5µm filter, followed by a UV filter to purify the water. The filtered water is then transferred to high-level water storage tanks (approx. 80 cm above the aquariums). In each room, a 144L (120x40x30 cm) water storage tank supplies 10 aquariums, and a second 72L (60x40x30 cm) tank supplies 5 aquariums. The storage tanks fill up when the water level drops and reaches a threshold, regulated by a "flush" system, i.e. a float and a rod that activates the water inlet opening if the float drops. A pipe connects these tanks to the aquariums and terminates in a small plastic tap in each aquarium. The Water supply to the aquariums is provided by an open-circuit system, with drip feed (1 L. h-1) and drainage in each aquarium. Each aquarium is equipped with a landing net for capture, a landing net for collecting dead fish, and a cleaning kit. For transfers, we use black 5L plastic tanks to limit stress due to light and colour. In addition, the water drain of each aquarium is filled with foam to prevent fish from slipping through. In each room, a camera is permanently connected to a server and can be consulted remotely, to check that the water in the aquariums is not rising. Outgoing water is also successively filtered by a 25µm filter and a second 5µm filter, followed by a UV filter. It ends in a concrete holding tank, more than 50 m away from the nearest watercourse. A portable spectrometer is used to measure nitrite and ammonia concentrations on a weekly basis. Oxygen content and pH are also measured at the same frequency. Oxygen concentration is maintained at 100% saturation by an air pump feeding bubbler-filters in each aquarium and the pH is maintained at 7.5. Each tank is equipped with an air filter to prevent high nitrite concentrations and maintain oxygen at saturation.

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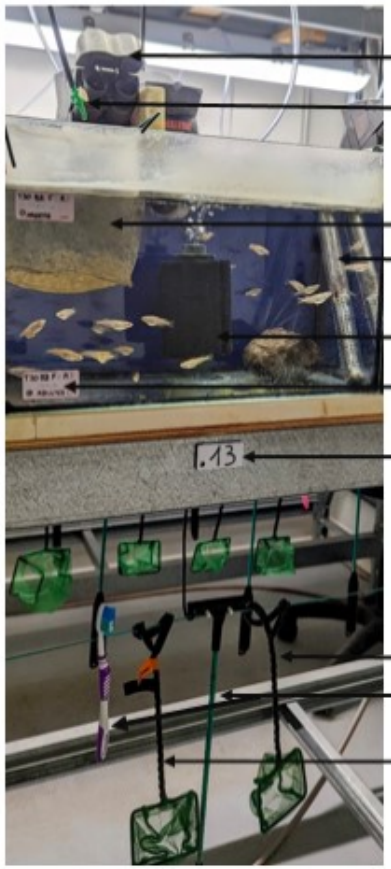
Storage tank

Neon light

Aquariums

Aluminium cabinet




automatic feeder

drip feed (1 L. h-1)

juvenile fish net

heaters

air pump bubbler-filters

Fish lineage ID

Aquarium number

landing net for capture

cleaning kit

landing net for collecting dead fish

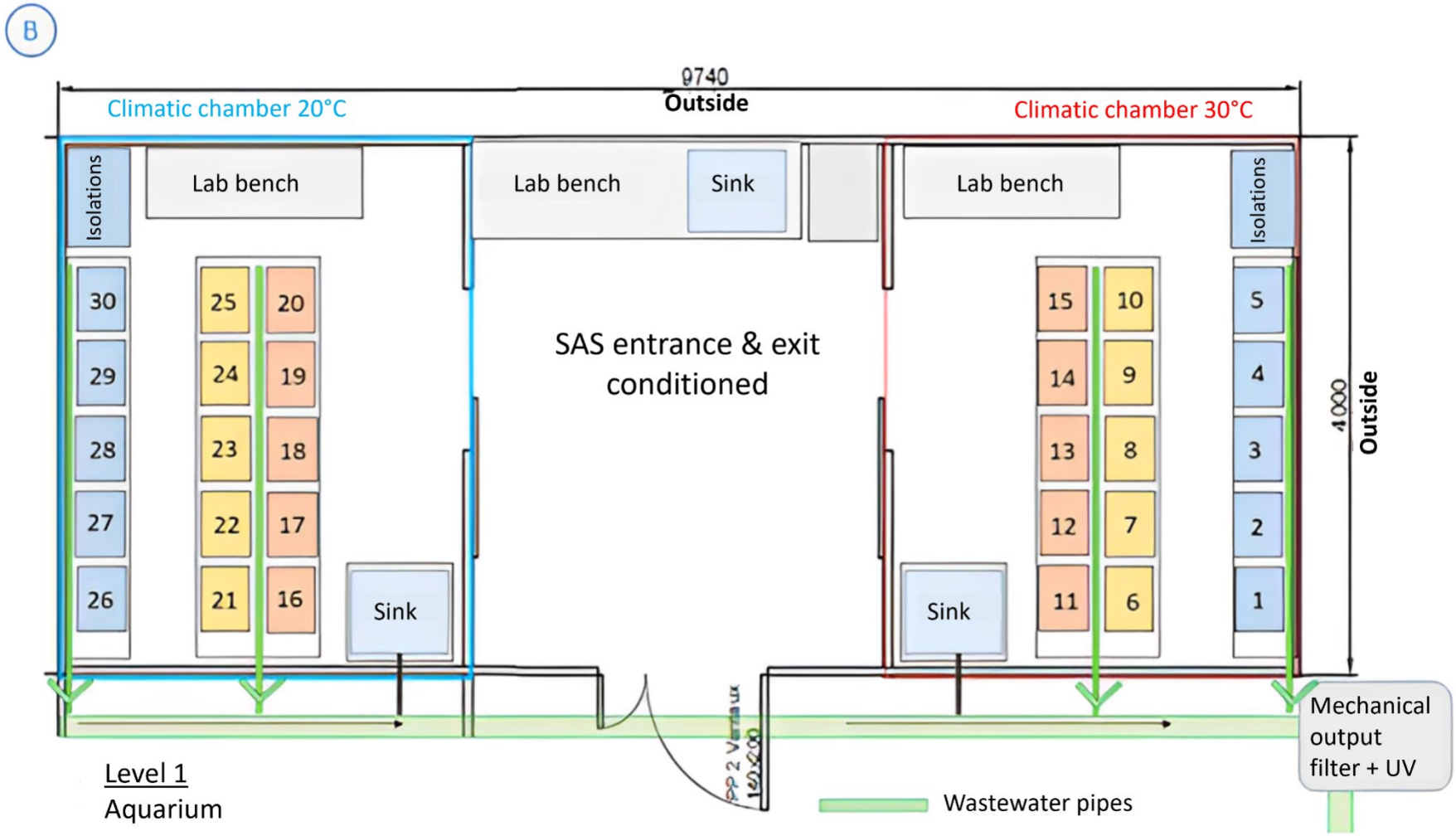



***Figure 16**: (A) Climatic chambers equipment details with aquariums and (B) climatic chamber plan (©Lassus. Rémy)*

## Medakas lineages

For each temperature treatment, two lineages were obtained from a starting $F_0$ population of 320 Medakas (160 females & 160 males) belonging to the CAB strain from Carolina Biological Supply Company (USA) and provided by AMAGEN© (France) (Fig.17). $F_0$ generation fish were maintained at 25°C into 20 L aquariums and were then progressively acclimatized to 20 or 30 °C at a rate of one degree every 2 days (Hemmer-Brepson, 2013; Loisel, 2019). To start a new fish generation, we collected eggs clutches using a brush (approximately 300 eggs) during the optimal fecundity period (i.e. when most females were laying eggs (100-200 for T30 and 150-300 for T20). The photoperiod was adjusted to 16h: 8h (day:night), which is optimal for medaka reproduction (Hirshfield, 1980) and eggs were placed in hatcheries (2.5 L) at the parental temperature (20 °C or 30 °C). We maintained the new born fish in climatic chambers at the same temperature as their parents and then placed them in five replicates aquariums (25 × 40 × 20 cm) until maturity, with a density of ~30 adult fish per aquarium. The fish were fed "ad libitum" with dry flakes food TétraMin© three times a day (at 9h, 13h and 18h). After 2 years of multigenerational exposure (fish rearing was already underway at the start of my thesis), fish from $F_3$ generations at 20°C and $F_4$ at 30°C were used in chapter 1 and fish from $F_3$ at 20°C and $F_5$ at 30°C were used in chapter 3. Finally, after 5 years of multigenerational exposition fish from the $F_6$ at 20°C and $F_{10}$ at 30°C were used in chapter 2 (Fig. 17).

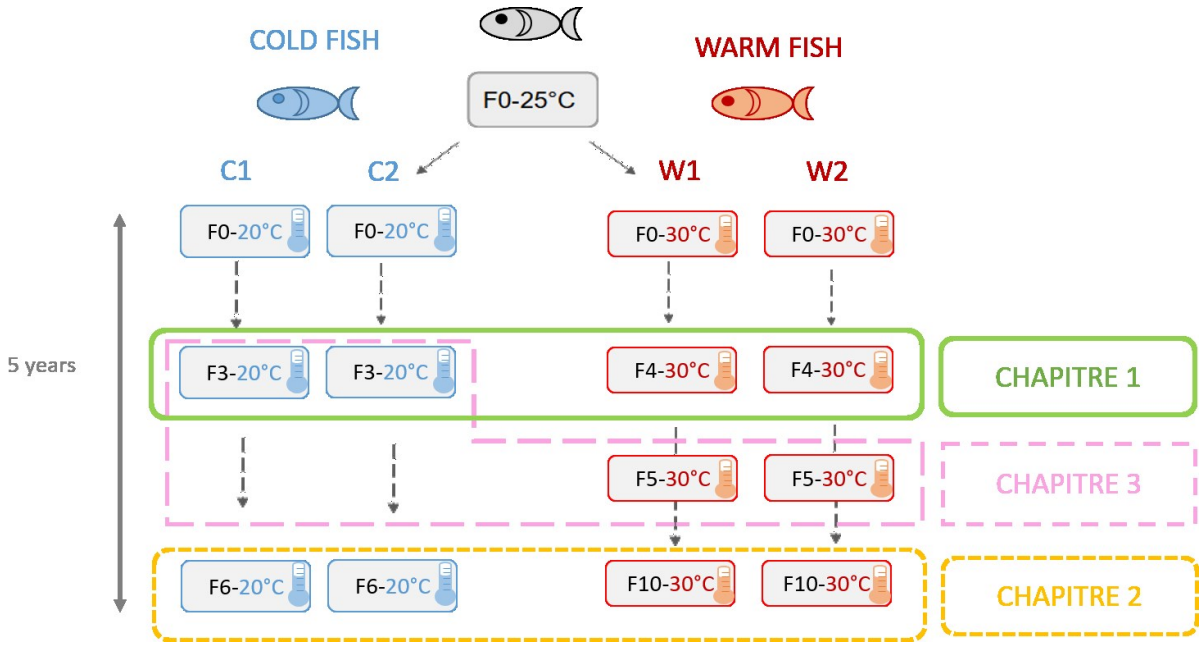



***Figure 17**: Medaka lineage number used during the thesis for warm and cold fish.*

To monitor medaka growth, all fish from each aquarium were placed on a Petri dish one after the other, not anesthetized (lasting only a few seconds out of water), with ± 0.5 mm precision graph paper, to measure their total length (Lt, from head to caudal fin). Size was measured at 30, 45, 60, 80, 100, 150, 200, 300 and 350 days post-hatching (Fig.18).

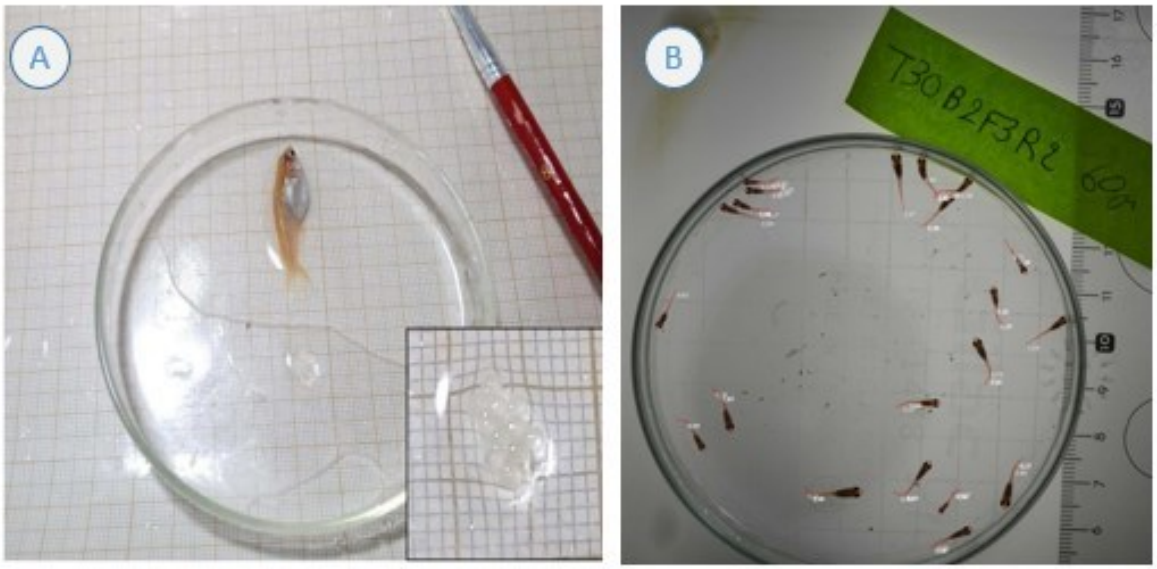



***Figure 18**: (A) size measurement of adult female medaka fish with eggs (© Loisel, 2019) and (B) size measurement of juvenile medaka fish on picture with piximetre software (©Remy.Lassus).*

# Chapter 1

# Multigenerational exposure to temperature influences mitochondrial oxygen fluxes in the Medaka fish (*Orizias Latipes*)

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STATUS: Under review in *Acta Physiologica*, with minor comments status

AIM: The first chapter of my thesis focuses on the mitochondria, also call the “powerhouse of cells" that plays a central role in the control of metabolism under thermal warming. In this chapter, I was interested in the cellular metabolism of Medaka fish (*Orizyas latipes*) raised over several generations (two years) at 20°C or 30°C. This chapter aims to determine the long-term mitochondrial response of freshwater ectotherms after several generations of exposure to elevated temperature, in order to improve our understanding of mitochondrial functioning under thermal pressure and to highlight potential adaptive phenomena. As part of the thesis, this chapter deals with the relationship between temperature and mitochondria and aims to add a multigenerational experimental approach to already existing studies, mainly on populations from natural environments or exposed to temperature only for one generation. These results also provide a basis for understanding the mitochondrial multigenerational response to elevated temperature in order to be able to link these cellular responses to temperature at the scale of whole organism and ecosystem.

MY CONTRIBUTION: I participated in setting up the experiment, designing the experimental protocol, measuring mitochondrial flows and collecting data with the help of Rémy Lassus, Julie Favre-Marinet, Arnaud Sentis and Martin Daufresne. I also carried out the statistical analyzes with the help of Arnaud Sentis and the writing of the first version of the paper as well as the writing of the revised versions in order to improve its quality over the course of the revisions carried out by Arnaud Sentis, Martin Daufresne and Karine Salin, and from Acta physiologica feedbacks.

# Article 1: Multigenerational exposure to temperature influences mitochondrial oxygen fluxes in the Medaka fish (*Orizias Latipes*)

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CONFLICT OF INTEREST STATEMENT: The authors declare they have no competing interests

DATA AVAILABILITY STATEMENT:

Data are available online: https://doi.org/10.5281/zenodo.7904757

ANIMAL ETHICAL DECLARATION: No ethical permit was required under French legal requirements to conduct this experiment because experimental temperatures are within the range of non-stressful temperature for this species (Leaf et al. 2011; Shima and Mitani 2004). Fish were maintained in the laboratory under permit number A1300101 by the French authority of the "Direction départementale de la protection des populations des Bouches-du-Rhones" under the articles R 214-87, R 214-122, and R 215-10 of the French law.

AUTHORS' CONTRIBUTIONS: Julie Morla: Conceptualization-Equal, Data curation-Equal, Formal analysis-Lead, Investigation-Lead, Methodology-Equal, Writing – original draft-Lead, Writing – review & editing-Lead. Karine Salin: Methodology-Supporting, Writing – review & editing-Supporting. Rémy Lassus: Data curation--Equal, Methodology- Equal. Julie Favre-Marinet: Data curation--Equal, Methodology- Equal. Arnaud Sentis: Conceptualization-Lead, Funding acquisition-Lead, Investigation-Supporting, Methodology-Equal, Project administration-Lead, Supervision-Lead, Validation-Lead, Writing – review & editing-Supporting. Martin Daufresne: Conceptualization-Lead, Funding acquisition-Lead, Investigation-Supporting, Methodology-Equal, Project administration-Lead, Supervision-Lead, Validation-Lead, Writing – review & editing-Supporting

## Abstract

Thermal sensitivity of cellular metabolism is crucial for animal physiology and survival under climate change. Despite recent efforts, the effects of multigenerational exposure to temperature on the metabolic functioning remain poorly understood. We aimed at determining whether multigenerational exposure to temperature modulate the mitochondrial respiratory response of Medaka fish. We conducted a multigenerational exposure with Medaka fish reared multiple generations at 20 and 30°C (COLD and WARM fish, respectively). We then measured the mitochondrial oxygen consumption of tail muscle homogenate at two assay temperatures (20 °C and 30 °C). Mitochondrial function was determined as the respiration supporting ATP synthesis (OXPHOS) and the respiration required to offset proton leak (LEAK(Omy)) in a full factorial design (COLD-20°C; COLD-30°C; WARM-20°C; WARM-30°C). We found that higher OXPHOS and LEAK fluxes at 30°C compared to 20°C assay temperature. At each assay temperature, WARM fish had lower mitochondrial oxygen fluxes than COLD fish. Interestingly, we did not find significant differences in respiratory flux when mitochondria were assessed at the rearing temperature of the fish (i.e. COLD-20°C vs WARM -30°C). The lower OXPHOS and LEAK capacities in warm fish is likely the results of the multigenerational exposure to warm temperature. This is consistent with a modulatory response of mitochondrial capacity to compensate for potential detrimental effect of warming on metabolism. Finally, the absence of significant differences in respiratory fluxes between COLD-20°C and WARM-30°C fish likely reflect an optimal respiration flux when organisms adapt to their thermal conditions.

Key words: cellular respiratory, ectotherms, global warming, long-term exposure, tail muscle.

## 1. Introduction

Temperature is one of the most important drivers of climate change. In particular, increasing temperature can result in (a) higher individual growth rates (Atkinson, 1994; Atkinson and Sibly, 1996), (b) earlier maturity (Atkinson, 1995, 1994), (c) smaller adult body size (Atkinson, 1994; Daufresne et al., 2009; Harman, 1956) and (d) shorter lifespan (Dembski et al., 2006). These responses are at least partly driven by processes at the metabolic scale (Beckman and Ames, 1998; Brown et al., 2004; Harman, 1956) which suggests that better understanding how temperature affects metabolism is key to determine the ecological consequences of climate change. It was early demonstrated that the metabolic rate of ectotherms increases with warming, accelerating cellular respiratory and oxygen demand following the Arrhenius law (Arrhenius, 1889; Abele et al., 2002; Hilton et al., 2010; Roussel and Voituron, 2020). Conversely, decreasing temperature reduces the production of adenosine triphosphate (ATP) in ectotherms mitochondria (Fangue et al., 2006; Guderley, 2004; Guderley and Gawlicka, 1992;

Lucassen et al., 2006; Schulte, 2015). These thermal effects may be particularly important for aquatic freshwater ectotherms, especially threatened by global warming (Adrian et al., 2009; Sala et al., 2000) and that have fewer possibilities for behavioural thermoregulation or dispersal compared to marine or terrestrial species (Beisner et al., 2006). Warming also decreases oxygen concentration in water (Truesdale et al., 1955) inducing a mismatch between the oxygen demand and oxygen concentration (Atkinson and Sibly, 1996; Pörtner, 2002; Verberk et al., 2016).

Mitochondria are the central organelle of metabolism and are responsible for almost 90% of the cellular oxygen consumption of an organism (Rolfe and Brand, 1997). They contain the enzymatic systems generating cellular energy in the form of ATP by oxidative phosphorylation (OXPHOS), i.e. the coupling between substrate oxidation requiring oxygen, and the phosphorylation of ADP (Adenosine diphosphate) into ATP (Mitchell, 1966). Mitochondrial responses to elevated temperatures are multiple (Chung and Schulte, 2020). According to previous studies, temperature leads to (a) an exponential increase in the rate of consumed oxygen molecules related to ATP production (Abele et al., 2002; Hilton et al., 2010; Roussel and Voituron, 2020), (b) the production of cellular damages (Abele et al., 2002; Roussel and Voituron, 2020; Woodward et al., 2010) and (c) a modification of the activities and functionalities of several mitochondrial enzymes, membrane proteins and gene regulatory mechanisms (Dahlhoff et al., 1991; Somero, 2010).

Climate is changing gradually, acclimation and evolutionary responses can be fast (Geerts et al., 2015; Wang et al., 2021), it is therefore essential to better understand the long term mitochondrial responses induced by the environmental temperature on aquatic freshwater organisms. Previous studies highlighted that warm and cold acclimation in ectotherms can lead to compensation in mitochondrial functions (Chung et al., 2017; Chung and Schulte, 2015; Seebacher et al., 2010; Yan and Xie, 2015). In particular, long-term exposure to higher temperature can increase mitochondrial efficiency and decrease mitochondrial metabolism through: (a) a decrease in mitochondrial respiration rates, (b) a decrease in mitochondrial density and (c) modifications of enzyme structure or compositional changes within mitochondrial membranes (i.e. phospholipid proportions and fatty acid composition) (Bielski et al., 1983; Chung et al., 2017; Chung and Schulte, 2020; Dhillon and Schulte, 2011; Ekström et al., 2016; Guderley and Johnston, 1996; Guderley and St-Pierre, 2002; Hazel, 1990; Le Roy et al., 2021; Seebacher et al., 2010; Shama et al., 2016; St-Pierre et al., 1998; Yan and Xie, 2015). Studies have reported that ectotherms from colder environments (higher latitudes or altitudes) have elevated metabolic rates compared to those from warmer climates (Clarke, 2003; Hazel and Prosser, 1974). This has been reported for fish where northern populations are increasing their mitochondrial oxidative capacities and mitochondrial density as a compensatory mechanism (Fangue et al., 2009; Lucassen et al., 2006; Schulte, 2015). In addition, seasonal exposure to cold temperature can enhance aerobic metabolism by increasing mitochondrial densities and/or capacities of mitochondrial enzymes (Guderley, 2004; Guderley and Gawlicka, 1992).

These adjustments may result both from genetic adaptation over generations or reversible plastic modifications in mitochondrial structure and function. Although there

is a growing interest for the study of mitochondrial adjustments in response to temperature, most studies focused on populations from thermally contrasted environment (Chung et al., 2017; Dhillon and Schulte, 2011; Ekström et al., 2016; Loisel, 2019) or on seasonal acclimatization (Guderley et al., 1997; Guderley and St-Pierre, 2002; Seebacher et al., 2003; St-Pierre et al., 1998). However, in these empirical studies, other environmental factors such as luminosity, photoperiod, pH and nutrients can covariate with temperature which makes it difficult to quantify the influence of temperature on mitochondrial response. In addition, the time required for thermal acclimation to be completed is often assumed rather than measured, which can biased conclusions regarding the acclimation status of the studied processes (Pichaud et al., 2017). In a rare study, Shama et al. (2016) (Shama et al., 2016) showed that, in marine sticklebacks, acclimation of grandmother to warming lead to lower and optimized offspring mitochondrial respiratory. However, there is no consensus on how acclimation within or across generations modulates mitochondrial oxygen flux in response to temperature. In addition, to our knowledge, there is no multigenerational exposure study on mitochondrial responses to temperature in freshwater aquatic organisms, despite their lower possibilities for behavioural thermoregulation or dispersal in comparison to marine or terrestrial species (Beisner et al., 2006). This investigation is thus important to improve our understanding of environmentally driven variation in mitochondrial responses (Chung and Schulte, 2020; Nord et al., 2021; Nord and Giroud, 2020) and by consequence, to understand how freshwater organisms can cope with climate change.

To determine whether multigenerational exposure to temperature can modulate mitochondrial thermal response, we investigated mitochondrial oxygen fluxes (JO2) in the Medaka fish *Oryzias Latipes* (Adrianichthyidae) after rearing them for two years at two contrasted temperatures (20 and 30°C). These represent non extreme temperatures falling within the natural range experienced by the species (Leaf et al., 2011; Shima and Mitani, 2004). Using a full factorial design with fish from the same parental group (F0) reared over several generations at 20 °C (F3) or 30 °C (F4) crossed with two assay temperatures (20 °C or 30 °C). We expected (H1) higher oxygen fluxes for mitochondria assessed at 30 °C compared to 20 °C. We also hypothesized that (H2) long-term exposure to temperature would lead to a modulation of mitochondrial response through modifications in (H2.i) mitochondrial efficiency, (H2.ii) mitochondrial density or (H2.iii) mitochondrial capacities. H1 is supported by previous studies showing that mitochondrial activities is higher at warmer assay temperatures compared to cooler ones (Abele et al., 2002; Brand, 2000; Guderley, 2004; Guderley and Gawlicka, 1992; Ricquier and Bouillaud, 1998; Sommer and Pörtner, 2004) whereas H2 is motivated by the hypothesis that thermal acclimations in ectotherms can results in compensation of mitochondrial functions (Chung et al., 2017; Chung and Schulte, 2015; Seebacher et al., 2010; Yan and Xie, 2015).

# 2. Materials and methods

## 2.1 Biological model and rearing conditions

The medaka fish (*Oryzias latipes)* is a small (20-40 mm) amphidromous freshwater fish from Southeast Asia. It is an eurythermal fish that can live in temperatures ranging from 0 to 40°C (Leaf et al., 2011; Shima and Mitani, 2004) with an optimum individual growth temperature at 25°C (Dhillon and Fox, 2007) and can reach sexual maturity within only 10–12 weeks at 27°C (Hirshfield, 1980), making it a good biological model for multigenerational thermal experimentation. It has an average lifespan of 1 year in the wild and up to 5 years in captivity (Shima and Mitani, 2004). Medaka used in this study were reared at 20 °C or 30 °C (non-stressful temperatures) (COLD and WARM fish, respectively) during two years over several generations under controlled experimental conditions in climatic chambers. WARM and COLD fish used in the experiments present differences in generation numbers (4 and 3, respectively) due to a shorter generation time at 30°C compared to 20°C. Difference in the number of generation is due to warming condition applied in this study, as fish grow faster and have an early sexual maturity when temperature increase (Alberto-Payet et al., 2022; Atkinson, 1995, 1994). To get F4 COLD fish, the experiment should have been run for an additional year. As a consequence, we preferred comparing mitochondrial respiration of fish at the same date rather than at the same generation to reduce the risk of changes in experimental conditions experienced by WARM and COLD fish (e.g. water quality or potential diseases) over a year, as well as potential changes in conditions of oxygen flux measurements.

The climatic chambers are equipped with temperature and humidity sensors to control room conditions. Water is controlled by mechanical, biological and UV filters and is supplied from a buffer tank via an open-circuit system, with drip feed (1 L. h-1). Photoperiod is controlled by timers and set at 12:12 (L:D). The two climate chambers are composed of two rows of five aquariums. The two lineages were obtained from a starting F0 population of 320 Medakas (160 females & 160 males) belonging to the CAB strain from Carolina Biological Supply Company (USA) and provided by AMAGEN© (France). F0 generation fish were maintained at 25°C (i.e. the temperature at which AMAGEN reared their strain) and were then progressively acclimatized to 20 or 30 °C at a rate of one degree every 2 days. To start a new fish generation, we collected egg clutches during the optimal fecundity period (i.e. when the number of laying females and the average number of eggs per female per day were at their maximum) and placed them in hatcheries at the parental temperature (20 °C or 30 °C). We maintained the newborn fish in climatic chambers at the same temperature as their parents (20 °C or 30 °C) and then placed them in five replicates aquariums (25 × 40 × 20 cm) until maturity, with a density of ~30 adult fish per aquarium. The fish were fed "ad libitum" with dry flakes food TétraMin© three times a day (at 9h, 13h and 18h). Oxygen concentration during the experimental period in our climatic chamber tanks were 8.32 ± 0.33 O2_mg/L (91,5%) at 20°C and 6.62 ± 0.59 O2_mg/L (88,4%) at 30°C. The oxygen concentration is maintained by means of an air pump feeding bubbler-filters in each aquarium. In our experiment, oxygen concentration was lower at 30°C but was not limiting and non-stressful.

## 2.2 Experimental design

After two years of multigenerational exposure to 20°C and 30°C, we aimed at measuring mitochondrial respiration using full factorial experimental design with two rearing temperatures: [20 °C (COLD; C) and 30 °C (WARM; W)] crossed with two assay temperatures (20 and 30 °C)(Fig. S1). To measure mitochondrial oxygen fluxes we used 18 COLD fish from F3: 10 adult females (mean age ± s.e: 326.8 ± 4.5 days) and 8 adult males (325. 5 ± 6.5 days) from F3. We used 19 WARM fish: 9 adult females (230.3 ± 6.6 days) and 10 adult males (227.4 ± 6.6 days) from F4.

We used two muscle samples of each fish for the two assay temperatures (one sample per temperature) in the oxygraph (i.e. 36 muscular samples for COLD fish and 38 muscular samples for WARM fish).

## 2.3 Mitochondrial respiratory measurement

Medakas were euthanized by percussion of the cranial cavity and then measured (mm), weighed (mg) and dissected to sample muscle from the two flanks of each fish. Dissections were conducted within five minutes following euthanasia on a freezer block to slow down cellular destruction. For each fish, 4 mg (Mean ± SEM = 4.02 ± 0.50 mg) of skeletal tail muscle from each of the two flanks were collected with a scalpel and fine forceps under binocular magnifying glass, then they were cut using micro-dissecting scissors and minced to obtain a homogenized solution of muscle buffered in 1 mL of 0.5M Respiratory Medium (MIR05) (Gnaiger et al., 2000) (EGTA 0.5mM, MgCl2 3 mM, Lactobionic acid 60mM, Taurine 20mM, KH2PO4 10 mM, HEPES 20 mM, D-Sucrose 110 mM). Tissue homogenates were then diluted in 1 mL MIR05 to obtain a final concentration of approximately (2mg muscle/mL of MIR).

Mitochondrial oxygen fluxes (Mitochondrial oxygen consumption in picomol.s-1.mg-1) were measured using two Oxygraph-2k high-resolution respirometers (Oroboros Instruments, Innsbruck Austria), each equipped with two independent measuring chambers (2.1 mL). Immediately following preparation, homogenized tissue from each fish were added to one of the two measurement chambers of the oxygraph. For each individual fish, we tested simultaneously one flank at 20 °C and the other at 30 °C. We established oxygen flux curves at different states of oxidative phosphorylation, corresponding to different functions of the respiratory chain (OXPHOS, LEAK(Omy), COX-capacity and ROX state, described hereafter). To fulfill this purpose we followed an injection protocol of different substrates enabling the activation or inhibition of ATP-producing enzyme complexes (following (Pesta and Gnaiger, 2012; Salin et al., 2016; Thoral et al., 2021)). The oxygen electrodes were calibrated at air-saturated MiR05 and zero oxygen after sodium dithionite addition. The homogenized muscle is added to the measurement chamber at 20 °C or 30 °C and a sequential substrate/inhibitor protocol is initiated. Several minutes after each substrate injection, a plateau is reached for the oxygen flux. The average flux value of the plateau is retrieved using Datlab software (Oroboros Instruments) in picomol.s-1.mg-1. First, the Krebs cycle and glycolysis are

simulated by adding 5 µL of 2 M (5 mM) Pyruvate, 2.5 µL of 400 mM (0.5 mM) Malate and 20 µL of 1 M succinate (10 mM). These substrates promote the electrons entry. Then, the OXPHOS state (state 3) is reached by adding a saturating concentration of ADP of 16 µL at 500 mM (4 mM). This state corresponds to the maximum oxidative phosphorylation capacity of the membrane (state 3) that comprises both proton leak rate and phosphorylating rate. The LEAK(Omy) state (state 4o) is then induced by the addition of 1 µL of Oligomycin at 0.01 mM (5 nM), a complex V inhibitor thereby prevent phosphorylation so that proton leak rate can be isolated from state 3. The residual oxygen consumption, ROX state, is obtained by the addition of 1 µL of 5 mM (2.5 µM) Antimycin A, an inhibitor stopping the functioning of the respiratory chain, in order to determine residual oxygen consumption. Finally, the COX capacity state is reached by adding 20 µL of of 800 mM Ascorbate (8 mM), and a 5µL of 200mM TMPD (0.5 mM) to obtain the Cytochrome C oxidase (COX) associated flux that is a proxy of mitochondrial density (Larsen et al., 2012). The auto-oxidation of Asc and TMPD was not evaluated in this protocol. The residual oxygen flux (ROX) is subtracted from each state (OXPHOS, LEAK(Omy) and COX capacity) to correct fluxes values.

## 2.4 Respiratory Control Ratio

The Respiratory Control Ratio (RCR) reflects the coupling of oxidative phosphorylation through the Electron Transport System flux and is thereby a measure of the efficiency of converting substrates to ATP (respiratory efficiency). It is calculated as the ratio of ATP-synthesizing mitochondrial respiration (OXPHOS state) over mitochondrial respiration in the absence of ATP-synthesizing (LEAK(Omy) state) (Brand and Nicholls, 2011; Estabrook, 1967).

## 2.5 Statistical analyses

We tested the effects of fish rearing temperature, assay temperature and their interactions on oxygen fluxes at the OXPHOS, LEAK(Omy), COX capacity state and on the RCR using linear mixed models (LMMs). We initially included the time from dissection to the start of the measurement, and the identification number of the measurement chamber as fixed effects in the LMMs but, in the absence of significant effects, they were finally excluded from the analyses. The measurement date was included as a random effect, as well as the identity of individual fish and their sex, to account for the repeated measurements (two measurements for each fish). Application conditions for the LMMs (homoscedasticity, normality, independence of residuals) were tested and the significance of the fixed model terms was assessed using analyses of deviance (Anova function from the car package). LMMs were computed using the lme4 package (Bates et al., 2015). To test for differences in mitochondrial density between the two fish rearing temperature, we used a linear mixed model with COX capacity flux as a response variable and rearing temperature as fixed independent variable. Assay temperature was included

in the model as random effect to account for the thermal sensitivity of the COX capacity. The measurement date, the identity of individual fish and their sex were also included as random effects. To test whether the OXPHOS and LEAK(Omy) fluxes remain similar across rearing temperature for the correlated COX capacity flux, we used a mixed ANCOVA model with OXPHOS or LEAK(Omy) fluxes as response variables and COX capacity flux, rearing temperature and their interactions as fixed independent variables. Assay temperature, the measurement date, the identity of individual fish and their sex were also included as random effects. This model allowed to standardize fluxes by COX capacity and thus removing the potential influence of changes in mitochondrial density on oxygen fluxes.

# 3. Results

## 3.1 Mitochondrial oxygen consumption

After two years of multigenerational exposure to 20 and 30°C, we measured mitochondria oxygen consumption from skeletal tail muscle of Medaka fish using two Oxygraph-2k high-resolution respirometers (Oroboros Instruments, Innsbruck Austria) for two states. These states corresponding to the rate of respiration supporting ATP synthesis (OXPHOS) and the respiration required to offset proton leak (LEAK), obtained using oligomycin (i.e. LEAK(Omy)). Finally, we measured the COX capacity (i.e. as a proxy of mitochondrial density of tissues). This choice is motivated by results from Larsen et al, 2012 who reported a strong correlation ($r = 0.94$, $p < 0.001$; Figure 3.H) between permeabilized fiber respiration rate using Ascorbate + TMPD as substrates, and the mitochondrial content (total mitochondrial volume) for skeletal muscle of human subject (Larsen et al., 2012).

For each respiratory state and the COX capacity, we used a linear mixed model (LMM) to test the effects of rearing temperature, assay temperature, and their interaction on the mitochondria oxygen fluxes (JO2 in picomol.s-1.mg-1). The statistical interaction between fish rearing temperature and assay temperature was non-significant for both OXPHOS (Chisq = 0.97, df = 1, p = 0.32) and LEAK(Omy) (Chisq = 0.80, df=1, p = 0.37) indicating that the effects of assay and rearing temperatures on oxygen fluxes were additive. Assay temperature significantly affected OXPHOS oxygen flux (Chisq =7.42, df =1, p = 0.005***) (Fig. 1A) and LEAK(Omy) oxygen flux (Chisq = 10.05, df = 1, p = 0.002 **) (Fig. 1B). For both WARM and COLD fish, we found that mitochondria assessed at 30°C have higher oxygen fluxes than those assessed at 20°C (Fig. 1A, Fig. 1B). Moreover, we found a significant effect of fish rearing temperature on oxygen fluxes at OXPHOS (Chisq = 20.76, df = 1, p = <0.0001***) (fig.1A) and LEAK(Omy) states (Chisq = 17.62, df= 1, p= <0.0001***) (Fig. 1B). At each assay temperature, the mitochondria of WARM fish had lower oxygen fluxes compared to the mitochondria of COLD fish (Fig. 1A, Fig. 1B). Finally, we compared oxygen fluxes between COLD fish assessed at 20°C and WARM fish assessed at 30°C to test if oxygen fluxes can converge to similar levels after multigenerational exposure. We found that no significant differences in their oxygen fluxes for both OXPHOS

(Chisq = 2.05, df = 1, p = 0.15) and LEAK(Omy) (Chisq = 2.006, df = 1, p = 0.16) despite a tendency towards lower flux for WARM fish.

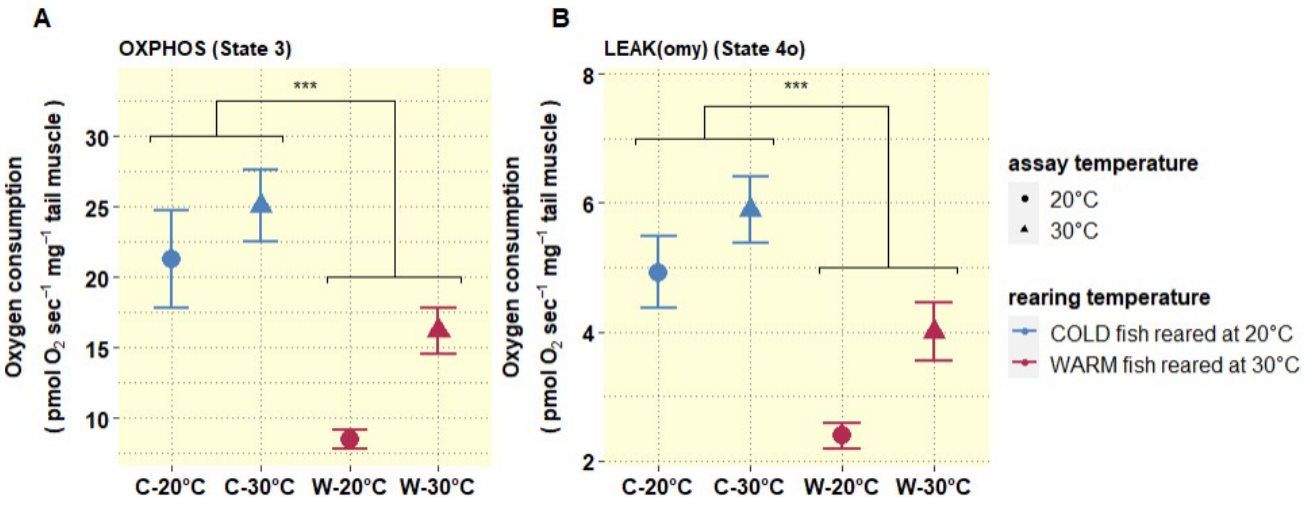


***Figure 1*** *: (A) OXPHOS respiration mean (± SE) O2 fluxes in picomol.s$^{-1}$.mg$^{-1}$ and (B) LEAK(Omy) respiration mean (± SE) O2 fluxes in picomol.s$^{-1}$.mg$^{-1}$ according to fish rearing temperature (COLD in blue or WARM in red) and assay temperature (20°C, dots or 30°C, triangles).*

## 3.2 Respiratory Control Ratio (RCR)

For each sample, we next computed the Respiratory Control Ratio (RCR) as the ratio of ATP-synthesizing mitochondrial respiration (OXPHOS respiration) over mitochondrial respiration in the absence of ATP-synthesizing (LEAK(Omy) respiration) (Brand and Nicholls, 2011; Estabrook, 1967). The RCR thus reflects the coupling of oxidative phosphorylation through the Electron Transport System flux and is thereby a measure of the efficiency of converting substrates to ATP (respiratory efficiency). We tested the effects of fish rearing temperature (20 °C or 30 °C), assay temperature (20 °C or 30 °C) and their interactions on the respiratory control ratio (RCR) using a LMM.

We found no significant effect of fish rearing temperature (Chisq = 0.21, df = 1, p = 0.65), assay temperature (Chisq = 2.78, df = 1, p = 0.09) or their interaction (Chisq = 0.87, df = 1, p = 0.35) on the RCR (Fig. 2).

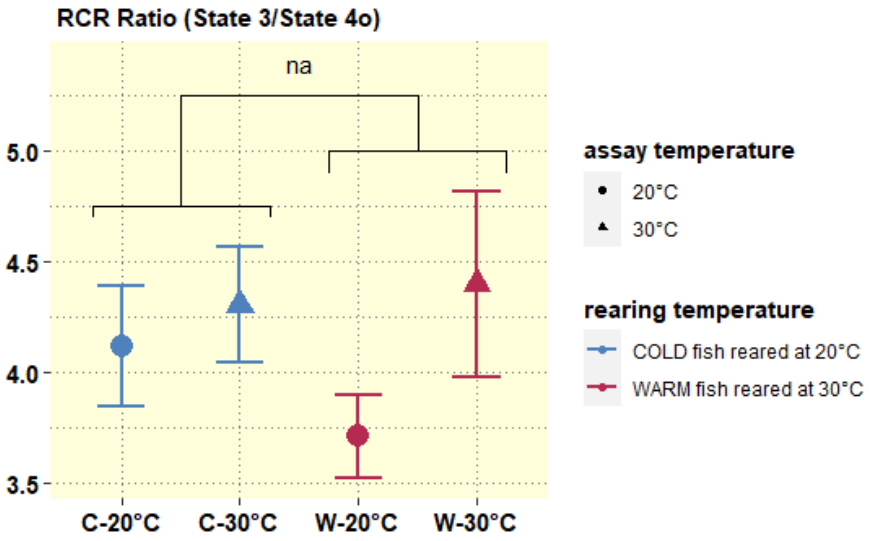


***Figure 2**: Mean (± SE) of RCR according to fish rearing temperature (COLD in blue or WARM in red) and assay temperature (20 °C, dots or 30 °C, triangles).*

## 3.3 OXPHOS and LEAK fluxes across COX capacity gradients

To test whether the OXPHOS (state 3) and LEAK(Omy) (state 4o) oxygen fluxes remain similar across fish rearing temperature along the COX capacity flux gradient, we used a mixed ANCOVA model with OXPHOS or LEAK(Omy) fluxes as response variables and COX capacity flux and fish rearing temperature as fixed independent variables.

We found no significant effect of the interaction between fish rearing temperature and COX capacity for both OXPHOS (Chisq = 0.99, df = 1, p = 0.32) and LEAK(Omy) fluxes (Chisq = 3.79, df = 1, p = 0.052). For both OXPHOS and LEAK(Omy) states, we found that WARM fish had a lower intercept than COLD fish but their slopes were not significantly different. (Fig. 3A, Fig. 3B).

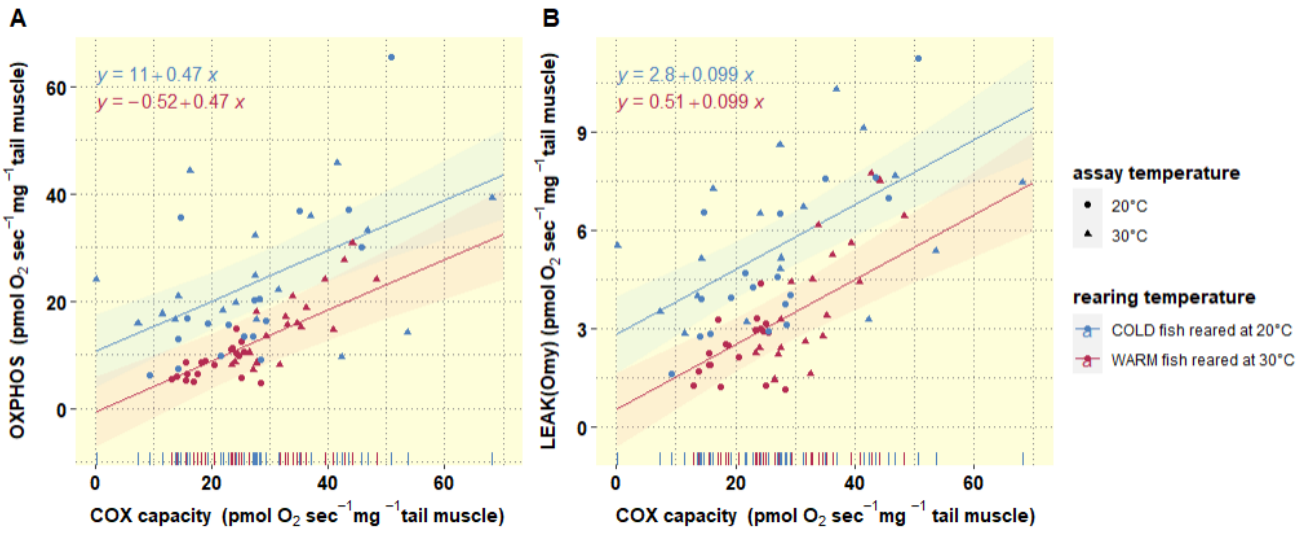


***Figure 3**: (A) OXPHOS O2 fluxes in picomol.s-1.mg-1 and (B) LEAK(Omy) O2 fluxes in picomol.s-1.mg-1 as a function of the COX capacity oxygen flux according to fish rearing temperature (COLD in blue or WARM in red) and Assay temperature (20°C, dots or 30°C, triangles).*

We found no significant impact of fish rearing temperature on oxygen fluxes for the COX capacity state (Chisq = 0.01, df = 1, p = 0.9). However, we found a significant impact of fish assay temperature on oxygen fluxes for the COX capacity (Chisq = 8.6, df = 1, p = 0.003**) reflecting the dependency of COX capacity to temperature (Fig. 4).

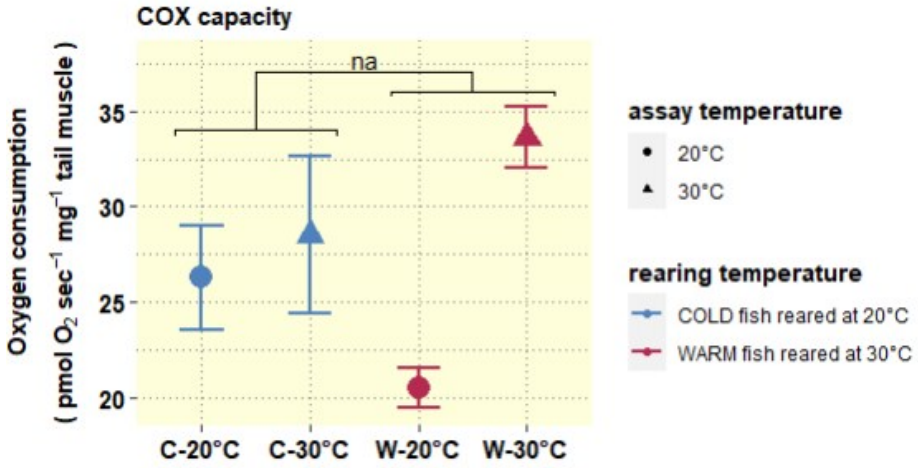


***Figure 4**: COX capacity mean (± SE) O2 fluxes in picomol O2.s-1.mg-1 according to fish rearing temperature (COLD in blue or WARM in red) and assay temperature (20°C, dots or 30°C, triangles).*

# 4. Discussion

The long-term impact of temperature on mitochondrial respiration is crucial for understanding how organisms can cope with temperature changes. It is well established that elevated temperature increases cellular oxygen demand which, in turn, increases mitochondrial oxygen consumption rates and ATP production (Arrhenius, 1889; Harman, 1956; Abele et al., 2002; Roussel and Voituron, 2020; Guderley and Gawlicka, 1992; Guderley, 2004). Some studies highlight that warm and cold acclimations in ectotherms can result in compensation of mitochondrial functions through modifications in mitochondrial respiration rates, density or compositional changes within membranes (Chung et al., 2017; Chung and Schulte, 2015; Seebacher et al., 2010; Yan and Xie, 2015). However, these responses can vary across studies depending on exposure duration and species studied. As a result, there is no consistent general pattern in the way within or across generation, acclimation to temperature modulates mitochondrial oxygen fluxes. Following these previous studies, our objective was to determine whether ectotherms could modulate their mitochondrial oxygen fluxes after a multigenerational (2 years) exposure to temperature.

We found that, for both COLD and WARM fish, OXPHOS and LEAK(Omy) respiration fluxes were higher for mitochondria assessed at 30°C than at 20°C. Our findings are in line with previous studies showing that temperature increases the rate of consumed oxygen molecules related to ATP production and accelerates the speed of biochemical reactions (Abele et al., 2002; Bielski et al., 1983; Brand, 2000; Guderley, 2004; Guderley and Gawlicka, 1992; Ricquier and Bouillaud, 1998; Roussel and Voituron, 2020; Sommer and Pörtner, 2004). This thermal impact can lead to a live fast-die young strategy where organisms live fast with higher reproduction and growth (maximizing fitness), which may counteract cellular damages accumulation that reduces performance with age (Harman, 1956; Rubner, 1908).

Interestingly, we found that, at each assay temperature, mitochondria from WARM fish had lower oxygen fluxes than those of COLD fish. These results are in line with our second hypothesis, suggesting a modulation of the mitochondrial response over the long-term which could be a mechanism to counteract short-term temperature exposure effects. Portner (2002) (Pörtner, 2002) highlights that cold adaptation may lead to an increasing temperature dependence of mitochondrial oxygen demand. This effect is at least partially explained by compensatory mechanism leading to an increase in mitochondrial oxidative capacities and/or mitochondrial density, as reported in previous studies on cold long-term exposure (Fangue et al., 2009; Guderley and Gawlicka, 1992; Lucassen et al., 2006; Schulte, 2015). According to Skulachev (1998) (Skulachev, 1998), mitochondrial uncoupling can decrease the production of cellular damage, and thus potentially counteract the effects of high temperatures in the long term (mechanism H2i). This phenomenon can reduce damage formation via phospholipids membrane composition and uncoupling proteins (UCPs) (Blanc et al., 2003; Brand and Nicholls, 2011; Ricquier and Bouillaud, 2000, 1998), leading to lower ATP production and mitochondrial efficiency (Skulachev, 1998). Studies suggest that UCPs may be differentially regulated in response to thermal long-term exposure in ectotherms and thus can modulate the mitochondrial

response (Dos Santos et al., 2013; Jastroch et al., 2007). Under the uncoupling hypothesis, we expected a low RCR for warm fish indicating low phosphorylating oxidation capacity relative to the oxidation required to compensate for proton leakage (mitochondrial coupling efficiency). However, we found no significant difference in RCR between treatments, which implies that fish maintained their respiratory efficiency regardless of the temperature treatments. To deepen our results understanding, it would be interesting to quantify uncoupling proteins (UCPs) even if their physiological functions are not yet clearly established in fish (Gaudry and Jastroch, 2019; Nègre-Salvayre et al., 1997), as well as phospholipids in the membrane and the ATP production/O2 consumption ratio.

A second mechanism that may explain a modulation of the mitochondrial response is related to mitochondrial density (H2.ii). Previous studies suggest that the extent of thermal tolerance results from limitations imposed by mitochondrial function and density (Pörtner, 2002; Pörtner et al., 1999). For instance, fish seasonal cold acclimatization can increase mitochondrial density or mitochondrial aerobic capacity (Guderley et al., 1997; Kawall et al., 2002), while fish populations living in warm environments often have lower mitochondrial densities (Keller et al., 2004). Thus, a lower mitochondrial density would decrease oxygen fluxes and could explain an overall decrease in oxygen consumption in warm exposed fish. Conversely, in cold acclimated fish, an increase in mitochondrial density would increase the amount of mitochondrial enzyme needed to maintain high reaction rates (Guderley and Seebacher, 2011) which may also contribute to explain the differences between WARM and COLD exposed fish in our study. Using COX capacity fluxes as a proxy of mitochondrial density, we investigated if the differences between WARM and COLD fish are associated to difference in mitochondrial densities. We found no significant effects of fish rearing temperature on oxygen fluxes for COX capacity state. This result could suggest that mitochondrial density was not influenced by the multigenerational exposure to temperature in our study. We also found that, for a similar mitochondrial density (i.e. at a given COX flux), WARM fish have lower oxygen fluxes that COLD fish for OXPHOS and LEAK(Omy) states. However, we need to nuance this results because the auto-oxidation of Asc and TMPD was not evaluated in this protocol (i.e. sodium-azide can be used to inhibit the complex IV and correct for auto-oxidation of TMPD at elevated temperature) and may have influenced our data and their interpretation. We therefore cautiously conclude that our results are probably not explained by changes in mitochondria density.

Overall, our findings suggest that uncoupling or mitochondrial density changes are not the main mechanisms that could explain the observed modulation in mitochondrial respiration. A third mechanism could be a modulation of mitochondrial metabolic capacity in response to multigenerational exposure to high and low temperatures (H2.iii). This suggests internal changes in mitochondrial function through the implementation of regulatory mechanisms over the generations through physiological changes, enzyme isoform, and protein structure and membrane fluidity modifications or in the degree of unsaturation of the fatty acids (Bielski et al., 1983; Cossins and Society for Experimental Biology (Great Britain), 1994; Hazel, 1990). However, it is difficult to determine when and where (internal or external to the mitochondria) this regulatory mechanisms occur. Another possibility could be (ii) a maintenance of the ATP production/$O_2$ consumption ratio. After several generation, WARM fish may produce less ATP and thus have lower

oxygen requirements while still having an efficient ATP/$O_2$ ratio. Conversely, COLD fish may produce more ATP and have higher oxygen requirements.

Understanding whether modifications in mitochondrial oxygen fluxes translate to changes in individual respiration rate is important for the integration of thermal effects across scales. Interestingly, a recent study showed that nine years of experimental warming did not influence the thermal sensitivity of individual respiration rate in medaka fish (Alberto-Payet et al., 2022) which contrasts with previous studies (Kawall et al., 2002; Moffett et al., 2018; Somero, 2012). This phenomenon of maintaining respiratory efficiency could also be related to the absence of significant differences in respiratory flux when fish were assessed at their rearing temperature (i.e. COLD fish assessed at 20°C vs WARM fish assessed at 30°C). This could reflect the existence of an optimal respiration flux when organisms have enough time to acclimate or adapt to local conditions. A next step would be to investigate whether these processes are linked to selection over several generations or to plasticity of individuals or of the mitochondria itself. In a global change context, our results indicate that exposure to temperature during two years over several generations can modulate the thermal responses at the mitochondria level. Interestingly, this lead to similar mitochondrial respiratory rates when comparing the mitochondrial respiration of fish assessed at their rearing temperature (i.e. COLD-20°C vs. WARM-30°C). This result suggests that multigenerational exposure to temperature should be taken into account to predict the ecological impacts of global warming that are assessed using the metabolic theory of ecology under which metabolic rate increases exponentially with temperature (Brown et al., 2004; Schulte et al., 2011; Yvon-Durocher et al., 2010). It would thus be interesting to investigate potential trade-offs between increasing energy allocation in life history traits and reducing the production of pro-oxydant, and, in turn, cell senescence. We also need to determine the origin of the changes in mitochondrial phenotypes because, after several generations, plasticity, genetic and/or epigenetic inheritance could have influenced the frequency of the phenotype in our populations (Boukal et al., 2019; Monaghan et al., 2009). Especially, epigenetic mechanisms such as histone acetylation, DNA and RNA methylation, can be passed from one generation to another and induce changes across generations (Burton and Greer, 2022; Hao et al., 2020; Holliday and Pugh, 1975; Matilainen et al., 2017; Razin and Riggs, 1980). It is possible that differences in the number of generations in our study can lead to a higher accumulation of epigenetic modifications for WARM fish compared to COLD fish and affect how mitochondrial traits are responding to assay temperature. However, in a previous study on Médaka (*Orizias Latipes*) testing the impact of warm multigenerational exposure on growth pattern (Loisel, 2019), we found that the effects of growth and reproduction were mainly plastic responses. We thus attribute our result to plastic acclimation effect although molecular analyses would be need to rule out the influence of genetic or epigenetic variations.

Another potential factor of metabolic stress is a lower oxygen concentration in warmer waters, while metabolic demand increases with temperature. In our experiment, oxygen concentration was lower at 30°C but was not limiting (88.4%) and non-stressful. Furthermore, the impacts of low oxygen concentration on fish are complex and the question of whether partial pressure or solubility limits oxygen supply is still poorly

understood (Verberk et al., 2011). This suggests that our results are probably not explained by limitations in oxygen availability.

To conclude, our results indicate that multigenerational exposure leads to modifications in mitochondrial respiration without modifications of the oxidative phosphorylation process effi-ciency or mitochondrial density. Our study highlights that multigenerational exposure can leads to a modulatory response to compensate for potential detrimental consequences of tem-perature and highlights that both responses (i.e. rearing temperature versus assay temperature) should be taken into account to better understand how organisms can cope with temperature changes. Finally, the absence of significant differences in respiratory flux between COLD fish assessed at 20°C and WARM fish assessed at 30°C in our experiment could reflect the exist-ence of an optimal respiration flux when organisms have sufficient time to acclimate or adapt to local conditions. Future studies are required to better understand the diversity of mecha-nisms by which organisms can modulate their mitochondrial capacity under temperature changes.

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# 6. Supplementary material

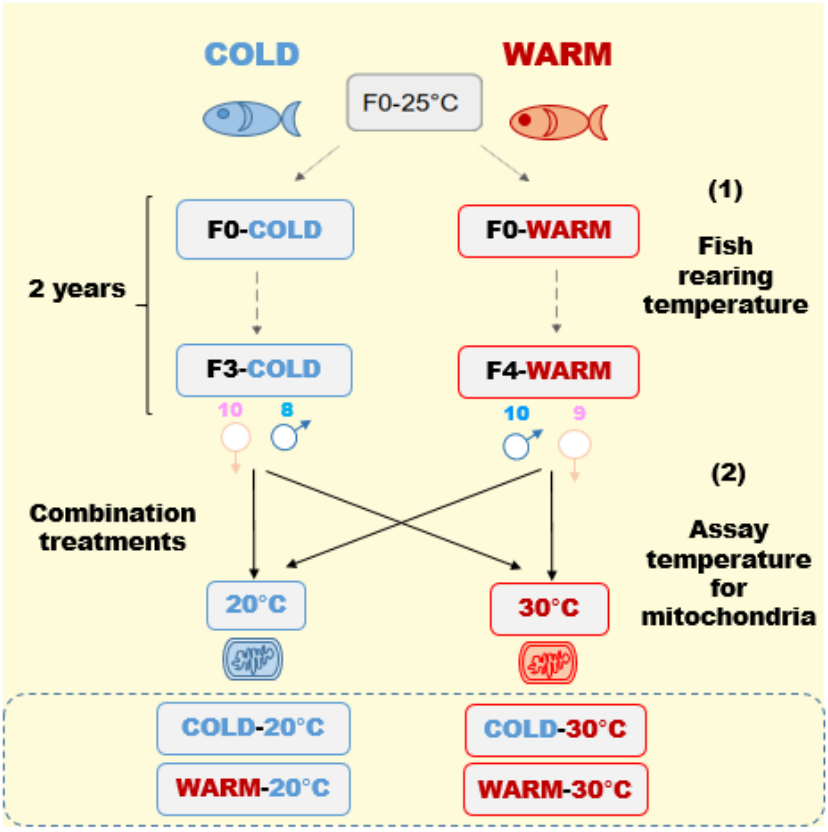


***Fig. S1**: Experiment design with the two crossed conditions: Ancestral temperature and assay temperature*

# Chapter 2

# Multigenerational effects of temperature exposure on upper thermal limit and mitochondrial functioning in Medaka (*Orizias Latipes*) brain

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AIM: The second chapter of my thesis focuses on the relationchip between whole organism thermal response and mitochondria fonctioning under thermal pressure. The aim of this chapter was to determined the upper thermal tolerance (CTmax) of medaka fish maintained over several generations (five years) at cold (20°C) and warm (30°C) temperatures and to highlight a link between dysfunction of fish brain mitochondria and whole organism thermal tolerance (CTmax). Mitochondria plays a central role in the control of metabolism and life-history traits and better understanding the relationship between mitochondria and thermal tolerance is particularly important in a multi-scale context because the thermal tolerance of organisms will play a major role in the distribution of populations and in trophic interactions in freshwater environments.

MY CONTRIBUTION: I participated in setting up the experiment, designing the experimental protocol, measuring mitochondrial flows and collecting data with the help of Rémy Lassus, Melissa Richard, Arnaud Sentis and Martin Daufresne. I also carried out the statistical analyses with the help of Arnaud Sentis and the writing of the first version of the paper as well as the writing of the revised versions in order to improve its quality over the course of the revisions carried out by Arnaud Sentis, Martin Daufresne and Nicolas Pichaud.

# Article 2: Multigenerational effects of temperature exposure on upper thermal limit and mitochondrial functioning in Medaka (*Orizias Latipes*) brain

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DATA AVAILABILITY STATEMENT:

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ANIMAL ETHICAL DECLARATION: Experimental protocols were approved and performed in agreement with the ethical permits #42422-2023040411317548 v3. The above-mentioned project has been ethically assessed by the ethics committee for animal experimentation n°071.

AUTHORS' CONTRIBUTIONS: M.D., A.S., J.M, N.P and R.L. conceived the ideas and designed methodology. J.M., R.L. & M.R. collected the data. J.M., A.S and M.R. analysed the data. J.M. wrote the first draft or the manuscript and N.P., A.S & M.D. contributed to its revision. All authors gave final approval for publication.

## Abstract

Determining the mechanisms underpinning the thermal limits of organisms is essential to anticipate the impact of climate change on their survival. As mitochondria activity is key for the production of energy to the cells, we aimed at investigating the link between mitochondrial dysfunction and the upper thermal limit (CTmax) at which neuromuscular functions are lost. We reared medaka fish (Orizyas latipes) over generations at 20°C (COLD) and 30°C (WARM) during five years and measured their CTmax and their mitochondrial oxygen consumption rates (OCRs) at 25°C and at their CTmax. We found that multigenerational exposure at elevated temperature increases the CTmax by 13%. We observed for all fish a general decrease in the OCRs and for the complex-I coupling efficiency between 25°C and their CTmax suggesting a link between CTmax and cerebral mitochondrial performance. Finally, we found an increase in the contribution of the mitochondria complex II to OCRs at elevated temperature suggesting that this complex underlie the plasticity in mitochondrial functioning at high temperature. Our study highlights the link between whole organism thermal tolerance and mitochondrial dysfunction and that multigenerational exposure can modify mitochondrial and thermal performance.

Keywords: Multigenerational exposure; Ectotherm; Temperature; CTmax; Mitochondrial respiration

## 1. Introduction

Temperature is a fundamental environmental factor highly affecting metabolic and physiological processes, through the increased the rate of biochemical reactions in ectotherms. Global warming and the expected increase in the frequency of extreme thermal events (IPCC 2023) is thus imposing strong constraints on ectothermic organisms. A rapid increase in temperature, particularly in shallow aquatic environments that are highly sensitive to temperature fluctuations, is a determining stress factor for aquatic ectotherms (Woodward, 2009). In the current context, a better understanding of the thermal tolerance of ectotherms is thus crucial to predict the vulnerability of aquatic ectotherms to these rapid changing environmental conditions.

Thermal vulnerability indices such as safety margins and warming tolerance have increased in popularity for predicting species vulnerability to climate change (Clusella-Trullas et al., 2021). For each species, we can define specific tolerated temperature ranges in which optimum temperature values represent maximum performance (T°Opt) as well as temperatures at which performance is negatively affected and where the species survival is jeopardized. Cowles and Bogert in 1944 defined the upper limit to thermal tolerance as "the thermal point at which locomotor activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death" (Cowles and Bogert, 1944). Such limit is generally refers as to the critical thermal maximal (CTmax; Martínez et al., 2016; O'Donnell et al., 2020). CTmax is considered as an excellent

proxy of upper thermal tolerance as it trigger similar behavioural responses across a diversity of species (Lutterschmidt and Hutchison, 1997).

Understanding the limiting physiological processes determining the CTmax has been the focus of several previous studies (reviewed in Chung & Schulte, 2020). For most organisms, thermal responses are determined by physiological processes at the metabolic level. Following the Arrhenius law, the metabolism of ectotherms increases with warming, accelerating cellular respiratory and oxygen demand (Arrhenius, 1889; Hilton et al., 2010; Sommer and Pörtner, 2004). Mitochondria are central to cellular respiration as they contain the enzyme systems required to transduce energy from reserves and nutrients in the form of adenosine triphosphate (ATP) needed to maintain essential body functions. Enzyme and protein complexes of the respiratory chain that allow cellular respiration are profoundly influenced by temperature (Fig. S1; Abele et al., 1998; Clotfelter et al., 2013; Somero et al., 2017). Previous studies reported that the different mitochondrial complexes have different thermal sensitivities and may play a role in thermal adaptation of ectotherms (Blier et al., 2014; Chung and Schulte, 2020). Especially, the first complex (complex I) of the respiratory chain has been suggested to be a site of thermal mitochondrial damage after a critical heat exposure (El-Wadawi and Bowler, 1996; Jørgensen et al., 2021; Menail et al., 2022). Some studies highlight that heat tolerance was correlated with failure of mitochondrial oxygen consumption for complex I, which increases intracellular waste (Reactive Oxygen Species, i.e. ROS) levels and enhances macromolecular damage (Genova et al., 2004; Jørgensen et al., 2021; Lenaz et al., 1997). Thus, the study of these complexes can help to explain the thermal limits of ectotherms, which holds important significance in the context of the current climate warming and species adaptation.

Although mitochondria can function at temperatures higher than the organism's CTmax (Somero et al., 1996; Somero, 2002; Pörtner, 2002; Chung & Schulte, 2020), recent studies suggested a link between mitochondrial dysfunction, heart and brain failures as well as the whole-body thermal tolerance (Chung and Schulte, 2020; Ekström et al., 2016; Fangue et al., 2009; Harada et al., 2019; Hraoui et al., 2020; Martínez et al., 2016). In particular, mitochondria may specifically mediate heart failure under heat stress and play a role in CTmax response (Ekström et al., 2016; Hunter-Manseau et al., 2019; Iftikar et al., 2014; Iftikar and Hickey, 2013; Pichaud et al., 2017). However, mitochondrial dysfunction could also underlie neuronal failure. Indeed, mitochondrial oxygen consumption measured in the brain could indicate greater mitochondrial thermal sensitivity compared to cardiac tissue in *Fundulus heteroclitus* (Chung et al., 2017). Furthermore, neuronal system dysfunction induced by temperature have been suggested to constrain whole-organism thermal limits (Ern et al., 2015; Somero and DeVries, 1967). Overall, these studies highlight the role of mitochondrial functions on the whole organism response even if the link between mitochondrial failure and failure of higher-level processes is not well established yet (Bilyk & DeVries, 2011; Clark et al., 2013; Ern et al., 2015; Schulte, 2015; Ørsted et al., 2022).

Several studies suggest that CTmax varies with the exposure duration and may thus be enhanced after a period of acclimation to elevated temperature (Angilletta, 2001; Bilyk

and DeVries, 2011). Some studies found that thermal acclimation can lead to plastic modifications of the structure and function of mitochondria in ectotherms (Guderley and St-Pierre, 2002; Seebacher et al., 2010). Warm acclimation generally results in a decrease in mitochondrial metabolism at elevated temperatures, due to a decrease in mitochondrial respiration rates, or a decrease in mitochondrial density or through compositional changes within mitochondrial membranes (Ekström et al., 2016; Schulte, 2015; Yan & Xie, 2015; Chung et al., 2017; Morla et al., under review). These adjustments may result both from reversible phenotypic plasticity, notably observable in mitochondria, and from genetic adaptation over generations (Guderley and St-Pierre, 2002; Seebacher et al., 2010) and can have an impact on population performance. In a recent study, Rank et al., 2020, reported that population of montane leaf beetle can exhibit latitudinal differences in mitochondrial and nuclear genotypes that are associated with differences in performance traits, suggesting that mitochondrial thermal limits can affect whole organism performance. Due to the complexity of the thermal acclimation process, the different components of mitochondrial metabolism affected by temperature are unclear although it can be crucial to predict and anticipate the impacts of rapid global warming on ectotherms.

In this study, our objective was to determine multigenerational temperature exposure effects on (1) the upper critical thermal maximum (CTmax) of the whole organism medaka fish (*Orizyas Latipes*), (2) the respiration in their brain mitochondria (i.e. mitochondrial oxygen consumption rates, OCRs) and (3) the link between mitochondrial dysfunction and CTmax. We hypothesized that CTmax can be increases by a multigenerational exposure at elevated temperature (Angilletta, 2001; Bilyk and DeVries, 2011). We also hypothesized that mitochondrial neural dysfunction can limit the CTmax, (Somero and DeVries, 1967; Ern et al., 2015). To test our hypotheses, we assessed the CTmax of medaka fish maintained over several generations to cold (20°C) and warm (30°C) temperatures by exposing fish of each thermal origin to a progressive increase in temperature until it loses its locomotor function. In a second step, we measured the mitochondrial OCRs of fish from the two lines at 25°C and at their mean CTmax focusing on differences between the contributions of the different mitochondrial complexes to respiration.

# 2. Materials & Methods

## 2.1 Biological model and rearing conditions

The Japanese medaka fish (*Oryzias Latipes*) is a small amphidromous freshwater fish from Southeast Asia. This species is eurythermic with a thermal range from zero to 40 °C (Dhillon and Fox, 2007; Hirshfield, 1980; Shima and Mitani, 2004), and a thermal optimum of 25°C (Kirchen & West, 1976; Hirshfield, 1980; Leaf et al., 2011). It has a short generation time of 3 months and can reach sexual maturity in about 10 weeks (Hirshfield, 1980), making it a good biological model for multigenerational thermal experimentation. In its native range, the medaka generally lives in small bodies of water such as rice paddies

and with important temperature fluctuations (Hirshfield, 1980). In small shallow water bodies, adult medaka can support up to 43°C (Sentis. A, personal observation). Medaka used in this study were reared at 20 °C (COLD fish) or 30 °C (WARM fish) over several generations under controlled experimental conditions in climatic chambers. Two lineages by temperature (W1, W2 for WARM fish and C1, C2 for COLD fish) were obtained from a starting F0 population of 320 Medakas (160 females & 160 males) belonging to the CAB strain from Carolina Biological Supply Company (USA) and provided by AMAGEN© (France). F0 generation fish were maintained at 25°C and were then progressively acclimatized to 20 or 30 °C at a rate of one degree every 2 days. To start a new fish generation, we collected eggs clutches during the optimal fecundity period and placed them in hatcheries at the parental temperature (20 °C or 30 °C). We maintained the new-born fish in climatic chambers at the same temperature as their parents (20 °C or 30 °C) and then placed them in five replicates aquariums (25 × 40 × 20 cm) until maturity, with a density of ~30 adult fish per aquarium. The fish were fed "ad libitum" with dry flakes food TétraMin© three times a day (at 9h, 13h and 18h).

## 2.2 Whole organism thermal critical maximum tolerance CT max

After 5 years of multigenerational exposure to 20°C and 30°C, we exposed fish from each thermal origin to a thermal tolerance dynamic test to estimate their CTmax. We used 32 fish from the two different thermal origin (W1 & W2, C1 & C2). Specifically, we used 16 fish maintained at 20°C for six generations (F6): 8 adults females (4 from C1 & 4 from C2) (mean age in days ± se: 215.1 ± 6.7; i.e. 4.302 degree days) and 8 adults males (4 from C1 & 4 from C2) (215.1 ± 6.7). We also used 16 fish maintained at 30°C for ten generation (F10): 8 adults females (4 from W1 & 4 from W2) (122.6 ± 3.8 days; i.e. 3.678 degree days) and 8 adults males (4 from W1 & 4 from W2) (122.6 ± 3.8 days). Ages of the studied fish were chosen accordingly to female reproductive optimum in terms of clutch size (i.e. number of eggs laid per day and per female; Loisel, 2019). Although fish had different ages in days, their physiological ages in degree-days were relatively close (i.e. 3.678 degree days for WARM fish and 4.302 degree days for COLD fish).

WARM and COLD fish used in the experiments present differences in generation numbers (10 and 6, respectively) due to a shorter generation time at 30°C compared to 20°C. Difference in the number of generation is due to warming condition applied in this study, as fish grow faster and have an early sexual maturity when temperature increase resulting in asynchronous generations between the two acclimation temperatures (Alberto-Payet et al., 2022; Atkinson, 1995, 1994). To get the same fish generation, the experiment should have been run for additional years. Therefore, we preferred comparing mitochondrial respiration of fish at the same date rather than at the same generation to reduce the risk of changes in experimental conditions experienced by WARM and COLD fish (e.g. water quality or potential diseases) over a year, as well as potential changes in conditions of oxygen flux measurements. A previous study on the medaka fish (Loisel, 2019) showed that there was few difference in fish growth between

generations over 10 generations of exposure at 20°C and 30°C and that the responses observed were mainly plastic (following a common garden experiment).

In addition, to test if the CTmax experiment could influence mitochondrial respiration, 16 control fish (i.e. 4 males and 4 females from each thermal origin) were also maintained under the same laboratory conditions except that they were not exposed to the CTmax test.

To estimate the CTmax, two water baths (WB) were used (WB1 and WB2) (Fig. S2). Each WB had a water pump and a water heater (1500 Watt). Within each WB, two aquariums were placed at each side of the WB (hereafter referred as to top and bottom positions T & B; Fig. S2). To reduce bias, the location of the fish in the aquariums (i.e. T or B) and in the WB (i.e. WB1 & WB2) was switched each day. The water for the experimental tanks came from the Canal de Provence (water from the Verdon River) and was filtered using two mechanical filters (25 µm then 5 µm) and a UV filter to eliminate microorganisms. Two cameras were installed at the front and at the top of the water-baths to observe fish behaviour remotely. Before the CTmax experiment, each fish was weighed, measured, and photographed.

For each thermal origin and lineage, pairs (one male, one female) were placed in an aquarium in the WB at their rearing temperature (20°C or 30°C). Control fish were placed in a recovery aquarium. We then exposed organisms to rapid temperature changes through a constant heating rate (~ 1.5°C every 10 minutes) that allows deep body temperature to follow ambient temperatures without a significant time lag (Hutchison 1961, 1976). Loss of equilibrium (LOE) was used as an end-point for this experiment (Ziegeweid et al., 2008). Fish were then immediately returned to a tank at their initial temperature to allow their total recovery. Oxygen concentration was measured before and after each temperature rise using an oxygen probe (HACH - HQ40d) and temperature was recorded continuously during the CTmax trial. For the recovery phase, fish were fed twice a day and monitored individually to ensure their recovery (normal behaviour, weight gain, absence of lesions) over the following 20 days (Tab. s1).

## 2.3 Measurement of mitochondrial oxygen consumption rates (OCRs)

After 20 days of recovery following the CTmax experiment, we measured their mitochondrial OCR at 25 °C (Topt) and at mean CTmax for each thermal origin (WARM and COLD), defined as $\overline{CTmax}$, to determine the effects of transgenerational long-term temperature on mitochondrial functioning. We used a full factorial experimental design (Fig. S3A,B) with (I) fish from the two ancestral temperatures (COLD or WARM) tested at (II) three assay temperatures: 25 °C (thermal optimum), $\overline{CTmax}$ of COLD fish and $\overline{CTmax}$ of WARM fish (obtained from the previous experiment).

Medakas were euthanized by percussion of the cranial cavity and then measured (mm), weighted (mg) and dissected to sample cerebral tissue of each fish. Dissections were

conducted within five minutes following euthanasia at 4 °C. For each fish, the brain was transferred in 0.5M Respiration Medium (MIR) (EGTA 0.5mM, MgCl2 3 mM, Lactobionic acid 60mM, Taurine 20mM, KH2PO4 10 mM, HEPES 20 mM, D-Sucrose 110 mM; Pesta and Gnaiger, 2012), gently mechanically permeabilized with fine forceps and placed on an orbital shaker at 4 °C for 5 min. The permeabilized tissue was split into three different parts and weighed before measurements. Mitochondrial OCRs (pmol O2.s-1.mg-1 of tissue) were measured using three Oxygraph-2k high resolution respirometers (Oroboros Instruments, Innsbruck Austria) equipped with two independent measuring chambers. For each fish, we tested simultaneously three assay temperatures (25 °C, $\overline{CTmax}$ COLD fish, $\overline{CTmax}$ WARM fish) and the oxygen electrodes were calibrated at air-saturated MiR05 and zero oxygen after sodium dithionite addition for each specific temperature tested. To establish OCR a sequential substrate/inhibitor protocol was used (Fig. S4). Specifically, the permeabilized cerebral tissue sample was added to the oxygraph chamber and we immediately injected 5mM Pyruvate, 2mM Malate and 5mM glutamate. These substrates allowed to determine the LEAK value for proton gradient created by electron transfer in complex I of the electron transport system, which represents the oxygen consumption compensating for the proton leak when ATP synthase is not active (non-phosphorylating state). Then, the oxidative phosphorylation at the level of complex I (OXPHOS-CI) was initiated by adding a 2.5 mM saturating concentration of ADP to couple the proton gradient created by electron transfer in CI to phosphorylation of ADP to ATP. Additional injection of cytochrome C (10µM) was used to verify the outer mitochondrial membrane integrity (CYTO-C). The oxidative phosphorylation at the level of complex I+II (OXPHOS-CI+II) was measured with the addition of 10mM succinate. Electron transport system capacity (ETS-CI+II) (reflecting the maximal mitochondrial respiration induced in a noncoupled state) was achieved with repeated additions of carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone 0.5µM (FCCP, 1 + 1 + 1 µL). This was followed by inhibition of complexes I and III through the sequential addition of 0.5µL rotenone and 2.5µM Antimycin A, respectively. The resulting residual oxygen consumption or non-mitochondrial oxygen consumption obtained was subtracted from the previous rates obtained to correct flux values and obtain only mitochondrial specific OCR. The COX-activity flux was then reached by adding 2mM of Ascorbate and a stepwise addition (5 µL + 5 µL + ...) of 0.5mM TMPD. Finally, we added (≥100mM) sodium azide to inhibit the complex IV and correct for auto-oxidation of TMPD. Several minutes after each injection, a plateau is reached for the oxygen flux. The average flux value of the plateau was retrieved using Datlab software (Oroboros Instruments) to obtain OCRs. We obtain 5 OCRs (LEAK-CI, OXPHOS-CI, OXPHOS-CI+II, ETS-CI+II and COX) representing different processes of the mitochondria respiratory chain allowing us to better understand mitochondrial functioning and performance (Fig. S1; Fig.S4).

## 2.4 Mitochondrial Ratio

The coupling efficiency of complex I (CE.CI) which reflects the coupling between electron transport and oxidative phosphorylation at the level of complex I was calculated as the ratio of ATP-synthesizing mitochondrial respiration (OXPHOS-CI) over mitochondrial

respiration in the absence of ATP-synthesizing (LEAK-CI) with complex I-induced respiratory substrates (Brand and Nicholls, 2011; Estabrook, 1967).

$$\text{CE.CI} = \frac{OXPHOS\ CI}{LEAKCI}$$

We also calculated the substrate relative contribution ratio (SCR) (i.e. the relative contribution to increased oxygen consumption rate when adding a new substrate) of complex I + II (succinate) to complex I only (Cytochrome-C) for OXPHOS state:

$$\text{SCR} = \frac{OXPHOS\ CI{+}II - CYTOC\ CI}{CYTOC\ CI}$$

Finally, for each fish, we calculated ratios of OXPHOS-CI, LEAK-CI and OXPHOS-CI+II values measured at their $\overline{CTmax}$ divided by 25 °C assay temperature values (i.e. OCR $\overline{CTmax}$/ OCR 25 °C) to characterize the fold change in mitochondrial oxygen consumption at the CTmax relative to the oxygen consumption at the optimal temperature.

## 2.5 Statistical analyses

Statistical analyses were performed using R software version 4.3.1 (R Foundation for Statistical Computing Platform, Free Software Foundation's, Boston, USA). For the CTmax experiment, we used a linear mixed effect model (LMM) to test the effects of fish ancestral temperatures (i.e. 20 °C or 30 °C), fish lineage, fish sex and their interactions on the $\overline{CTmax}$ values. The aquarium position nested in the experimental day (i.e. the same information as WB) were included as random effects, to account for potential spatial and temporal effects.

For OCRs at the mitochondrial level, we tested the effects of fish ancestral temperatures, assay temperatures, treatment (CTmax test or control fish), fish sex, fish lineage, and their interactions for LEAK-CI, OXPHOS-CI, OXPHOS-CI+II, ETS-CI+II and COX activity as well as on the CI coupling efficiency and SCR using LMMs. The fish identification number and the couple pair code nested in the Date experiment were included as random factors, to account for the repeated measurements (three measurements for each fish) and for potential temporal effects. For each model, when the interaction was significant between the two experimental conditions tested, we divided the datasets by the experimental condition using LMMs. OCRs presenting anomaly during measurements or not having reacted to injections were excluded from the analyses.

We then compared OCRs between the COLD fish at their assay temperature $\overline{CTmax}$ (C-38.9) and the WARM fish at their assay temperature $\overline{CTmax}$ (W-43.8) using LMMs to

better understand if their mitochondria function similarly when exposed to their respective CTmax temperatures.

We finally compared distribution pattern of the mitochondrial OCRs at $\overline{CTmax}$ assay temperature as a function of relative CTmax for each fish for OXPHOS-CI, LEAK-CI and OXPHOS-CI+II. We also compared distribution pattern for the OCR at $\overline{CTmax}$ divided by the OCR at 25°C for OXPHOS CI, LEAK CI & OXPHOS CI+II as a function of CTmax whole-organism values for each thermal origin and tested the differences in the Ratio (flux CTM/ flux 25°C) between the two fish thermal origins using LMMs.

Application conditions for the LMMs (homoscedasticity, normality, independence of residuals) were tested. The significance of the fixed model terms was assessed using analyses of deviance (Anova function from the car package) and non-significant ($p > 0.05$) variables or interactions were removed from the final model. LMMs were computed using the lme4 package (Bates et al., 2015).

# 3. Results

## 3.1 Whole organism thermal critical maximum (CTmax)

The $\overline{CTmax}$ values (Fig. 1) were not influenced by the lineage and the sex of fish (Tab. S2). $\overline{CTmax}$ values were however significantly influenced by fish ancestral temperature (Chisq = 163, df = 1, $p < 0.001$ ***, Fig. 1, Tab. S2). Specifically, WARM fish had a $\overline{CTmax}$ (43.8 °C ± 0.27) significantly higher than COLD fish (38.9 °C ± 0.32) representing a 12.6% increase.

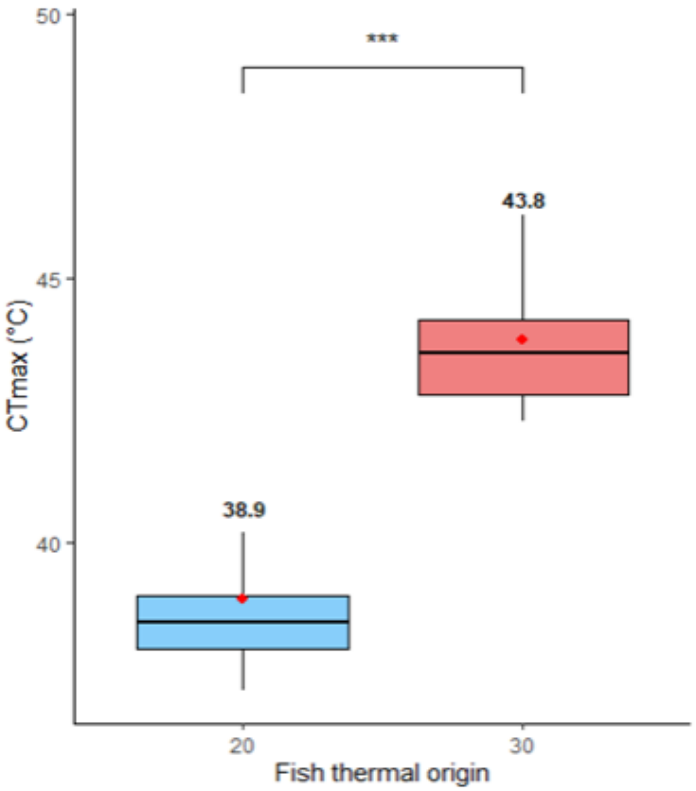


***Figure 1**: Boxplot with mean (red dots) of the CTmax (°C) according to fish ancestral temperature (20 for COLD fish & 30 for WARM fish) (N= 32). *** represents highly significant differences between WARM and COLD fish (P < 0.001).*

## 3.2 Mitochondrial oxygen consumption rates (OCRs)

LEAK-CI (Fig. 2a, Tab. S3) was not influenced by the sex, the treatment, and the lineage (Tab. S4) nor by the interaction between ancestral temperatures and assay temperatures (Chisq = 4.9, df = 2, p = 0.09). However, LEAK-CI OCR was significantly influenced by assay temperature (Chisq =16.5, df =2, p < 0.001 ***) through increased OCR with temperatures. We found no significant effect of ancestral temperatures on LEAK-CI (Chisq = 1.05, df = 1, p = 0.31).

OXPHOS-CI (Fig. 2b, Tab. S3) was not affected by the treatment, the sex, and the lineage (Tab. S4). However, it was significantly influenced by the interaction between ancestral temperature and assay temperature (Chisq = 9.94, df = 2, p = 0.006 **). When we split the dataset by experimental conditions, COLD and WARM fish OXPHOS-CI state was significantly affected by assay temperature. For COLD fish, we observed a decrease with increased assay temperature (Chisq = 12.39, df = 2, p < 0.005**) and for WARM fish we observe an increase between 25 °C and 38.9 °C followed by a decrease between 38.9 °C and 43.8 °C (Chisq = 12.16, df = 2, p < 0.005**). Moreover, we found a significant difference between COLD and WARM fish at the 38.9 °C assay temperature (Chisq = 4.8, df = 1, p <

0.05*) and at 43.8 °C (Chisq = 4.21, df = 1, p < 0.05*) but no difference for 25 °C (Chisq = 0.3, df = 1, p =0.59).

We found no significant effect of the sex, treatment, and lineage for OXPHOS-CI+II (Fig. 2c, Tab. S3) (Tab. S4). OXPHOS-CI+II was significantly influenced by the interaction between ancestral temperature and assay temperature (Chisq = 6.14, df = 2, p =0.05*). When we split the dataset by experimental conditions, OXPHOS-CI+CII was significantly influenced by assay temperature for COLD and WARM fish. For COLD fish, we observed a decrease with increased assay temperature (Chisq = 6, df = 2, p =0.05*), and for WARM fish, an increase between 25 °C and 38.9 °C followed by a decrease between 38.9 °C and 43.8 °C (Chisq = 21.3, df = 2, p < 0.001***) . We found no significant difference between COLD and WARM fish for each assay temperature (25 °C: Chisq = 1e-04, df = 2, p =0.99; 38.9 °C: Chisq = 1.47, df = 2, p =0.23; 43.8 °C: Chisq = 0.42, df = 2, p =0.52).

ETS-CI+II (Tab. S2; Fig. 2d) was not significantly affected by the sex, the treatment, and the lineage (Tab. S4). It was not significantly influenced by the interaction between ancestral temperature and assay temperature (Chisq = 5.93, df = 2, p = 0.051) even if we are close to the interaction and we are seeing similar trends to the OXPHOS fluxes. ETS-CI+II significantly decreased with assay temperature (Chisq =46.33, df =2, p = p < 0.001 ***). Finally, we found no significant effect of ancestral temperature on ETS-CI+II (Chisq = 0.36, df = 1, p = 0.55).

The COX-activity (Tab. S2; Fig. 2e) was not significantly affected by the sex, the treatment, and the lineage (Tab. S4). It was not significantly influenced by the interaction between ancestral temperature and assay temperature (Chisq =3.48, df = 2, p =0.18). This OCR significantly increased with assay temperature (Chisq =23.4, df =2, p = p < 0.001 ***). We also found a significant effect of ancestral temperature (Chisq = 11.5, df = 1, p < 0.001 ***).

For all OCRs, we observed a non-significant general trend towards lower fluxes for WARM fish at 25 °C than for COLD fish at 25 °C.

## 3.3 Mitochondrial Ratio

The CI coupling efficiency (CE.CI) was generally low for all conditions tested (Tab. S3; Fig. 2f). The CI coupling efficiency was not significantly affected by the sex and the treatment, but we observed a significant effect of lineage on CE.CI (Tab. S5). We found the same tendency for the two lineages through lower fluxes for COLD fish than WARM fish. This tendency was non-significant for C1 and W1 but (W2) and COLD (C2) displayed significant differences at 25 °C (Chisq = 7.7, df = 2, p = 0.005**) and 38.9 °C (Chisq = 5.2, df = 2, p = 0.03*) (Fig. S5). CE.CI was not significantly influenced by the interaction between ancestral temperature and assay temperature (Chisq = 0.95, df = 2, p = 0.62), but was significantly influenced by assay temperature (Chisq =94.2, df =2, p < 0.001 ***) through a decrease with increased assay temperature. We found no significant effect of ancestral temperature on the CE.CI (Chisq = 1.76, df = 1, p = 0.18).

The SCR (Tab. S2; Fig. 2g), reflecting the succinate relative contribution to respiration, was not significantly affected by the sex, the treatment, and the lineage (Tab. S5). The statistical interaction between ancestral temperature and assay temperature was non-significant (Chisq = 1.13, df = 2, p = 0.57). However, the SCR was significantly influenced by assay temperature (Chisq =15.5, df =2, p < 0.001 ***) through an increase with assay temperature. We found no significant effect of ancestral temperature on SCR (Chisq = 0.11, df = 1, p = 0.73).

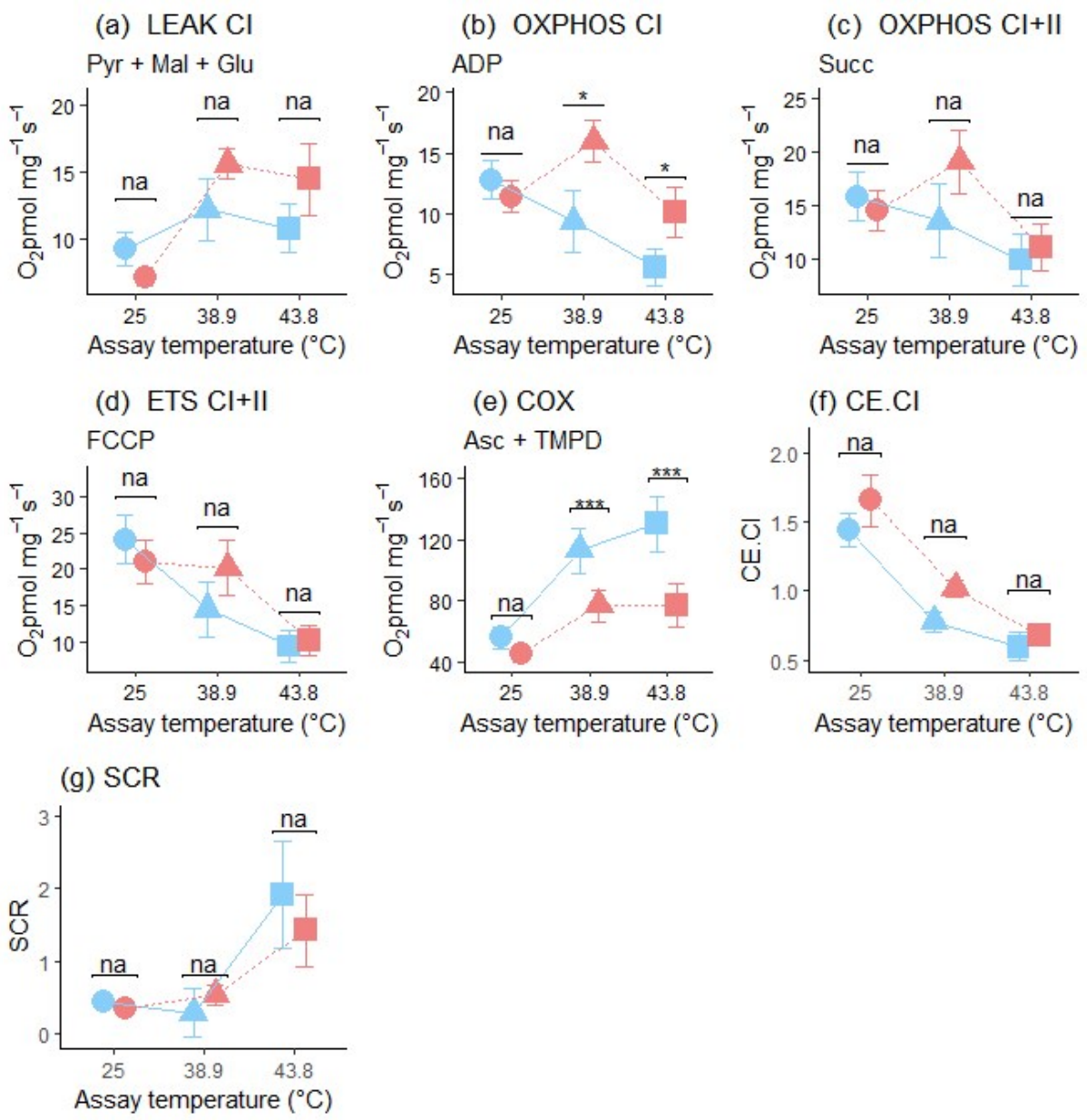


***Figure 2****: (a) LEAK-CI, (b) OXPHOS-CI, (c) OXPHOS-CI+II, (d) ETS-CI+II, (e) COX, (f) CE.CI and (g) SCR mean (± SE) oxygen consumption rates (OCRs) in picomol.s$^{-1}$ .mg$^{-1}$ according to the ancestral temperature (COLD 20 °C fish in blue or WARM 30 °C fish in red) and assay temperature (25 °C, dots; 38.9 °C, triangles; 43.8 °C, square). * represents significant differences between WARM and COLD fish (P < 0.05).*

## 3.4 OCRs comparisons between warm and cold fish at their respective CTmax

To highlight mitochondrial OCRs differences at $\overline{CTmax}$ temperature between thermal lineages, we compared OCRs between COLD fish at $\overline{CTmax}$ 38.9 °C and WARM fish at $\overline{CTmax}$ 43.8 °C (Tab. S3; Fig. 3). We found that OCRs did not significantly differ between COLD and WARM fish at their respective $\overline{CTmax}$ for LEAK-CI (Fig. 3a) (Chisq = 0.91, df = 1, p = 0.34), OXPHOS-CI (Fig. 3b) (Chisq = 0.79, df = 1, p = 0.37), OXPHOS-CI+II (Fig. 3c) (Chisq = 0.25, df = 1, p = 0.62), ETS-CI+II (Fig. 3d) (Chisq = 1.17, df = 1, p = 0.28), CE.CI (Fig. 3f) (Chisq = 3.49, df = 1, p = 0.38) and for SCR despite slightly higher SCR for WARM fish (Fig. 3g) (Chisq =3.5, df =1, p = 0.06). However, we found that COX activity significantly differed between COLD and WARM fish at their $\overline{CTmax}$ (Fig. 3e) (Chisq = 4.38, df = 1, p = 0.03*).

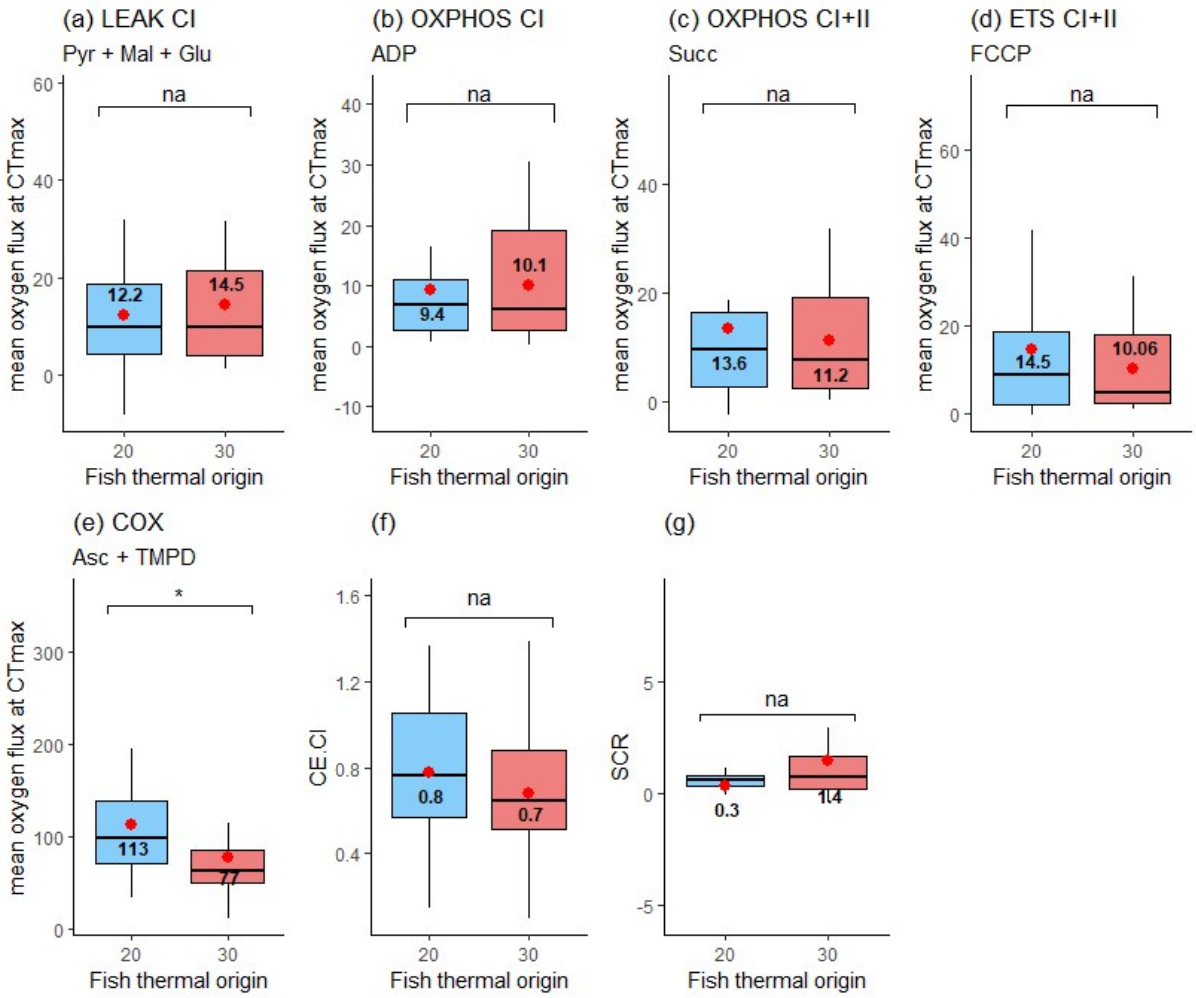


***Figure 3**: Boxplot with mean (red dots with black text) of OCR in picomol.s$^{-1}$.mg$^{-1}$ at $\overline{CTmax}$ assay temperature for (a) LEAK-CI, (b) OXPHOS-CI,(c) OXPHOS-CI+II,(d) ETS-CI+II, ( e ) COX activity, (f) CE.CI, and (g) SCR for each thermal origin (COLD in blue or WARM in red). * represents significant differences between WARM and COLD fish (P < 0.05).*

## 3.5 Links between mitochondrial OCRs and whole organism CTmax

For a better understanding of the link between whole organism CTmax and mitochondrial OCRs at $\overline{CTmax}$ assay temperature, we plotted the $\overline{CTmax}$ assay temperature according to the whole-organism real CTmax values for each fish against individual mitochondrial OCRs in picomol.s$^{-1}$ .mg$^{-1}$ (Fig. S6) as well as for relative OCRs (i.e. OCR $\overline{CTmax}$/ OCR 25 °C; Fig. S7) $\overline{CTmax}$. We did not observe a distribution pattern of OCR or ratio values flux at $\overline{CTmax}$ assay temperature for OXPHOS-CI, LEAK-CI and OXPHOS-CI+II (Fig. S6 and Fig. S7). We also illustrated the fold change in mitochondrial OCR at $\overline{CTmax}$ temperature relative to the OCR at the optimal temperature (OCR $\overline{CTmax}$/ OCR 25 °C ratio) for LEAK CI, OXPHOS CI & OXPHOS CI+II according to the whole-organism real CTmax values for each fish from both thermal origins (Fig. 4). We found no significant difference in the Ratio (flux $\overline{CTmax}$/ flux 25 °C) between the two fish thermal origins for LEAK-CI (Chisq = 0.02, df = 1, p = 0.9), OXPHOS-CI (Chisq = 0.10, df = 1, p = 0.74) and OXPHOS-CI+II (Chisq = 1.12, df = 1, p = 0.29) (Fig.4).

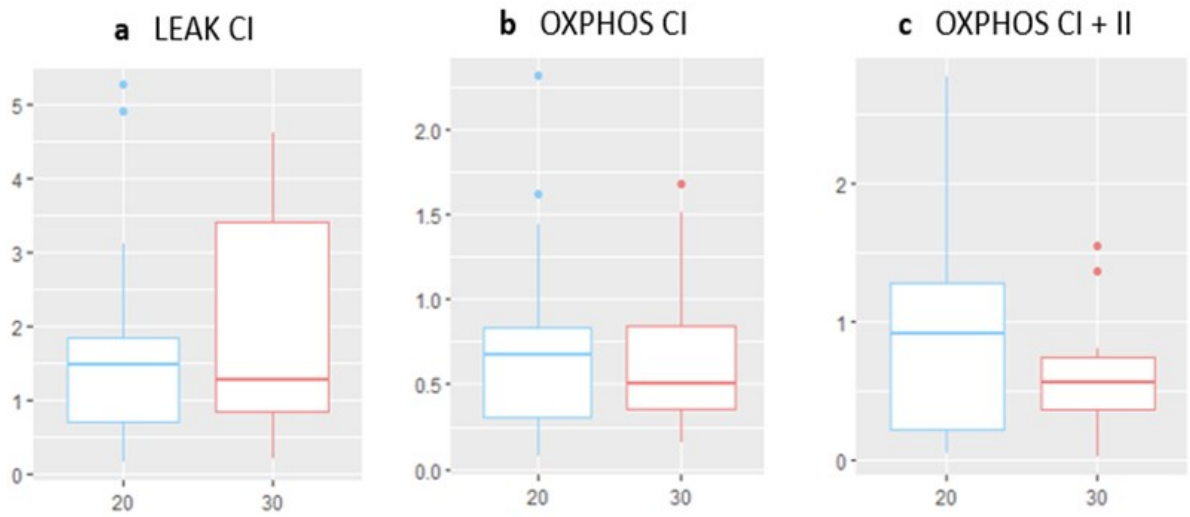


***Figure 4**: Boxplot of the Ratio (OCR $\overline{CTmax}$/ OCR 25 °C) for (a) LEAK-CI, (b) OXPHOS-CI and (c) OXPHOS CI+II, according to fish thermal origin (i.e. ancestral temperature of 20 °C for COLD fish in blue or 30 °C for WARM fish in red).*

# 4. Discussion

The aim of this study was to investigate whether acclimation over several generations (multigenerational exposure) of freshwater Medaka fish to an elevated, non-stressful temperature (30°C) can result in changes in whole-organism thermal limits (CTmax) and brain mitochondrial functions. Moreover, we aimed at determining whether there is a link between whole-organism CTmax and brain mitochondrial performance at elevated temperature in the same fish. These questions are motivated by studies showing that higher thermal tolerance can be improved after a period of exposure to elevated

temperature (Bilyk and DeVries, 2011; Angiletta 2001) and by studies showing that mitochondrial neural dysfunction can limit the upper thermal tolerance of the entire organism (Somero and DeVries, 1967; Ern et al., 2015).

## 4.1 Whole organism upper thermal tolerance (CT max)

In line with studies suggesting that the CTmax varies with the exposure duration (Podrabsky & Somero, 2006; Bilyk and DeVries, 2011; Angilletta 2001), we found that thermal acclimation over several generations increases the maximum thermal tolerance of the whole organism. Fish from multigenerational exposure at 20 °C (COLD fish) had a $\overline{CTmax}$ of 38.9 °C, compared to 43.8 °C for those exposed to 30 °C (WARM fish) representing a 12.6% increase (Fig.1).

Our results thus highlight the influence of an acclimation period spanning several generations (long-term exposure) on the thermal tolerance of the whole organism. Similar results have already been observed for studies performing single-generation exposure. For example, Fangue et al (2006) obtained significant differences in CTmax for common killifish (*Fundulus heteroclitus*) after 21 days of acclimation. Killifish acclimated at 5 °C had a CTmax of 31 °C, while fish acclimated at 33 °C had an average CTmax around 41 °C (representing a 10°C increase). Li et al., 2015 also demonstrated that the CTmax of marine medaka (*Oryzias melastigma*) larvae increases significantly with acclimation temperatures. Fish were subjected to stepwise temperature change at a rate of 1 °C/h, increasing or decreasing from a starting temperature of 25 °C (the control) to reach six target temperatures (12, 13, 15, 20, 28 and 32 °C) with a range of maximum thermal tolerance variation from 39.9 °C at 13 °C to 42.8 °C at 32 °C. In their study, they found a CTmax of 40.53 °C at 20 °C and a CTmax of 42.56 °C at 30 °C (Li et al., 2015). Since acclimation took place over several generations in our case, it is even possible that this difference in thermal tolerance reflects adaptation to different thermal regimes considering that temperature might be a driving force for species adaptation. Genetic and genomic data would however be essential to evaluate the relative contribution of phenotypic plasticity (acclimation) versus genetic adaptation for the observed changes in CTmax.

## 4.2 Mitochondrial performances

The link between acute warming and mitochondrial function is already well established. Several studies have shown that acute exposure to elevated (non-stressful) temperatures leads to an increase in mitochondrial respiration (Abele et al., 2002; Bielski et al., 1983; Brand, 2000; Ricquier and Bouillaud, 1998; Roussel and Voituron, 2020; Sommer and Pörtner, 2004). However, this increase in mitochondrial respiration can also lead to higher levels of ROS (Reactive oxygen species) production at temperatures close to the thermal limit, which can fasten ectotherms senescence and mortality (Christen et al., 2018; Woodward, 2009). In this study, we observed a general increase in OCRs for

OXPHOS-CI, OXPHOS-CI+II and LEAK-CI states between assay temperatures of 25°C and 38°C for WARM fish (38°C is considered as non-stressful because lower than $\overline{CTmax}$ value for WARM fish), confirming that assay elevated temperature exposure leads to an increase in oxygen consumption (Fig. 2abcd).

There is evidence of thermal mitochondrial plasticity in ectotherms following acclimation (Guderley and St-Pierre, 2002; Seebacher et al., 2010; Morla et al., under review). Our results indicate a trend towards lower fluxes for WARM fish at their optimal temperature (25°C assay temperature). Similar results have been observed previously in other ectotherms (Chung et al., 2017; Ekström et al., 2016; Khan et al., 2014; Schulte, 2015; Yan & Xie, 2015) and on the same medaka lineage after two years of temperature exposure (Morla et al., under review). This decline in mitochondrial respiration with acclimation would allow counteracting the increasing oxygen consumption inherent to faster reaction rates at higher temperature and associated cellular damages (Abele et al., 2002; Hochachka and Somero, 2002).

Interestingly, regarding LEAK-CI, mitochondrial fluxes increased between assay temperatures of 25°C and $\overline{CTmax}$, regardless of the thermal origin of fish (Fig. 2a). Previous studies have reported mechanisms allowing to reduce the metabolic cost at elevated temperatures by decreasing mitochondrial efficiency in term of ATP production through increasing proton leakage (LEAK) and decreasing the proton gradient for ATP production (Brand, 2000; Ricquier & Bouillaud, 1998; Skulachev, 1998). In our case, the CI coupling efficiency values were very low (< 1) (Fig.2f) for assay temperatures above 25°C. Considering that almost no increased oxygen consumption was detected after addition of cytochrome c attesting mitochondrial integrity, the low values for the CI coupling efficiency may reflect that substrates used were not optimal to stimulate oxygen consumption or generally low due to low oxygen fluxes as previously reported in studies on brains of Atlantic killifish and triplefin fish (Chung and Schulte, 2017; Willis et al., 2021). Nevertheless, our results showing that LEAK flux are more important than OXPHOS flux suggest a low substrate oxidation capacity at complex I relative to what is required to compensate for the proton leak (Brand & Nicholls, 2011b). This could also be the result of a mismatch between ATP demand and supply because pyruvate and malate oxidation at elevated temperature is associated with a collapse in ATP synthesis and a drop in the ATP/O efficiency (Roussel et al., 2023). This result can be interpreted as a strong indication of mitochondrial dysfunction (i.e. reduced efficiency in terms of ATP production) but measuring ATP production would be required to confirm this interpretation.

There was no difference in OXPHOS and LEAK between WARM and COLD fish at $\overline{CTmax}$ temperature (Fig. 3abcdfg), suggesting a convergence towards a similar response of the mitochondrial OCRs at temperature reaching the $\overline{CTmax}$. We did not observe neither significant difference in the Ratio (OCR $\overline{CTmax}$/ OCR 25°C) between the two thermal origins of the fish (Fig.4) suggesting limits in thermal plasticity at stressful elevated temperature for mitochondrial respiration.

However, our results concerning the relative contribution of complex II (SCR) (Fig. 2g) show an increase between assay temperatures of 25°C and $\overline{CTmax}$ only for WARM fish, suggesting that the relative contribution of complex II at higher stressful temperatures is more important for these fish. This result suggests that WARM fish may exhibit significant mitochondrial flexibility in complex II at temperatures reaching the thermal limit of the organism. A possible explanation for this result would be that WARM fish have developed plastic or genetic strategies to avoid the problem of substrate oxidation capacity at the complex I level (Jørgensen et al., 2021; Menail et al., 2022). This mechanism due to plasticity, genetic and/or epigenetic inheritance (Boukal et al., 2019; Monaghan et al., 2009) could appear after a period of acclimation over several generations at high temperature, in order to compensate for the high thermal sensitivity of complex I identified as the primary site for heat failure in mitochondria (El-Wadawi and Bowler, 1996; Chung et al., 2018; Martinez et al., 2016; Iftikar et al., 2010).

## 4.3 Links between mitochondrial performances and whole organism CTmax

Due to the thermal sensitivity of mitochondria, studies have suggested a link between mitochondrial neuronal dysfunction under thermal pressure and the failure of whole-organism (Somero and DeVries, 1967; Pörtner, 2002; Iftikar and Hickey, 2013; Iftikar et al., 2014; Ern et al., 2015). In this study, we did not observe a distribution pattern of ratio or OCR values (Fig. S6 & S7) as a function of relative CTmax for each fish measured for LEAK-CI, OXPHOS-CI, and OXPHOS-CI+II.

Our results suggest a decrease (not always significant) in the flux of OXPHOS-CI, OXPHOS-CI+II and ETS-CI+II between assay temperatures of 25°C and $\overline{CTmax}$, for WARM and COLD fish (Fig. 2 bcd). Chung et al. (2017) found similar results for mitochondrial OCRs of fish acclimatized to 15°C, which decreased significantly as they approached $\overline{CTmax}$, suggesting a link between whole-organism CTmax and mitochondrial performance at critical temperature. A more important decrease was observed for WARM fish, suggesting greater thermal sensitivity (Fig. 2bcd). However, the link between whole-organism CTmax and mitochondrial performance at elevated temperatures needs to be clarified. Our results also suggest that the mitochondria of COLD fish continue to function (flux > 0) beyond average whole organism mean heat tolerance. In line with this finding, recent studies suggests that mitochondria are more tolerant than the whole organism (Chung and Schulte, 2020; Jørgensen et al., 2021; Syromyatnikov et al., 2019). However, mitochondrial responses to extreme temperatures can also affect other functions, such as ATP production, ROS production, coupling efficiency (ATP/O) and membrane fluidity (Willis et al 2019; Biederman et al., 2019; Roussel et al 2023) which we have not measured in this study and which could also explain our results.

To conclude, our results indicate that 5 years of multigenerational exposure to elevated temperature significantly increases the CTmax of individuals. Although cerebral mitochondria continue to function beyond the average heat tolerance of the whole organism, our results also suggest a link between whole-organism CTmax and cerebral

mitochondrial performance, particularly in terms of complex I oxidation capacity, highlighting the importance of brain mitochondria in the context of thermal tolerance. Finally, we suggest that WARM fish may exhibit mitochondrial flexibility at the level of complex II at temperatures reaching the thermal limit of the whole organism. Our study highlights the complexity of the link between mitochondrial thermal sensitivity and whole-organism thermal tolerance, as well as the fact that acclimation can modify mitochondrial and thermal performance, which can have tremendous impacts on natural populations considering the increased average environmental temperature as well as the frequencies and durations of heatwaves brought forth by climate change. These capacities are nevertheless rarely taken into account in species distribution models (Clusella-Trullas et al., 2021).

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# 6. Supplementary material

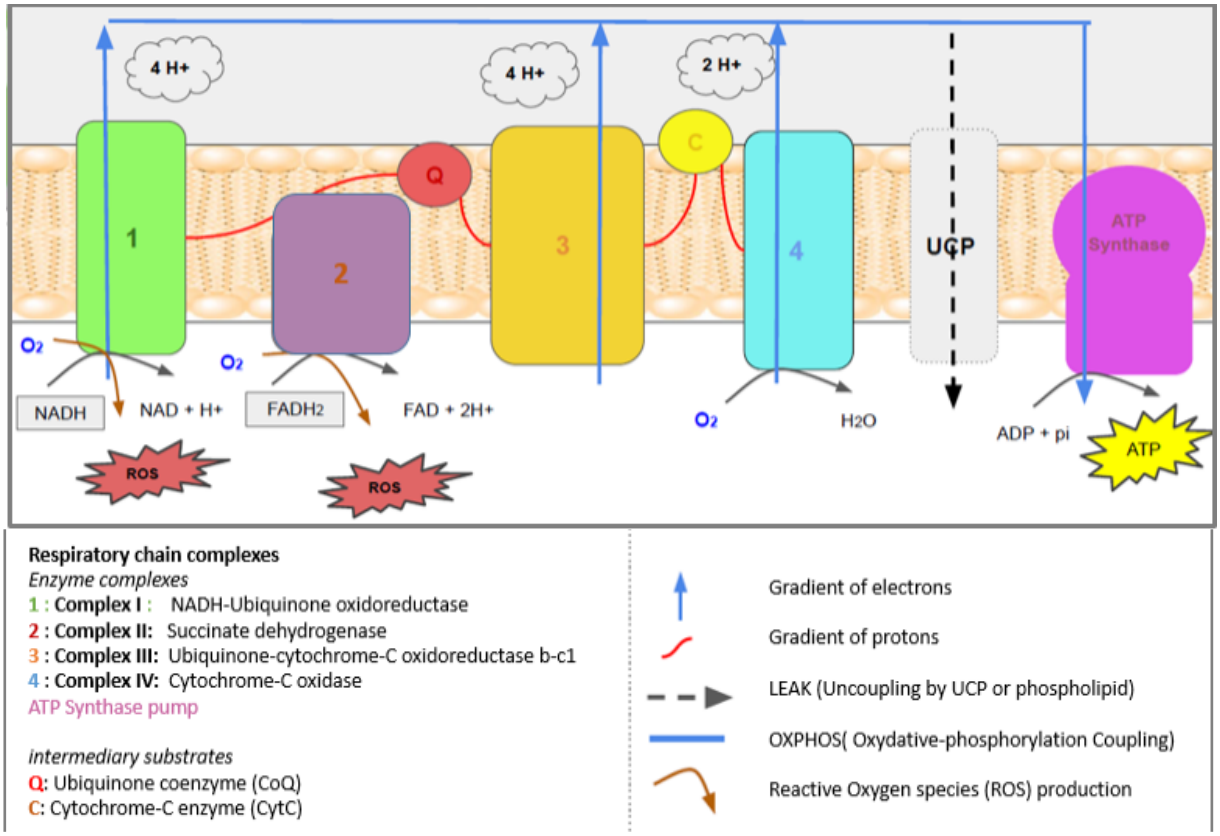
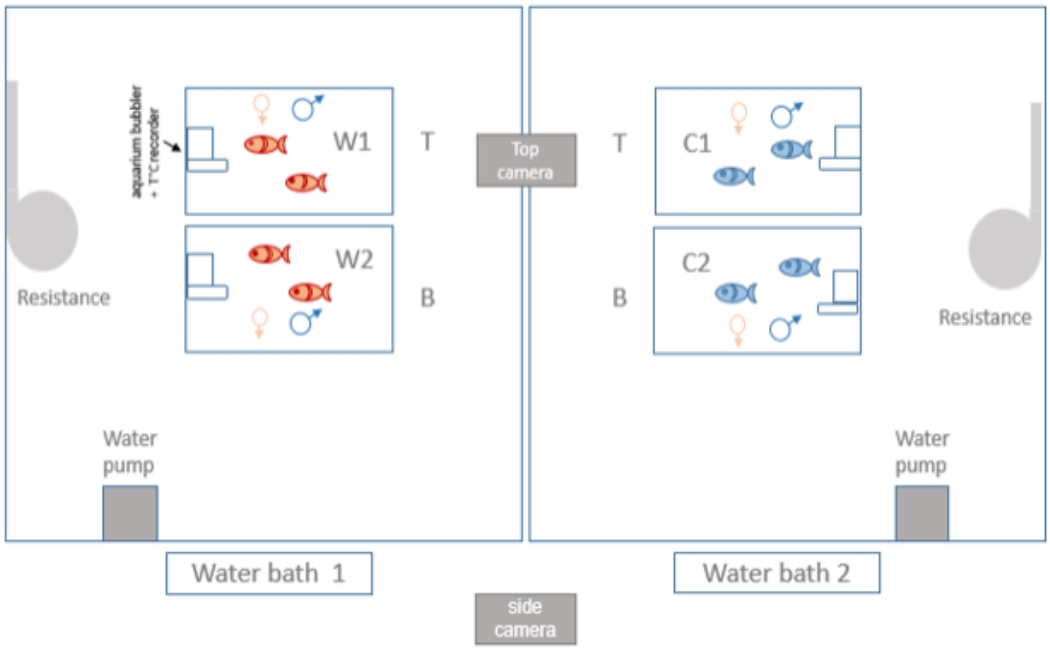



***Fig. S1:*** *The oxidative phosphorylation process in the mitochondria respiratory chain: coupling between cellular respiration and phosphorylation of ADP to ATP. The flow of electrons from NADH and $FADH_2$ is guided by the redox potentials of the four complexes and by the coenzymes (Q and C) to the final acceptor, the oxygen which is reduced to $H_2O$. Each of these complexes, except 2, couples the flow of electrons with a proton pump. The energy of the proton gradient is used to phosphorylate ADP into ATP. Protons can sometimes return to the matrix independently of ATP synthase, via uncoupling proteins (UCPs) or through the membrane phospholipids (mitochondrial uncoupling). The electron transport leads to the production of ROS, reactive oxygen species.*

A




B

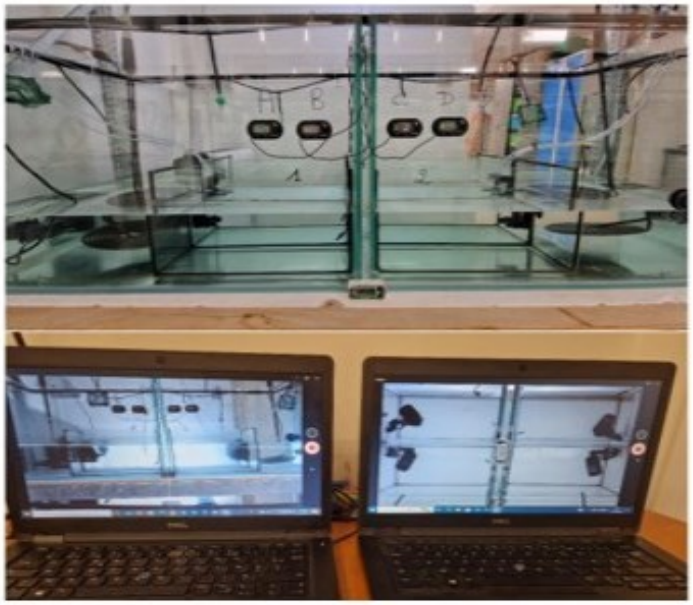
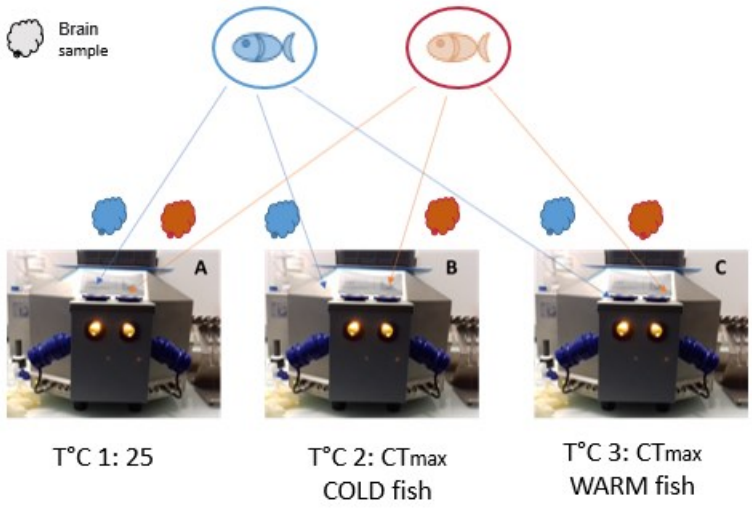


***Fig. S2****: (A) Schematic top view of the experimental design of the* CTmax *experiment with C1 & C2 for COLD lineages and W1 & W2 for WARM lineages; water baths 1 and water baths 2; T or B for top or bottom aquariums. Red fish represent fish reared over several generations at 30°C and blue fish represent fish reared over several generations at 20°C. Fish position was interchanged between aquaria and water baths. (B) Photo of the experimental structure of the water baths and the fish observation device.*

***Tab. S1:*** *health score grid for evaluating clinical condition during 20 day after* CTmax *experiment for each fish.*

| Date: | Time | Fish n°.: | Score (from 1 to 4) |
|---|---|---|---|
| Appearance | Body condition | Weight (overweight, skinny, emaciated, cachectic) | |
| | | inflated, e.g. swim bladder, eggs retention | |
| | | Altered shape, e.g. spinal abnormalities | |
| | | Modified or missing fins | |
| | | Modified or missing gill covers | |
| | | Eye lesions | |
| | | Weight loss (5, 10, 15, 20%) | |
| | Scales and skin condition | Scale/skin changes | |
| | | Skin reddening | |
| | | Lighter/darker pigmentation | |
| | | Other skin color changes | |
| | | Ulcers | |
| | | Localized protuberance/tumor | |
| Body function | Breathing | Increased opercular frequency | |
| | | Surface inspiration | |
| | Food intake | Quantity of food consumed | |
| | Other | Please specify | |
| Spontaneous and provoked behaviors | Swimming and balance | Loss of balance, loss of equilibrium | |
| | | Increase/decrease in activity | |
| | | Swimming in circles/corkscrew/spiral | |
| | | Rubbing against aquarium wall | |
| | | Swimming at bottom of aquarium | |
| | | Swimming at surface | |
| | Responses to stimulation | Feeding activity | |
| | | Avoidance of mechanical stimulation | |
| | | Avoidance of light beam | |
| | Social interaction | Aggressiveness | |
| Free observation | Body condition | Record observations of unexpected negative effects on well-being here. | |
| | | | Score total : |

(A)




(B)



***Fig. S3:*** *(A) full factorial mitochondrial experimental design. Blue fish were reared at 20 °C over several generations (COLD) and red fish were reared at 30 °C over several generations (WARM). A, B, C represent the three oxygraphs with two independent measuring chambers at T°C 1: 25 °C (thermal optimum), T°C 2: $\overline{CTmax}$ of fish from 20°C and T°C 3: $\overline{CTmax}$ of fish from 30°C. (B) Experimental room and material for dissection and mitochondria respiratory measurement.*

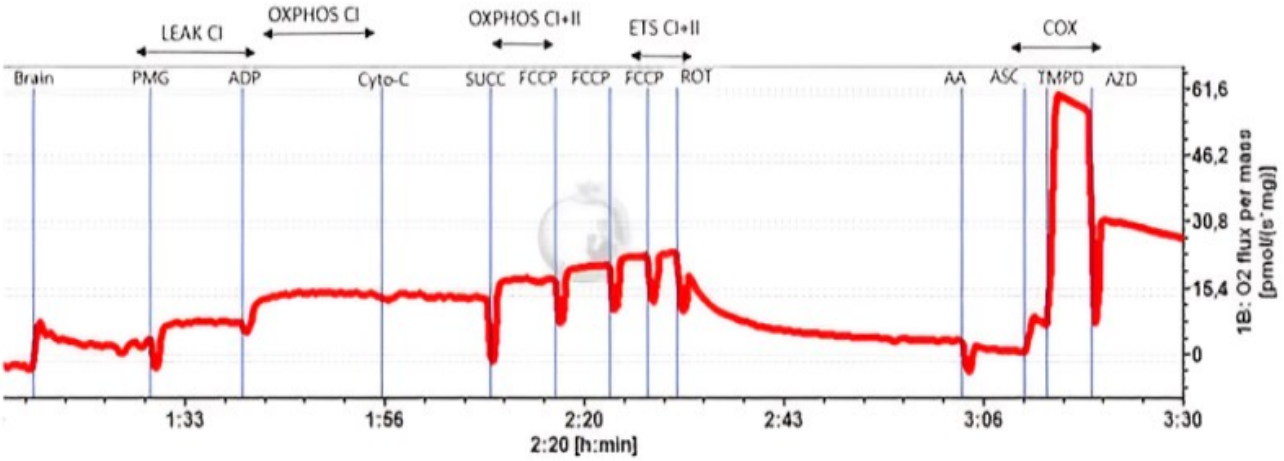



***Fig. S4****: Oxygen flux (picomol.s$^{-1}$.mg$^{-1}$) according to time (h :min) through the sequential activator/inhibitor injection of the respiratory chain.*

***Tab. S2:*** *Output of the Analysis of Deviance Table (Type II Wald chisquare tests) on the most parsimonious models for $\overline{CTmax}$ variable.*

| RESPONSE VARIABLE | PREDICTORS (Fixed effets) | DF | CHISQ | P |
|---|---|---|---|---|
| $\overline{CTmax}$ | *Lineage* | *1* | *0.06* | 0.81 |
| | *Sex* | *1* | *0.44* | *0.50* |
| | *Ancestral temperature* | *1* | *163* | *< 0.001 **** |
| | *sex x Lineage* | *1* | *1.05* | *0.30* |
| | *sex x ancestral temperature* | *1* | *0.95* | *0.33* |
| | *lineage x ancestral temperature* | *1* | *0.34* | *0.56* |
| | *sex x ancestral temperature x lineage* | *1* | *0.47* | *0.49* |

***Tab. S3:*** *mean (± SE) O2 fluxes in picomol.s$^{-1}$ .mg$^{-1}$ according to the ancestral temperature (COLD C or WARM W) and assay temperature (25°C, 38.9°C, 43.8°C) for LEAK-CI, OXPHOS-CI, OXPHOS-CI+II, ETS-CI+II, COX activity state and CE.CI & SCR ratios. Nechant corresponds to the number of tissue samples for each condition.*

| | Tissue | condition | Nechant | Mean | ± SE |
|---|---|---|---|---|---|
| LEAK CI | brain | C-25 | 21 | 9.22 | 1.21 |
| | | C-38.9 | 20 | 12.20 | 2.31 |
| | | C-43.8 | 18 | 10.75 | 1.83 |
| | | W-25 | 23 | 7.11 | 0.61 |
| | | W-38.9 | 20 | 15.59 | 1,17 |
| | | W-43.8 | 21 | 14.46 | 2.69 |
| OXPHOS CI | brain | C-25 | 21 | 12.78 | 1.55 |
| | | C-38.9 | 20 | 9.36 | 2.54 |
| | | C-43.8 | 18 | 5.56 | 1.49 |
| | | W-25 | 23 | 11.41 | 1,33 |
| | | W-38.9 | 20 | 15.95 | 1.72 |
| | | W-43.8 | 21 | 10,12 | 2.03 |
| OXPHOS CI+II | brain | C-25 | 21 | 15.85 | 2.23 |
| | | C-38.9 | 20 | 13.61 | 3.44 |
| | | C-43.8 | 18 | 9.99 | 2.38 |
| | | W-25 | 23 | 14.56 | 1,87 |
| | | W-38.9 | 20 | 19.12 | 2.98 |
| | | W-43.8 | 21 | 11.20 | 2.14 |

| | Tissue | condition | Nechant | Mean | ± SE |
|---|---|---|---|---|---|
| ETS CI + II | brain | C-25 | 21 | 24.09 | 3.41 |
| | | C-38.9 | 20 | 14.49 | 3.85 |
| | | C-43.8 | 18 | 9.31 | 2.25 |
| | | W-25 | 23 | 20.97 | 2.99 |
| | | W-38.9 | 20 | 20.12 | 3.81 |
| | | W-43.8 | 21 | 10.06 | 2.03 |
| COX | brain | C-25 | 21 | 55.42 | 7.3 |
| | | C-38.9 | 20 | 112.95 | 14.88 |
| | | C-43.8 | 18 | 130.20 | 18.49 |
| | | W-25 | 23 | 44.87 | 5.56 |
| | | W-38.9 | 20 | 76.69 | 10.40 |
| | | W-43.8 | 21 | 77.00 | 14.29 |

| | Tissue | condition | Nechant | Mean | ± SE |
|---|---|---|---|---|---|
| CE.CI | brain | C-25 | 21 | 1.44 | 0.12 |
| | | C-38.9 | 20 | 0,77 | 0.08 |
| | | C-43.8 | 18 | 0.59 | 0.10 |
| | | W-25 | 23 | 1.65 | 0.19 |
| | | W-38.9 | 20 | 1.02 | 0.06 |
| | | W-43.8 | 21 | 0.68 | 0.07 |
| SCR | brain | C-25 | 21 | 0.44 | 0.06 |
| | | C-38.9 | 20 | 0.28 | 0.34 |
| | | C-43.8 | 18 | 1.92 | 0.74 |
| | | W-25 | 23 | 0.34 | 0.03 |
| | | W-38.9 | 20 | 0.53 | 0.13 |
| | | W-43.8 | 21 | 1.42 | 0.50 |

***Tab. S4:*** *Analysis of Deviance Table (Type II Wald chisquare tests) for LEAK CI, OXPHOS CI, OXPHOS CI+II, ETS CI+II and COX activity. When the interaction between predictors was significant we spliced the dataset by PREDICTORS variables.*

| RESPONSE VARIABLE | PREDICTORS (Fixed effets) | DF | CHISQ | P |
|---|---|---|---|---|
| **LEAK-CI** | Lineage | *1* | *0.28* | 0.59 |
| | Treatment | *1* | *0.005* | *0.94* |
| | Sex | *1* | *1.04* | 0.31 |
| | *Assay* | *2* | *16.5* | *< 0.001 **** |
| | *Ancestral* | *1* | *1.05* | *0.31* |
| | *Ancestral x Assay* | *2* | *4.9* | *0.09* |
| **OXPHOS-CI** | Lineage | *1* | *0.12* | *0.74* |
| | Treatment | *1* | *0.09* | *0.76* |
| | Sex | *1* | *0.9* | *0.34* |
| | *Assay* | *2* | 14.1 | < 0.001*** |
| | *Ancestral* | *1* | *5.28* | *0.03** |
| | *Ancestral x Assay* | 2 | 9.94 | 0.006 ** |
| **OXPHOS-CI+II** | Lineage | *1* | *1.30* | *0.25* |
| | Treatment | *1* | *0.10* | *0.75* |
| | Sex | *1* | *1.38* | *0.24* |
| | *Assay* | *2* | *17.2* | < 0.001 *** |
| | *Ancestral* | *1* | *1.08* | *0.30* |
| | *Ancestral x Assay* | *2* | *6.14* | 0.05* |
| **ETS-CI+II** | Lineage | *1* | *0.52* | *0.22* |
| | Treatment | *1* | *0.14* | *0.7* |
| | Sex | *1* | *0.75* | *0.38* |
| | *Assay* | *2* | *46.33* | < 0.001 *** |
| | *Ancestral* | *1* | *0.36* | *0.55* |
| | *Ancestral x Assay* | *2* | *5.93* | *0.051* |
| **COX-activity** | Lineage | *1* | *0.51* | *0.48* |
| | Treatment | *1* | *1.03* | *0.31* |
| | Sex | *1* | *2.04* | *0.15* |
| | *Assay* | *2* | *23.4* | < 0.001 *** |
| | *Ancestral* | *1* | *11.5* | < 0.001 *** |
| | *Ancestral x Assay* | *2* | *3.48* | *0.18* |

***Tab. S5:*** *Analysis of Deviance Table (Type II Wald chisquare tests) for CE.CI and SCR variables. When the interaction between predictors was significant we spliced the dataset by PREDICTORS variables.*

| RESPONSE VARIABLE | PREDICTORS (Fixed effets) | DF | CHISQ | P |
|---|---|---|---|---|
| **CE.CI** | Lineage | *1* | *4.73* | *0.03** |
| | Treatment | *1* | *0.10* | *0.75* |
| | Sex | *1* | *0.04* | 0.84 |
| | *Assay* | *1* | *94.2* | *< 0.001 **** |
| | *Ancestral* | *1* | *1.76* | *0.18* |
| | *Ancestral x Assay* | *2* | *0.95* | *0.62* |
| **SCR** | Lineage | *1* | *0.38* | *0.54* |
| | Treatment | *1* | *0.04* | *0.83* |
| | Sex | *1* | *1.76* | *0.18* |
| | *Assay* | *2* | 15.5 | < 0.001*** |
| | *Ancestral* | *1* | *0.11* | *0.73* |
| | *Ancestral x Assay* | 2 | 1.13 | 0.57 |

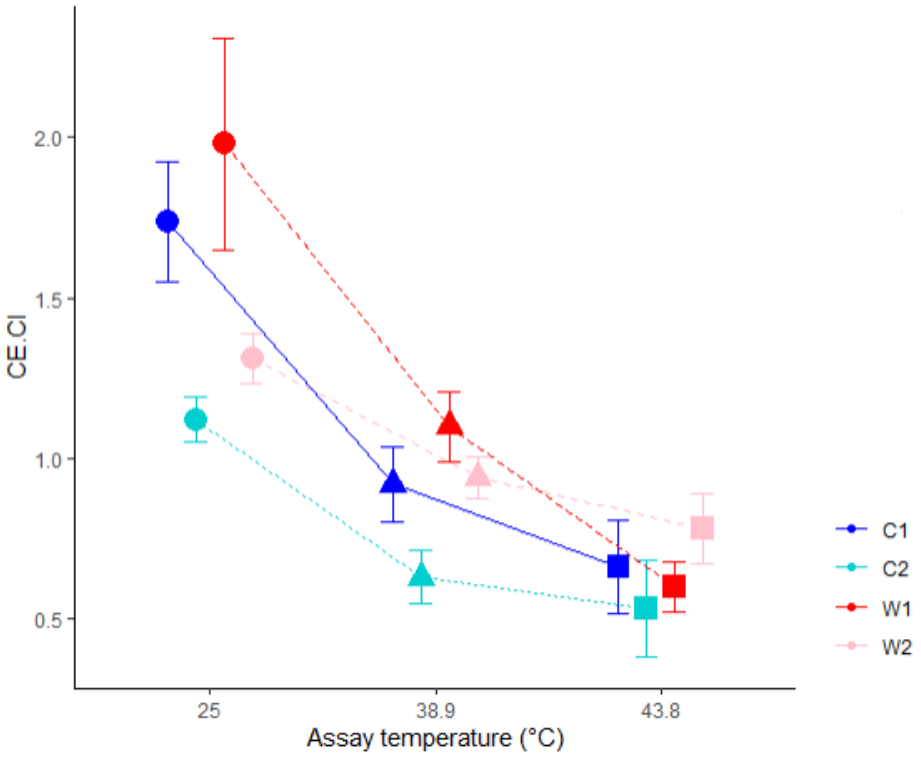



***Fig. S5**: Mean (± SE) of CE.CI ratio according to the ancestral temperature with the two lineages C1 & C2 for COLD and W1 & W2 for WARM fish (COLD 20°C fish C1 in blue; C2 in cyan; WARM 30°C fish W1 in red; W2 in pink) and assay temperature (25°C, dots; 38.9°C, triangles; 43.8°C, square).*

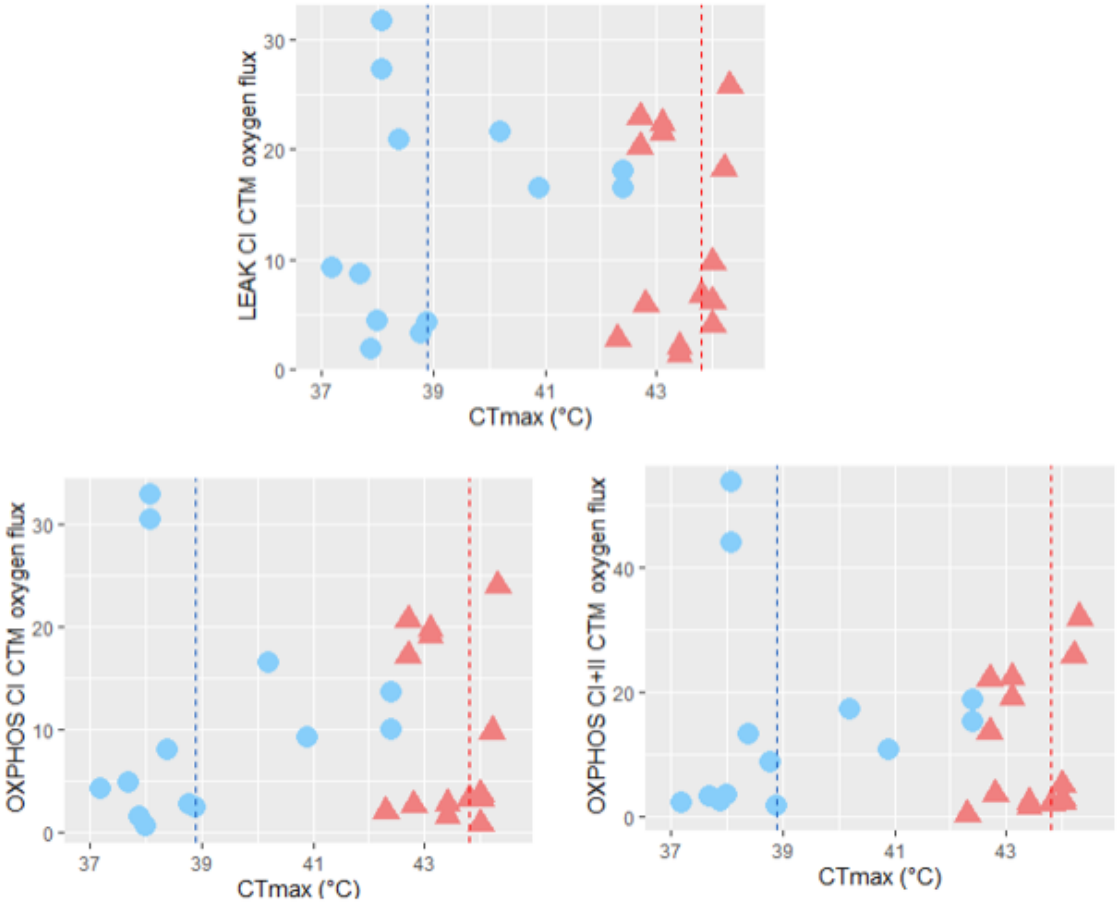



***Fig. S6:*** *OCR in picomol.s$^{-1}$ .mg$^{-1}$ at $\overline{CTmax}$ assay temperature for LEAK-CI, OXPHOS-CI, & OXPHOS-CI+II as a function of* CTmax *whole-organism values for each thermal origin (COLD in blue circles or WARM in red triangles). Red and blue lines are $\overline{CTmax}$ assay temperatures for WARM and COLD fish, respectively.*

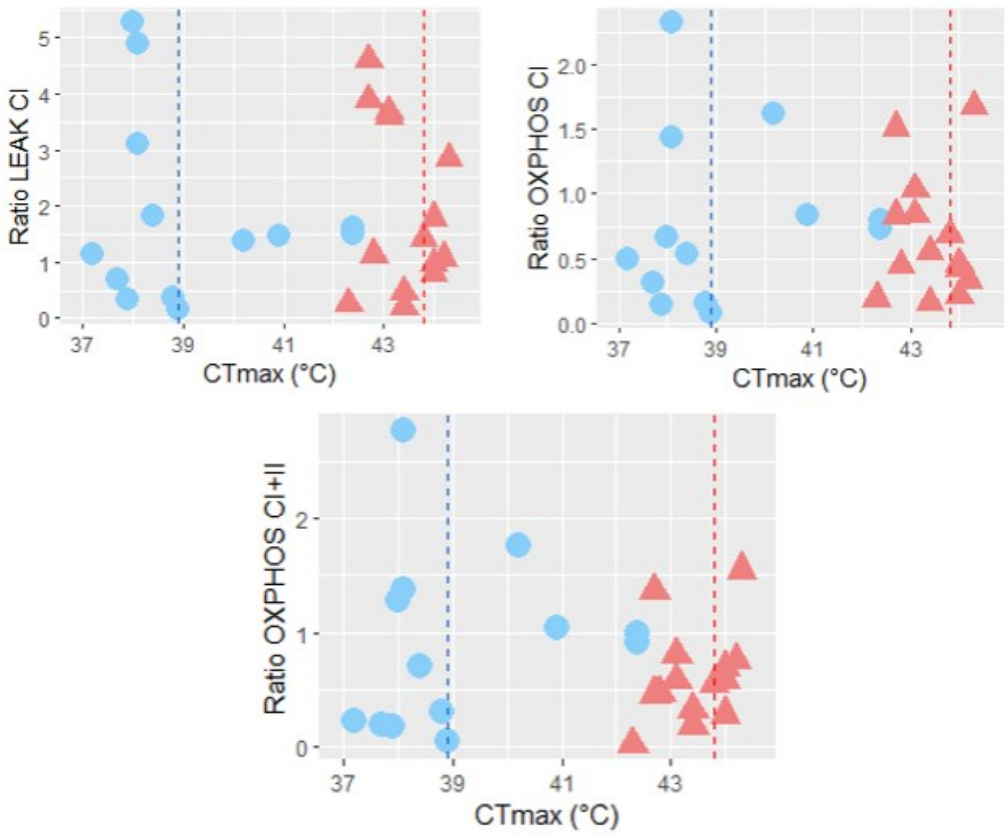



***Fig. S7:*** *Ratio (OCR at $\overline{CTmax}$ / OCR at 25 °C) for LEAK-CI, OXPHOS-CI, & OXPHOS-CI+II as a function of CTmax whole-organism values for each thermal origin (COLD in blue circles or WARM in red triangles). Red and blue lines are $\overline{CTmax}$ assay temperatures for WARM and COLD fish, respectively.*

# Chapter 3

# Impacts of temperature and body size reduction on ecosystem functioning

J. Morla[1], S. Bazin[1], M. Campana[1], T. Zambeaux[1], V. Diouloufet[1], A. Molina[1], J. Favre[1], G. Fremont[1], N. Armaing[1], Stéphan. Jacquet[2]; Serena. Rasconi[2]; D. Milesi[1], C. Leclerc[1], R. Lassus[1], T. Peroux[1], F. Colas[3], M. Daufresne[1], A. Sentis[1]

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STATUS: Draft

AIM: Freshwater ecosystems are particularly threatened by increasing water temperatures. Yet there are still few empirical assessments of how global warming might affect freshwater ecosystem functions beyond the relationship between temperature, size and metabolism exposed by the metabolic theory of ecology. This third and final chapter aims to link observations obtained at the cellular and biological scale with the functioning of the entire ecosystem. It is based on a three-month experiment in mesocosms where I manipulated the temperature (+4°C; IPCC, 2023) and the presence of fish (with contrasted thermal origin and TSR patterns). The objective is to determine whether effects induced by temperature on the body size of organisms and their metabolism, have repercussions on on all trophic levels and ultimately on ecosystem functions (primary production, decomposition, respiration and green-house gas).

MY CONTRIBUTION: Arnaud Sentis conceived the idea of studying the ecological consequences of warming and the reduction in the body size of a top predator on communities and on the functioning of ecosystems via a mesocosm experiment. Before the experiment, I contributed to the scientific discussions and set up the experimental protocol, in collaboration with Simon Bazin. With all technicians, I participated in the sample of the biological and physicochemical data. I sorted, cleaned, and weighed data relating to the decomposition and recovered data relating to primary production. I carried out the gas measurements with the help of Marina Campana and Tiphaine Peroux. Data concerning the identification and sorting of invertebrates and zooplankton were obtained by Simon Bazin, Thalia Zambieux and Marina Campana. The data concerning the microorganisms were obtained and transmitted by Stéphan Jacquet and Serena Rasconi. I carried out all the statistical analyzes with the help of Arnaud Sentis. I wrote the original draft of the article under the supervision of Martin Daufresne and Arnaud Sentis.

# Article 3: Impacts of temperature and body size reduction on ecosystem functioning

J. Morla[1], S. Bazin[1], M. Campana[1], T. Zambeaux[1], V. Diouloufet[1], A. Molina[1], J. Favre[1], G. Fremont[1], N. Armaing[1], Stéphan. Jacquet[2]; Serena. Rasconi[2]; D. Milesi[1], C. Leclerc[1], R. Lassus[1], T. Peroux[1], F. Colas[3], M. Daufresne[1], A. Sentis[1]

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ANIMAL ETHICAL DECLARATION: No ethical permit was required under French legal requirements to conduct this experiment because tested temperatures are within the range of non-stressful temperature for this species (Leaf et al., 2011b; Shima and Mitani, 2004b). Fish were maintained in the laboratory under permit number A1300101 by the French authority of the "Direction départementale de la protection des populations des Bouches-du-Rhones" under the articles R 214-87, R 214-122, and R 215-10 of the French law.

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## Abstract:

Freshwater ecosystems are particularly sensitive to increase in water temperature and associated stressors such as oxygen availability. Yet, there is still little empirical evidence to assess how global warming might affect freshwater ecosystem functions. Temperature, modifies the speed of metabolic biochemical reactions, the energy demand and the consumption rates of ectotherms thus modulating matter and energy fluxes. Moreover, global warming affects individual phenotypes and often leads to a reduction in individual body size, the latter structuring trophic interactions in aquatic food webs. Changes in temperature in combination with body size reduction are thus likely to have profound ecological impacts that will ramify through the entire ecosystem. In this study, our aim was to distinguish such direct and indirect effects of temperature on ecosystems functions. We conducted a mesocosm experiment manipulating (1) body size reduction in fish (*Oryzias Latipes*) using two populations raised for several generations under contrasted temperature and (2) temperature (artificial warming of +4°C) to test their independent and interactive effects on organic matter decomposition, primary production, respiration and greenhouses gas concentrations. Our results reveal no significant effect of artificial warming and fish modalities for decomposition rates. This may be explain by the observation that warming hasten microbial litter decomposition but reduces detritivores decomposition; as a result decomposition rate may remain unchanged by compensation phenomenon. In addition, we found an increase in primary production concentrations in the presence of fish as a function of water temperature in heated mesocosms. In line with previous studies, gross primary productivity increases with temperature and in presence of fish through a positive trophic cascade for phytoplankton Finally, although $CH_4$ concentrations were higher in warmed mesocosms, $T_{15}$ (i.e water temperature) generally decreased gas concentrations,. The well-known negative relationship between water temperature and gas solubility may largely explain such patterns. In contrast, heated mesocosms had higher $CH_4$ concentrations. Overall, our work demonstrates that the presence and size differences in fish had lower effects on ecosystem functions than direct effects of water temperature and artificial warming, and highlights the complexity of the link between temperature increase and freshwater ecosystem functions that can involve various compensatory phenomena.



Key-words: mesocosm experiment, artificial warming, ecosystem functions, freshwater organism, temperature-size rule, metabolism.

# 1. Introduction

Concerns about the impact of temperature on ecosystems have reached the top of the global environmental agenda due to climate change observations and predictions (Calvin et al., 2023; Harvey et al., 2023). In one of their most pessimistic scenario, the IPCC (2023) has predicted a global temperature increase of 4°C by 2100 and climate change will become one of the most impactful driver of biodiversity loss in the coming decades (Jaureguiberry et al., 2022). The extinction of species is likely to accelerate with increasing temperatures and 15 to 37% of species could be extinct by 2050 (Harvey et al., 2023; Thomas et al., 2004; Urban, 2015).

Freshwater ecosystems are particularly sensitive to increase in water temperature and associated stressors such as lower oxygen concentration and modification in nutrient supply (Jackson et al., 2016; Sheldon et al., 2021; Walther, 2010; Winder and Schindler, 2004; Falfushynska et al., 2016; Woodward et al., 2010). Ecosystem functions have received considerable attention since the 2000s and are often used as indicators of environmental pressure on ecosystems (Bergfur, 2007; Del Giorgio et al., 1999; Gessner and Chauvet, 2002; Gücker et al., 2006; Loreau, 2009; Malmqvist et al., 2001; Oliver and Merrick, 2006; Wallace et al., 1996). However, there is still little empirical evidence to assess how global warming might alter directly and indirectly ecosystem functioning, despite theoretical predictions of changes in the magnitude of energy flows, food-web topology and in the size distribution of organisms (Woodward and Warren, 2007).

Temperature can modify the rate of biochemical reactions, the energy demand, and the resource consumption rates of organisms that are individual processes contributing to matter and energy fluxes (Brown et al., 2004; Gillooly, 2001). As a consequence, changes in environmental temperature and thus in internal temperature for ectotherms, are likely to have profound effects on individuals that will ramify through the entire ecosystem (Brown et al., 2004). Actually, the idea that biogeochemical processes within ecosystems are consequences of the metabolic activity of all organisms that compose them is at the core of the metabolic theory of ecology (MTE; Brown et al., 2004). Since this seminal publication, this theory has been applied and extended to predict changes in ecosystem processes under global warming (Petchey et al., 2010; Woodward et al., 2010; Yvon-Durocher et al., 2010b).

The metabolic processes and biological activity of organisms (i.e. photosynthesis, respiration, decomposition, growth and nutrient uptake; Chisholm, 1992) increases with temperature. As a consequence, primary production is generally expected to increase with warming (Allen et al., 2005; Brylinsky and Mann, 1973; Davison et al., 1991; Lee et al., 2007). However, several studies focusing on the carbon cycle demonstrated that ecosystem respiration increases faster with temperature than photosynthesis, leading to an overall reduction in net primary production and an increase in carbon emissions to the atmosphere (Tait and Schiel, 2013; Yvon-Durocher et al., 2010b). Primary production also determines energy fluxes through the food web, which can affect higher trophic levels due to altered resource availability. Moreover, warming can reduced trophic transfer efficiency by up to 56% after several years, reducing the heterotroph-autotroph balance

(Barneche et al., 2021). Warming can also alter bottom-up control, increasing rates of decomposition and the abundance of heterotrophic bacteria (Morán et al., 2015; Widden et al., 1989). According to Follstad Shah et al., 2017, leaf litter decomposition is expected to increase exponentially by up to 20% considering both microbial and invertebrate taxa within a projected 4°C increase in average temperature water (Follstad Shah et al., 2017), even if, in some cases, litter decomposition may be inhibited or decrease under warming pressure, modifying the well-established relationship between temperature increase and metabolic. In fact, some studies suggest that litter decomposition in streams (i) can be limited by the low availability of dissolved nutrients, (ii) can decrease due to energetic mismatch induced by warming or finally (iii) can remain unchanged by compensatory phenomenon linked to opposite effects of warming on decomposers communities (Boyero et al., 2011; Medeiros et al., 2009; Réveillon et al., 2022). Finally, temperature will increase effluxing as $CO_2$ and $CH_4$ of ecosystems through its effects on the balance between plant growth and decomposition but also through a significant increase in respiration rates (Yvon-Durocher et al., 2010b; Yvon-Durocher et al., 2011).

Global warming also affects individual phenotypes, which may lead to altered biotic interactions. One of the most common phenotypic changes induced by warming is a reduction of individual body size. Atkinson first proposed the "*Temperature-Size Rule*" (TSR) based on the observation that ectotherm individuals developing in a warm (non-stressful) environment have lower adult sizes compared to those developing in a cold environment (Atkinson, 1995, 1994). Fifteen years later, reduction in individual body size was proposed as *the third universal response of species to warming* (Daufresne et al., 2009; Gardner et al., 2011; Martins et al., 2023; Sheridan and Bickford, 2011). Given the much lower availability of oxygen and the greater efforts required to increase uptake in water compared to air, TSR is stronger for aquatic organisms than for terrestrial ones, especially for larger species with a lower surface area/body mass ratio (Forster et al., 2012). Body size structures trophic interactions in aquatic food webs, which are particularly important for matter and energy fluxes within and between ecosystems (DeLong et al., 2015). These thermal effects on body size, particularly in predators such as fish, may lead to changes in prey-predator dynamics but the consequences of these size-induced changes at the ecosystem level remain largely unexplored. Furthermore, variation in body size of dominant predators induces trophic cascades altering biomass and mean body size of lower trophic levels (Jochum et al., 2012). The increased metabolic demands for a warmer environment can also lead to reduced community biomass (Fussmann et al., 2014; Petchey et al., 1999). Such changes can lead to even stronger cascading top-down effects due to changing predation levels, affecting the flow of energy through the food web (Barnes et al., 2014).

Overall, the impacts of temperature on ecosystem functions is complex because it depends on the metabolism of organisms, their size but also on numerous interactions at different trophic levels (e.g. Beveridge et al., 2010; Hall et al., 2000; Ives, 1995; Sterner and Elser, 2003; Vanni, 2002). Additionally, there is a negative relationship between temperature and the amount of oxygen available in water while aerobic organisms (bacteria, plankton, vertebrates and invertebrates) depend on the oxygen concentration in the environment to perform their biological and ecological processes. Moreover, the long-term effects (through plastic, genetic and epigenetic inheritance) of temperature can

have cascading effects on communities and key ecosystem functions (Boukal et al., 2019; Sentis et al., 2015; Wang et al., 2021). We thus need to distinguish both the direct and indirect effects of temperature on ecosystems functions to understand and predict how climate change impacts ecological processes.

In this study, we investigated how temperature and temperature-induced reduction in body size of a predatory vertebrate species, the medaka fish (*Oryzias Latipes*), can affect ecosystem processes. We conducted a mesocosm experiment in which medaka fish exposed for several generations to 20°C or 30°C (i.e. exhibiting TSR patterns with large body fish and small body fish, respectively) were introduced into mesocosms maintained at ambient temperature or artificially heated at +4°C, in line with the predictions for 2100 (IPCC 2023). We hypothesized: (I) that temperature increases oxygen demand and metabolism thereby increases processes related to ecosystem functions (i.e. respiratory, primary production and decomposition rate) (II) that the presence of fish modifies the trophic cascade by predating on zooplankton leading to released grazing pressure on primary producers (i.e. increasing primary production) (III) that the multigenerational thermal contrasted origin of fish can modify their metabolism, and predation pressure (i.e. a lower mitochondrial activity for small body fish, Morla et al., Submitted) as long as water temperature remains within the tolerated range of large body fish and a top down effect of small body size fish that can focus on smaller prey which can reduce strength of the cascading effects from fish to phytoplankton.

# 2. Materials & Methods

## 2.1 Biological model

The Japanese medaka fish (*Oryzias Latipes)* is a small (20-40mm) amphidromous freshwater fish from Southeast Asia. This species is eurythermic with a thermal range from zero to 40 °C (Dhillon and Fox, 2007; Hirshfield, 1980; Shima and Mitani, 2004a; Yamamoto, 1975) and a thermal optimum of 25°C (Dhillon and Fox, 2007; Kirchen et al., 1976; Leaf et al., 2011a; Yamamoto, 1975). It has a short generation time of 3 months and can reach sexual maturity in about 10 weeks (Hirshfield, 1980) making it a good biological model for multigenerational thermal experimentation. It has an average lifespan of 1 year in the wild and up to 5 years in captivity (Shima and Mitani, 2004a). In its native range, the medaka generally lives in small bodies of water such as rice paddies, with temperatures ranging from 5 to 35°C (Kirchen et al., 1976) and displaying important fluctuations (Hirshfield, 1980). Medaka lives in the upper part of the water column or in shallow habitats areas that are often sensitive to strong short-term temperature variations. The medaka is an omnivorous species that feeds primarily on zooplankton but also on diatoms, algae, and aquatic insects (i.e. larvae and adults on the surface) (Edeline et al., 2016; Kinoshita et al., 2009; Mano and Tanaka, 2012; Shima and Mitani, 2004a).

## 2.2 Rearing conditions

Medaka used in this study were reared at 20°C (cold exposed fish) or 30°C (warm exposed fish) (non-stressful temperatures) over several generations under controlled experimental conditions in climatic chambers. Two lineages by temperature were obtained from a starting F0 population of 320 Medakas (160 females & 160 males) belonging to the CAB strain from Carolina Biological Supply Company (USA) and provided by AMAGEN© (France). F0 generation fish were maintained at 25°C into 20 L aquariums (25 x 40 x 20 cm) and were then progressively acclimatized to 20 or 30°C at a rate of one degree every 2 days. To start a new fish generation, we collected eggs clutches (approximately 300 eggs) during the optimal fecundity period (i.e. when most females were laying eggs) and placed them in hatcheries (2.5 L) at the parental temperature (i.e. 20°C or 30°C). We maintained the new born fish in climatic chambers at the same temperature as their parents and then placed them in five replicates aquariums (25 × 40 × 20 cm) until maturity, with a density of ~30 adult fish per aquarium. The fish were fed "ad libitum" with dry flakes food TétraMin© three times a day (at 9h, 13h and 18h). Drippers (1 L.h-1) controlled the water supply of the open water system. The input water quality was maintained with mechanical, biological and UV filtration with a pH of 7.5. Each aquarium was equipped with an air filter to prevent high nitrite concentrations and maintain oxygen at saturation. The photoperiod was adjusted for medaka reproduction to 16h: 8h (day:night), which is optimal (Hirshfield, 1980).

For the mesocosm experiment, only males were used to avoid reproduction since we were not directly interested in their population dynamics. Cold exposed fish from the 3th generation were 453 days old (i.e. 9060 degree days) and warm exposed fish from the 5rd generation were 273 days old (i.e. 8190 degree days). The generation time of warm exposed fish was shorter because of faster development at 30°C compared to 20°C, resulting in asynchronous generations between the two temperatures. Although they had different ages in days, their physiological ages were relatively close (i.e. 9060 vs. 8190 degree days). When they were introduced in the mesocosms, warm fish were on average about 10% (4.0 mm) smaller and 30% (180 mg) lighter than cold fish (TSR patterns, Fig.1).

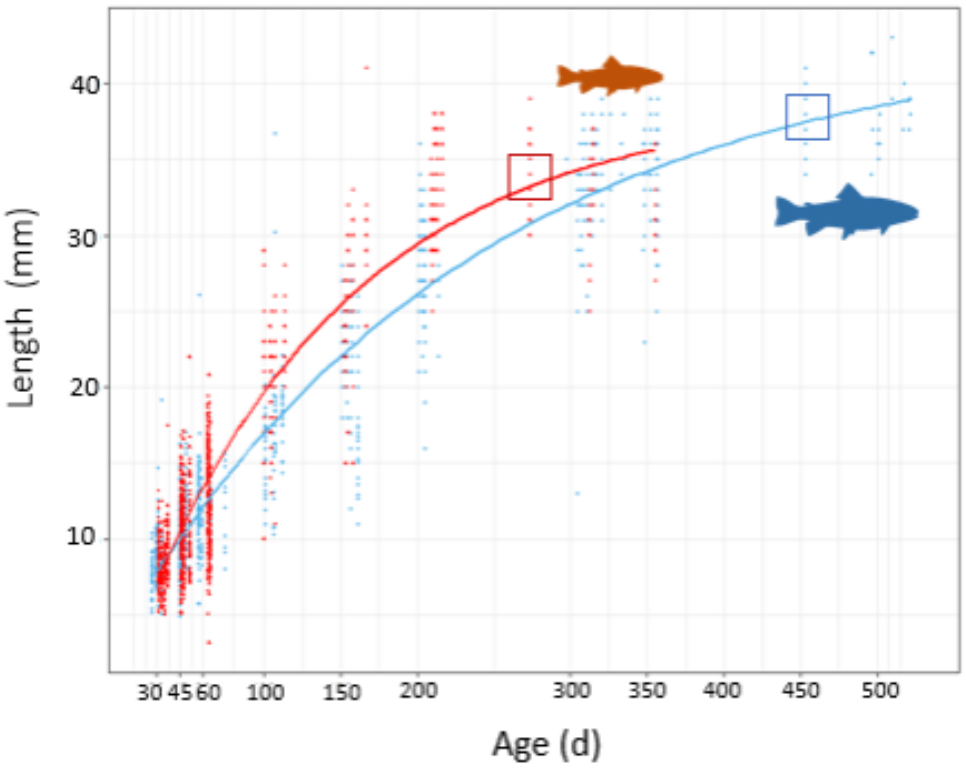


***Figure 1**: Fish body sizes (mm) according to their age (in days) from both warm (red) and cold (blue) fish lines. The squares represent the size and age of the fish used in mesocosms. Growth curve parameters are obtained for each line (T20-F3, T30-F5) (i.e. L∞: asymptotic size and k: Initial growth rate) using the von Bertalanffy growth equation (1938): L∞20= 42.2, L∞30=40.39 & k20: 0.0045, k30=0.0064. Only a sub-sample of the fish lineages from the climatic chamber were placed in the mesocosms and we continued to monitor growth on remaining medakas in the aquarium. This explains why the curves continue after the squares.*

## 2.3 Mesocosms experiment & design

The experiment was carried out at the INRAE Aix-en-Provence experimental platform (43° 31' 25'' N; 5° 30' 40'' E) that includes 25 outdoor freshwater mesocosms (1.20 m in diameter; 0.70 in depth; 1 $m^3$ capacities; Fig. 2, APP 1: Fig. S1). Mesocosms can be heated relative to a reference mesocosm, which follows the natural temperature fluctuations of Aix-en-Provence.

Each mesocosm was filled with 1 $m^3$ of freshwater from an irrigation canal originating in a nearby freshwater reservoir (Bimont Lake). They were then inoculated with 10 g of white poplar dry leaves and with zooplankton and phytoplankton collected from a local lake (Aulnes lake, 43° 35' 30'' N; 4° 47' 30'' E). The mesocosms were left undisturbed and opened to natural colonization and dispersal for 18 months to allow the communities to stabilize and diversify, through with the colonization of aquatic insects.



***Figure 2**: experimental platform of 25 mesocosms at INRAE Aix-en-Provence*

We used a full factorial design (Fig. 3) with two experimental temperatures treatments (ambient temperature; ambient temperature +4°C) and three fish treatments (no fish; warm exposed fish (30°C), cold exposed fish (20°)). Half of the mesocosms (n = 12) were at ambient temperature and followed natural temperature fluctuations (hereafter NW for no artificial warming) while the other half (n = 12) were heated by thermal resistors to + 4°C (hereafter W for artificial warming) compared to the ambient temperature. Water temperature was monitored throughout the experiment every 5 minutes by HOBO loggers to record the temperature differences between NW and W mesocosms and record temperature fluctuations associated to season (the experiment was conducted in spring when temperature was increasing). We added 4 cold fish (F20) on one third of the mesocosms (n = 8), 4 warm fish (F30) in another third (n = 8) and the last third (n = 8) was left without fish. Fish were acclimated to the water temperature of the mesocosms (i.e. around 15 degrees at the beginning of the experiment) in climatic chambers by decreasing the water temperature by 2°C per day to approximately 14°C. We measured and weighed all the fish at the beginning and the end of the experiment (Fig. S2). We found that growth rates in terms of size and weight were higher in small-bodied fish than in large-bodied fish ($Chi^2$ = 55.5, df = 1, $p < 0.001$ and $Chi^2$ = 43.3, df = 1, $p < 0.001$, respectively) and that artificial warming had no significant effect on the growth rates of fish (S3). At the end of the experiment, the total biomass of the fish in the mesocosms no longer depended on our fish treatments or on artificial warming (Fig. S3). Fish mortality was monitored weekly. Dead fish were not replaced. 8 large-bodied and 2 small-bodied fish died during the experiment. Mortality was 12.5% for all fish and occurred mainly at the end of the experiment. Nevertheless, we found no significant effect of fish origin (p = 0.055) or artificial warming (p = 0.998) on the fish survival probability during the experiment (Fig. S4).

Each of the 6 treatments (no fish – no warming, hereafter “NF_NW” ; no fish – warming “NF_W” ; fish 20°C - no warming “F20_NW“; fish 20°C - warming “F20_W” ; fish 30°C - no

warming “F30_NW” ; fish 30°C - warming “F30_W”) were assigned randomly to 4 mesocosms, resulting in 24 experimental communities (Fig. 3). The experiment lasted 3 months, from March 23, 2021 to June 15, 2021 during which we sampled monthly (i.e. April 20, 2021; May 18, 2021; June 15, 2021) macroinvertebrates, zooplankton, and bacterial communities. We also collected data related to ecosystem processes such as organic matter decomposition rate (monthly), benthic and pelagic primary production (weekly), greenhouse gas (monthly) and oxygen concentration (weekly) in each mesocosms.

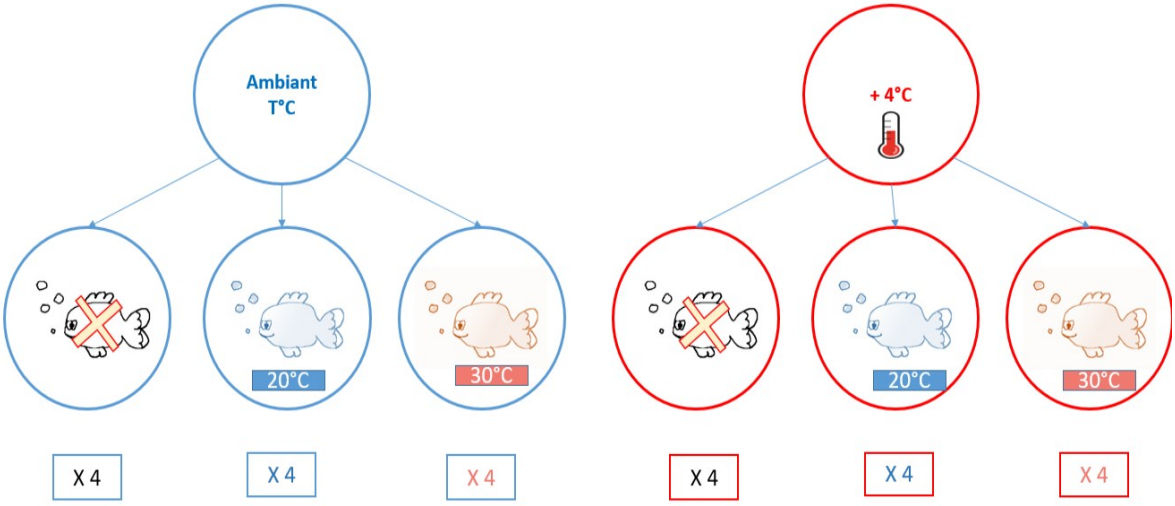


***Figure 3**: Experimental design with 2 temperatures x 3 fish modalities applied to the 24 mesocosms with four replicates per treatment.*

At the beginning of the experiment, water temperature was about 14°C in the non-heated mesocosms (Fig. 4). Water temperature strongly varied until mid-April, and then increased more or less continuously until reaching 29°C on average at the end of the experiment in June (Fig. 4). The heated mesocosms followed the same pattern with a significant artificial warming of + 3.35 °C ($p < 0.001$).

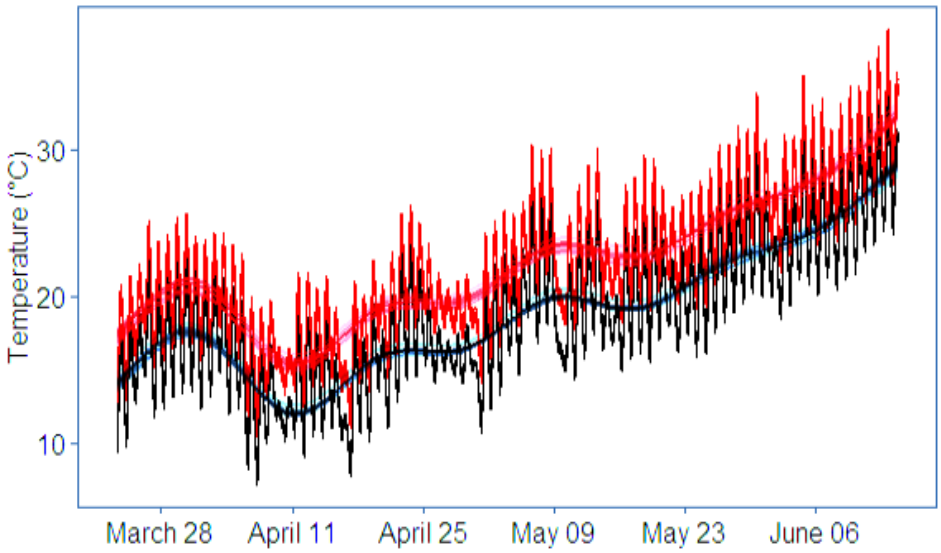


***Figure 4**: Mesocosms water temperature (°C) during the experiment. Blue and red colours represent ambient and heated mesocosms, respectively.*

## 2.4 Biological communities sampling and processing

### 2.4.1 Macroinvertebrates

Benthic and pelagic macroinvertebrate traps were randomly placed at the bottom of each mesocosms. Traps were made from a mesh net of 250 µm with a surface area of 225 $cm^2$ (15 x 15 cm), a tile and a brush of 50 $cm^2$ (10 cm x 5 cm) composed of stiff silicone bristles to create habitats likely to be colonized by invertebrates. Three weeks before introducing the fish and starting the artificial warming, we placed four traps by mesocosms. The first trap was recovered after three weeks when we introduced the fish and warmed the mesocosms (T0). The following traps were recovered at after one, two or three months of experience in order to have a follow-up over the whole experimental period. At these same dates, pelagic macroinvertebrates were sampled using a 250 µm mesh dip net (surface area 375 $cm^2$, 15 x 25 cm) with two net sweeps per mesocosms (filtered volume = 145 L). Macroinvertebrates were preserved in a 70° ethanol solution before being counted and identified to the lowest possible taxonomic level (the family in most cases). Each individual was photographed and body length was measured. Carbon biomass was then obtained by assuming that 40% of the dry mass is made of carbon following Feller and Warwick (1988). Published body length – weight relationships were used to estimate individual dry mass (Baumgärtner and Rothhaupt, 2003; Benke et al., 1999; Sabo et al., 2002; Smock, 1980).

### 2.4.2 Prokaryote like particle

Prokaryote-like particles (PLP) counts were obtained using a FACSCalibur (Becton Dickinson) benchtop flow cytometer, equipped with a blue laser beam fixed at 488 nm and with the standard filter setup (Jacquet et al., n.d.). MilliQ water was used as a sheath fluid since no significant differences were recorded with either filtered lake water or the FACSFlow provided by the manufacturer (not shown). Samples were run at medium speed (i.e. about 60 µl.min-1, the flow rate being checked before each analysis), and all parameters were acquired in log mode. To favour detection and avoid coincidence, the number of events was limited to between 100 and 1000 per sec by diluting the sample using 0.02 µm filtered Tris-EDTA (prepared at Ph = 8) and/or by raising the threshold of the instrument. PLP (e.g. mainly heterotrophic bacteria and archaea) were detected from dot plots of right angle light scatter (SSC) versus the green fluorescence of the acid nucleic dye (e.g. SYBR-Green I used at 1:10,000) complex (FL1 channel: 530 ± 15 nm) and the red fluorescence of phytoplankton (FL3 channel: > 630 nm) versus FL1. Data were collected in listmode files and then mesocosm on a separate PC using the custom-designed software CYTOWIN. Densities are reported as cells.Ml-1.

### 2.4.3 Zooplankton

Once a month, 10 samples were taken at random from the water column using a 500 Ml PVC column. The 10 samples were then pooled (5 L of water) and filtered through a 200 µm sieve to recover the fraction larger than this mesh size. This fraction was preserved in 70° ethanol for each sample. For each sample, a Zooscan© (HYDROPTIC Inc.) was used at the Mediterranean Institute of Oceanology in Marseille (MIO) to subsequently obtain 1 label per particle associated with numerous size variables (major and minor axis of the most suitable ellipse, area, volume, etc.) and metadata (date, GPS data, mesocosms ID). The 5L of fraction less than 200 µm from the meso- and macro-zooplankton sampling were filtered using a 30 µm mesh net (fraction between 30 and 200 µm to study the micro-zooplankton) and then preserved in lugol. This was then mesocosm using a Flowcam at the MIO laboratory to obtain size variables. Zooplankton and micro-zooplankton taxa were automatically identified using ecotaxa software, and total abundance calculated (number of individuals/mesocosms).

## 2.5 Ecosystem functions

### 2.5.1 Organic matter decomposition

Litterbags were added before the beginning of the experiment (March 17, 2021) at the bottom of each mesocosms to measure decomposition rate over time. Before the experiments, white poplar leaves were air-dried and then weighed in dry weight. We then placed them in leaf litter bags with 3-4g of leaves per bag, We used Coarse-mesh (CM) (size: 15 x 20 cm, 30 x 20 cm patterns, mesh approx. 10 mm) litter bag for macro-organisms (made 1 month before the experiment), and fine-mesh (FM) (size: 15 x 20 cm,

mesh approx. 500 µm) to determine the decomposition linked to micro-organisms or bacteria. Controls were carried out to take into account the leaching of the leaves and the difference in mass linked to drying. The bags were rehydrated and then fixed on bricks and placed in each mesocosm: 9 CM bags per mesocosm (3 replicate per date and 3 main sampling dates) and 4 FM bags per mesocosm (2 FM replicas for 2 additional sampling dates: the middle (May 18, 2021) and the end of experience (June 15, 2021)). Fine mesh litter bags were not presented in the results due to random invertebrate intrusions inside bags creating a bias. The macro-organisms present in the bags were recovered and the leaves were gently rinsed with water to clean them. Then the samples were placed in an oven for 48 hours at 105°C to determine the remaining dry mass and calculate decomposition rate k (Lecerf et al., 2005).

The remaining dry mass (RDM) in bags on each date was calculated, corresponding to the ratio between the remaining mass and the initial mass of the leaves introduced into the bag. The decomposition rate was then calculated according to the exponential model using GLMs taking into account the proportion of remaining dry mass and the kinetics of dates in days using the following equation: (Eq.1)

$$MSr = Mi \times e^{(-kT)} \quad \text{(Eq.1)}$$

With:

• MSr = remaining dry matter (in g)

• Mi = initial dry mass (in g). Mi is estimated based on the relationship between the mass of air-dried and oven-dried leaf litter (i.e. We correct the mass of air-dried leaves before introduction into mesocosms by the mass of control leaves air-dried at the same time, not placed in the mesocosms and oven-dried at the same time as leaves from the mesocosms experiment, in order to determine the loss of mass between drying in the air and in the oven).

• T = time in day

• K = decomposition rate

### 2.5.2 Primary production

Chlorophyll A in the water column was measured weekly during the experiment using an ANHYDRE-EXO multi-parameter probe (EXO-2). To measure benthic primary production three phytobenthos supports were placed at the bottom of each mesocosms and 3 others were suspended in the water column on Feb 18, 2021. They were sampled weekly using a benthic probe (bbe bento-torch Moldaenke GmbH Schwentinental, Germany) allowing measurement of Phytobenthos fluorescence. The use of a benthic probe allows quantifying the total biomass of algae by measuring [Chl A] for three major phytobenthos groups (i.e. cyanobacteria, green algae and diatoms) in µg/cm$^2$. The bottom benthic Phytobenthos measurements are not taken into account, as they may have been strongly

influenced by allochthonous inputs (pollen, microorganisms…) that covered tem and prevented colonisation by phytobenthos.

### 2.5.3 Gas concentrations:

The EXO multi-parameter probe allows us to monitor weekly the dissolved oxygen concentration in %sat and mg/L pressure in each mesocosm. To measure the greenhouse gases dissolved in each mesocosms we monthly sampled water at the beginning (April 12-13, 2021), the middle (April 26-27, 2021) and the end of the experiment (May 18-19, 2021). These measurements were preferably taken at the same time of the day (i.e. around midday for uniform luminosity). We used a gas analyser from the LGR brand composed of an optical chamber (Microportable Analyzers GLA131, ABB Inc, Quebec, Canada). Depending on the absorbance measured from the laser present in the LGR we obtain the mole fraction of greenhouse gases emissions for methane ($CH_4$) and carbon dioxide ($CO_2$) in the atmosphere and dissolved in water, in ppm. The averages of the plateaus of the various measurements in ppm are obtained after stabilization of the flow (few minutes) and extracted with the measuring time and the water temperature to be able to subsequently calculate metabolic balances. A drifting floating chamber (FC; volume: 47 L; area: 0.24 m2; water penetration: 6 cm) was used to calculate the transfer rate which allows the gas exchange flux to be calculated, using the concentration in the water and the atmospheric concentration. The transfer rate can change with temperature as the latter can modify gas solubility (the more insoluble/poorly soluble is the gas, the greater is the transfer rate). However, there was no transfer rate observed because the transfer surface was weak and calm (little mixing and wind). Thus, we worked on an approach taking into account the departure from saturation (Δgas saturation concentration in µM) (mean ± SD). Gas saturation was calculated using henry constant (1803), atmospheric concentrations of the sampling day in ppm for $CO_2$ and for $CH_4$ and temperatures corrected saturation values.

## 2.6 Physical-chemical data

Water physical-chemical datas were sampled at the beginning and the end of the experiment and analysed by an external laboratory (Eurofin France laboratory). We measured Biochemical Oxygen Demand (BOD5), Suspended Solids (MES), turbidity, Phosphorus (P), Dissolved Organic Carbon (COD), Total Organic Carbon, Chlorophyll a, Chlorides (Cl), Nitrates (in N), Nitrites, Sulfates $(SO_4)^{2-}$, Ammoniacal nitrogen, Ammonium ($NH_4$), Calcium (Ca), Magnesium (Mg), Potassium (K) and Sodium (Na). In addition, the EXO probe allowed us monitoring of turbidity, conductivity, fDOM, pH, pressure and temperature; weekly on each mesocosms (results of the physical-chemical datas are presented in the supplementary material Fig. S9:S13 and mentioned in the discussion).

## 2.7 Statistical analyses

Because the temperature changed across the experiment due to the spring warming, we defined the water temperature as the mean temperature of the 15 days prior to the sampling dates ($T_{15}$) for each mesocosms. Integrating the last 15 days is more representative than considering only the temperature at the sampling date. We computed linear mixed-effects model to determine the effects of artificial warming, fish treatments and their interactions on ecosystems functions (the date and the mesocosm number were included as random factors except for analyses on the remaining dry mass of leaf litter where experimental duration was included as a fixed factor). When statistical interactions among fixed variables were significant, we performed post-hoc analyses by splicing the dataset by the variable of interest and fitting again the mixed model to the data subsets. Application conditions for the LMMs (homoscedasticity, normality, independence of residuals) were tested. The significance of the fixed model terms was assessed using analyses of deviance (Anova function from the car package) and non-significant ($p > 0.05$) variables or interactions were removed from the final model. All mixed models were computed using the lmer function in the R package "lme4" (Bates et al. 2015).

For all ecosystem functions studied (organic matter decomposition, primary production and gas concentrations) we also computed piecewise structural equation models (SEMs) in order to take into account direct and indirect effects of biological communities, experimental conditions and other ecosystem functions on the different ecosystem processes (SEM links are illustrated below for each ecosystem process).

For decomposition rate we considered artificial warming, fish treatments and the biological communities' contributing to the decomposition function (i.e. bacteria, macro-organisms) by taking, for each mesososm, the average of the three dates for each of our variables. For primary production and gas concentrations we considered water temperature ($T_{15}$), artificial warming, fish treatments and the biological communities' effects on three sampling dates (i.e. at the beginning, the middle and the end of the experiment). We also take into account the trophic predatory interactions between our compartments (e.g. fish can eat invertebrates, fish and invertebrates can eat zooplanktons, and invertebrates and zooplanktons can eats bacteria and primary producers (Fenoglio et al., 2020; Hall et al., 1996; Hessen and Andersen, 1990; Work, 2003). SEMs were analysed using piecewise structural equation models (SEMs) and using as.psem function from the piecewiseSEM package.

All statistical analyses were performed with R software (version 4.3.1; R development Core Team).

# 3. Results

## 3.1. Organic matter decomposition

The organic matter decomposition rate in days (K(d)) was influenced neither by the presence/absence of fish (F =0.1434, df = 1, P= 0.70) nor by warming (F =2.4, df = 1, P = 0.12) (Fig. 5). Only the temporality (experimental duration in days) significantly affects the evolution of the remaining dry mass over the experiment (F =9451, df = 3, P <0.001***) (Fig. S6).

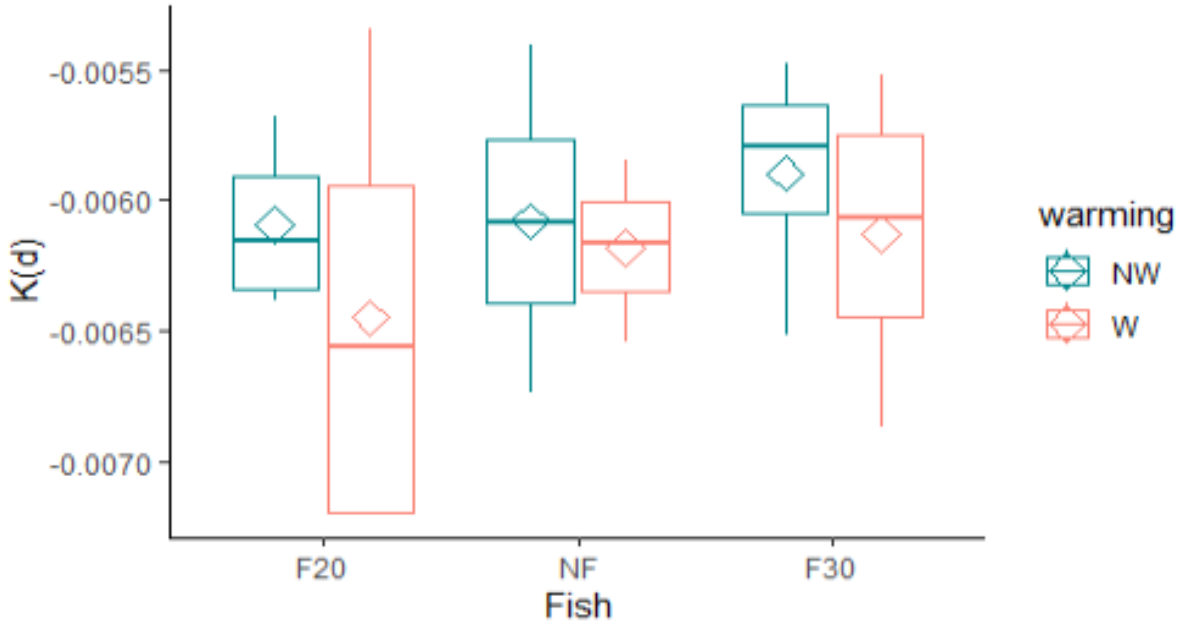


***Figure 5****: Boxplot of K decomposition rates (decomposition kinetics calculated from the three sampling dates) according to experimental treatments. Heated mesocosms are denoted (W) and unheated (NW). F20 indicates mesocosms with fish from exposure at 20°C, F30 fish from exposure at 30°C and NF mesocosms without fish.*

Using SEMs comparison based on linear mixed models, we found no significant direct effect of warming (P = 0.7), fish treatments (P=0.94), invertebrate (P= 0.9) and PLP (Prokaryote like particle) (P=0.22) on decomposition rate (K) (Fig.6).

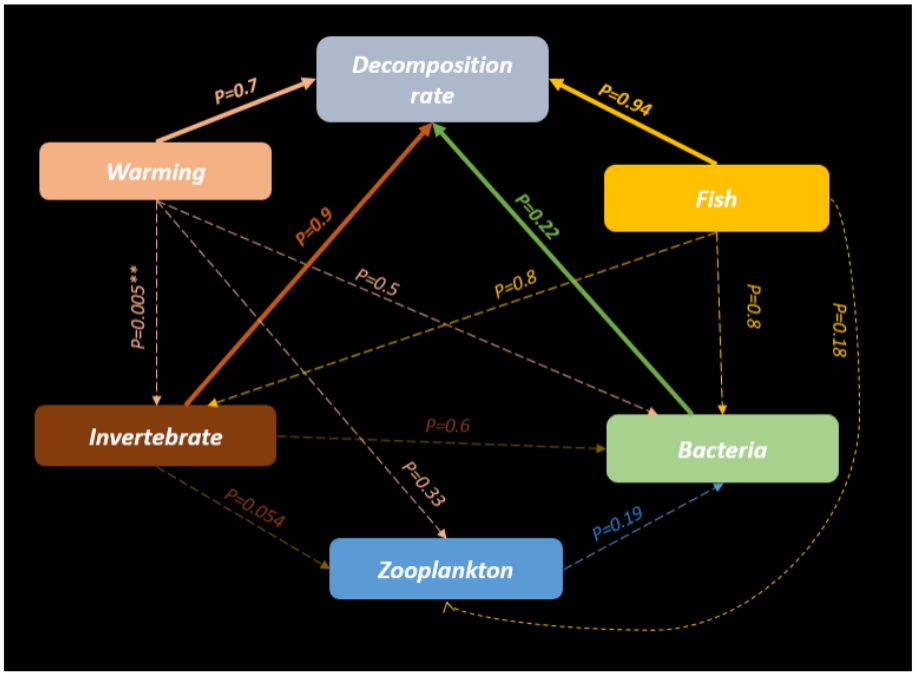


***Figure. 6****: Illustration of links included in the SEM analyse between the different trophic compartments and the experimental treatments for decomposition rate (only one date used). The solid lines correspond to the direct links with the ecosystem function studied, the dotted lines correspond to the relationships between the different trophic compartments. The values correspond to the p-values obtained.*

## 3.2 Primary production

Cyanobacteria concentrations was significantly affected by the interaction between warming and $T_{15}$ ($F = 36.5$, $df = 1$, $P < 0.001$***) and between fish treatments and $T_{15}$ ($F = 8.2$, $df = 2$, $P = 0.01$*). Cyanobacteria concentrations increase with $T_{15}$ increase, for heated mesocosms and in presence of fish of the two thermal origins (Fig. 7A).

Green-algae concentrations was significantly affected by the interaction between fish, warming and $T_{15}$ ($F = 6.2$, $df = 2$, $P = 0.04$*). We conducted separate analyses by fish treatments and found a significant interaction between $T_{15}$ and warming for mesocosms with small body fish (F30) ($F = 10.3$, $df = 1$, $P = 0.001$**) and mesocosms without fish (NF) ($F = 5.2$, $df = 1$, $P = 0.02$*). Green-algae concentration increases with $T_{15}$ and are higher in heated mesocosms without fish (NF) and with small body fish (F30). There was no effect of $T_{15}$ ($F = 1.2$, $df = 1$, $P = 0.28$) and warming ($F = 0.8$, $df = 1$, $P = 0.36$) for mesocosms with large body fish (F20) (Fig. 7B).

We found a significant interaction between fish, warming and $T_{15}$ ($F = 14.6$, $df = 2$, $P < 0.001$***) for Diatoms concentrations. We conducted separate analyses by fish treatments and found a significant interaction between $T_{15}$ and warming for mesocosms with small body size fish (F30) ($F = 11$, $df = 1$, $P < 0.001$***). Diatoms concentration are higher in heated mesocosms with small body size fish and tended to increase with $T_{15}$. Diatoms concentration significantly increased with $T_{15}$ ($F = 12$, $df = 1$, $P < 0.001$***) in mesocosms

with large body size fish (F20). Finally, there were no effects of $T_{15}$ (F =0.3, df = 1, P = 0.6) and warming (F =1.7, df = 1, P = 0.19) for mesocosms without fish (Fig. 7C).

Chlorophyll A concentration was significantly affected by the interaction between fish and $T_{15}$ (F =8.2, df = 2, P = 0.02*) and warming and $T_{15}$ (F =18.2, df = 1, P < 0.001***). Chlorophyll A concentration increase with $T_{15}$ for mesocosms with large body size fish (F20) or small body size fish (F30). Heated mesocosms with large body size fish (F20) and without fish (NF) have lower concentrations compared to unheated ones (Fig. 7D).

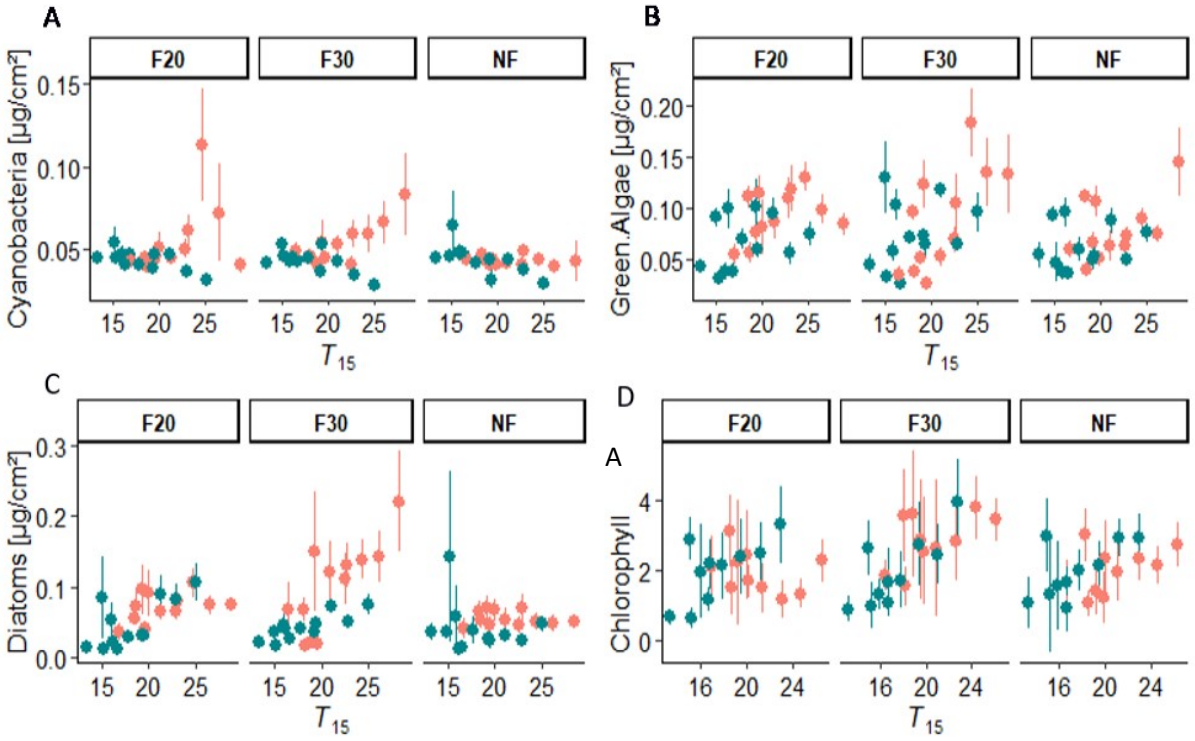


***Figure 7**: Concentration in µg/cm² mean (± sd) measured in the water column for Cyanobacteria (A), Green-algae (B), Diatoms (C) and chlorophyll A (D) according to $T_{15}$, for heated (red) and unheated (blue) mesocosms. F20 indicates mesocosms with fish from exposure at 20°C, F30 fish from exposure at 30°C and NF mesocosms without fish.*

SEMs comparison based on linear mixed models on three dates revealed a positive direct effect of warming on cyanobacteria concentrations (P= 0.02**). (Fig. 8A). There were no effects of $T_{15}$ (P = 0.28), warming (P = 0.87), fish treatments (p = 0.87), zooplankton (P = 0.8) and invertebrate (P = 0.76) on green algae concentration (Fig. 8B). SEMs comparison also revealed a positive effect of fish treatments on diatoms concentrations (P= 0.046*) (Fig. 8C). Finally there was no direct effect on chlorophyll A using SEMs (Fig. 8).

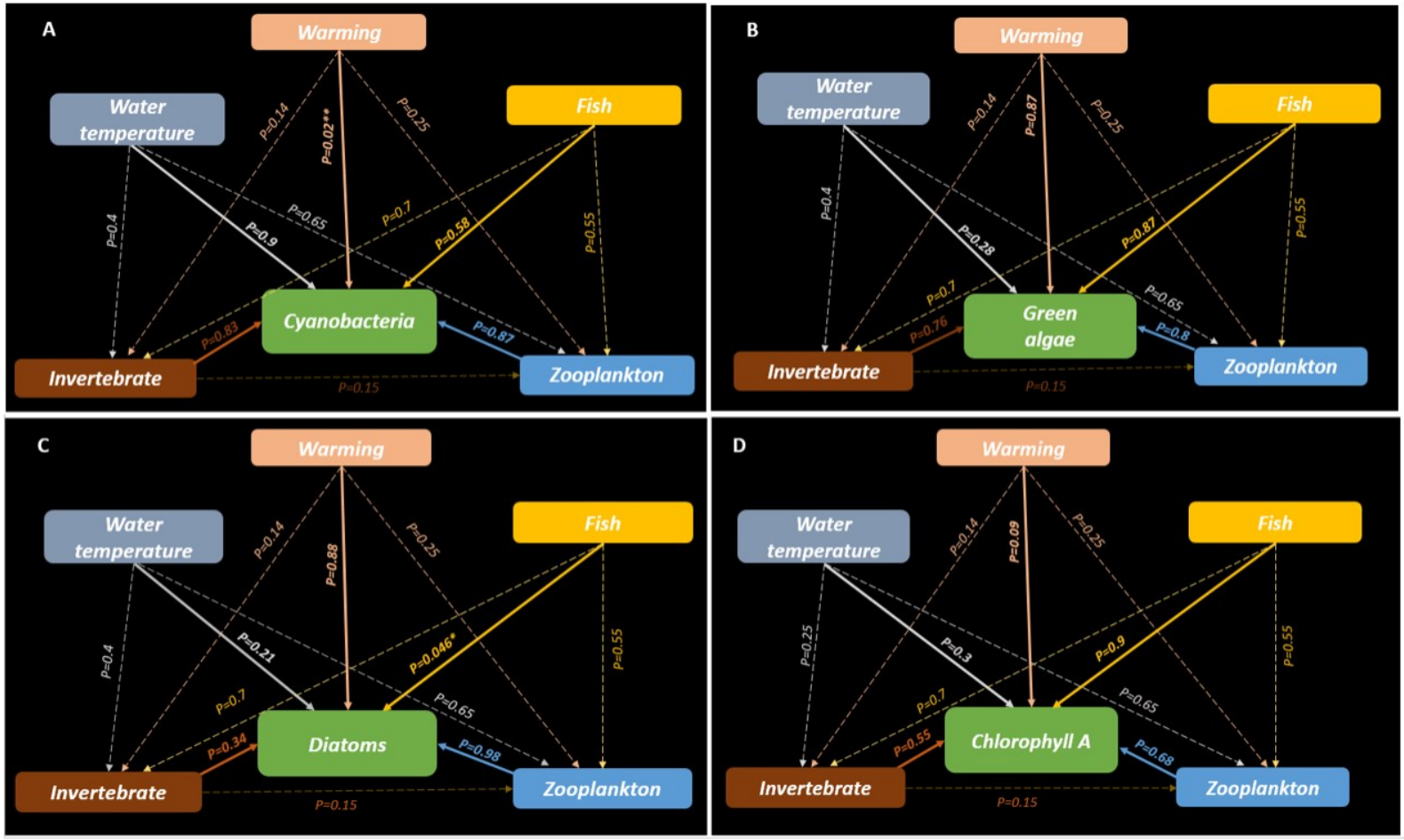


***Figure 8:*** *Illustration of links included in SEM analyses between the different trophic compartments and the experimental test conditions for Cyanobacteria (A), Green-algae (B), Diatoms (C) and chlorophyll A (D) (three dates used). The solid lines correspond to the direct links with the ecosystem function studied, the dotted lines correspond to the relationships between the different trophic compartments. The values correspond to the p-values obtained.*

## 3.3 Gas concentrations

We found a significant interaction between $T_{15}$, fish and warming (F =12.5, df = 2, P=0.001**) for dissolved Oxygen concentration. We conducted separate analyses for fish treatments. We found a significant interaction between warming and $T_{15}$ (F =4.04, df = 2, P = 0.04*) for mesocosms with large body fish (F20) through higher concentration for unheated mesocosms according to $T_{15}$. For small body fish (F30) we found no effects of $T_{15}$ (F =1, df = 2, P = 0.32) and warming (F =0.9, df = 2, P = 0.33) as well as for mesocosms without fish ( F =1.8, df = 2, P = 0.2 and F =2.6, df = 2, P = 0.1, respectively) (Fig. 9).

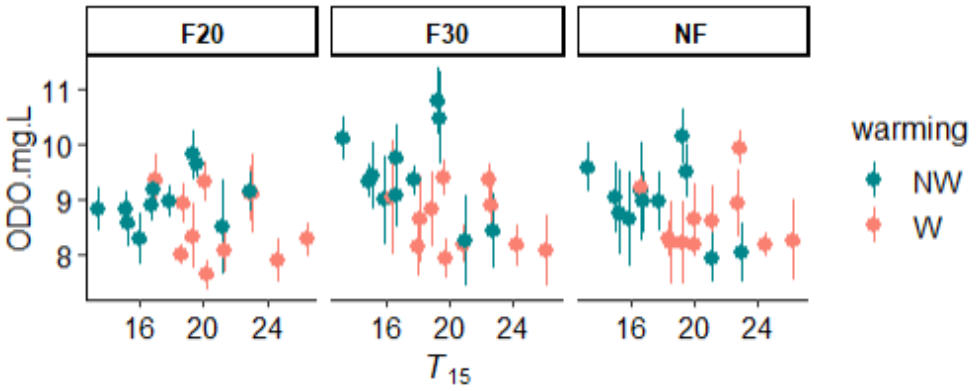


***Figure 9:*** *Mean (± sd) dissolved oxygen concentration in mg/L as a function of $T_{15}$. Heated mesocosms are shown in red (W) and unheated mesocosms in blue (NW). F20 indicates mesocosms with fish from exposure at 20°C, F30 fish from exposure at 30°C and NF mesocosms without fish.*

The SEM analysis on three dates indicated a negative direct effects of $T_{15}$ (P = 0.009**), and effects of invertebrates (P = 0.005**) and bacteria (p=0.03*) on dissolved oxygen concentration (Fig. 10).

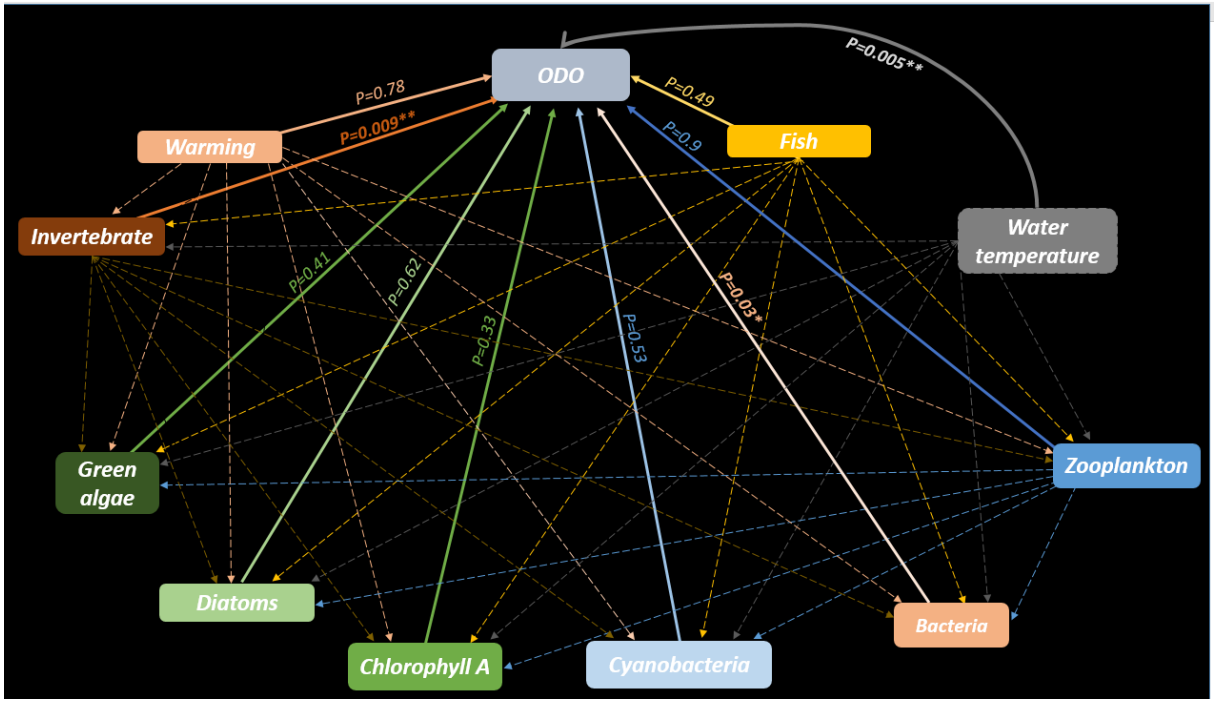


***Figure. 10**: Illustration of links included in the SEM analyse between the different trophic compartments and the experimental test conditions for ODO (three dates used). The solid lines correspond to the direct interactions with the ecosystem function studied, the dotted lines correspond to the relationships between the different trophic compartments. The values correspond to the p-values obtained.*

We found a significant interaction between $T_{15}$, fish and warming for $CO_2$ concentration ($F = 9.9$, $df = 2$, $P = 0.007^{**}$). We conducted separate analyses for fish treatments. There were a significant interaction between $T_{15}$ and warming ($F = 5.8$, $df = 1$, $P = 0.9$) for mesocosms with small body fish (F30). It tended to decrease with $T_{15}$ mainly for unheated mesocosms. We found a significant decrease of $CO_2$ concentration with $T_{15}$ ($F = 6.05$, $df = 1$, $P < 0.01^{*}$) for mesocosms without fish (NF). There were no effects of $T_{15}$ ($F = 1.7$, $df = 1$, $P = 0.2$) and warming ($F = 0.4$, $df = 1$, $P = 0.5$) in mesocosms with large body fish (F20) (Fig. 11A).

$CH_4$ concentrations were significantly affected by the interaction between $T_{15}$ and warming ($F = 4.9$, $df = 2$, $P = 0.03^{*}$). $CH_4$ concentration were higher for heated mesocosms and decreased with $T_{15}$ (Fig. 11B).

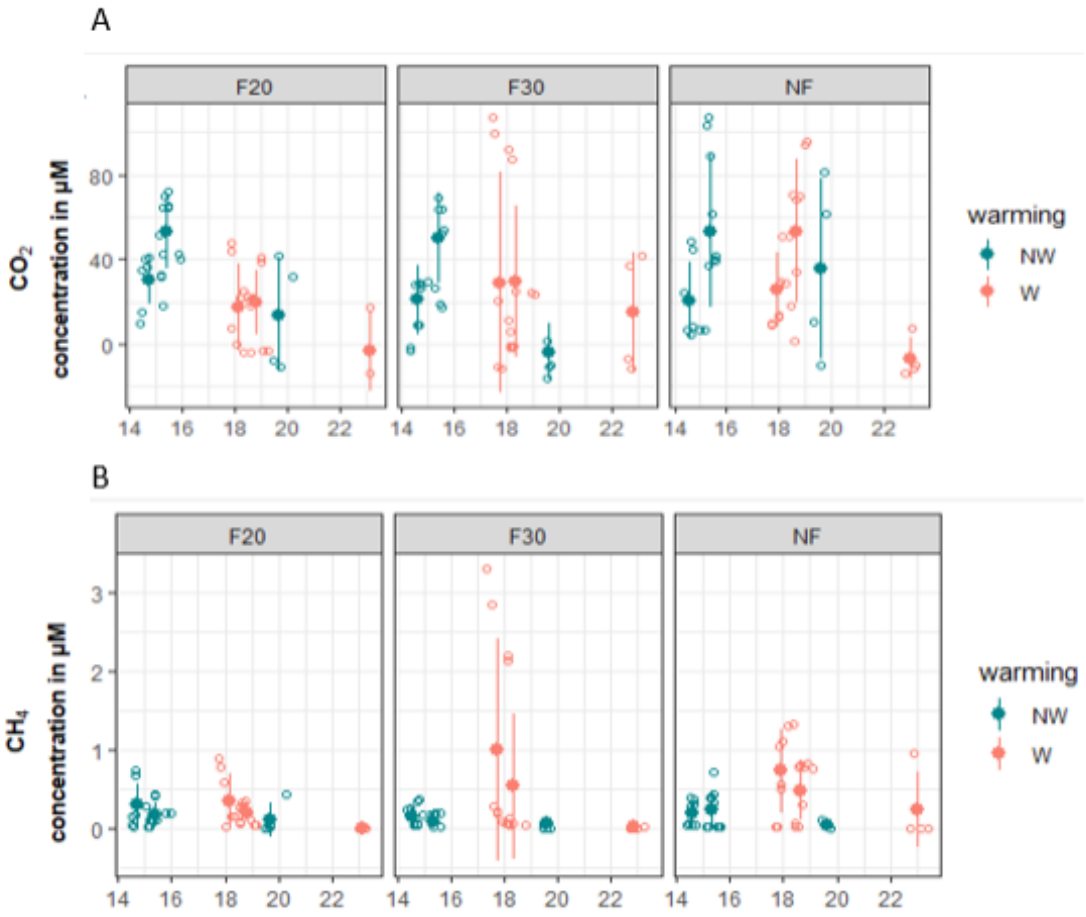


***Figure 11**: Mean (± sd) dissolved concentrations in µM of (A) $CO_2$ and (B) $CH_4$ according to $T_{15}$. Heated mesocosms (W) are shown in red and unheated mesocosms in blue (NW). F20 indicates mesocosms with fish from exposure at 20°C, F30 fish from exposure at 30°C and NF mesocosms without fish.*

SEMs comparison based on linear mixed models revealed a direct effect of Diatoms (P= 0.03*) on $CO_2$ concentration. The SEM analysis indicated only a direct positive effect of $CO_2$ concentration (p<0.001***) on $CH_4$ (Fig. 12 & 13).

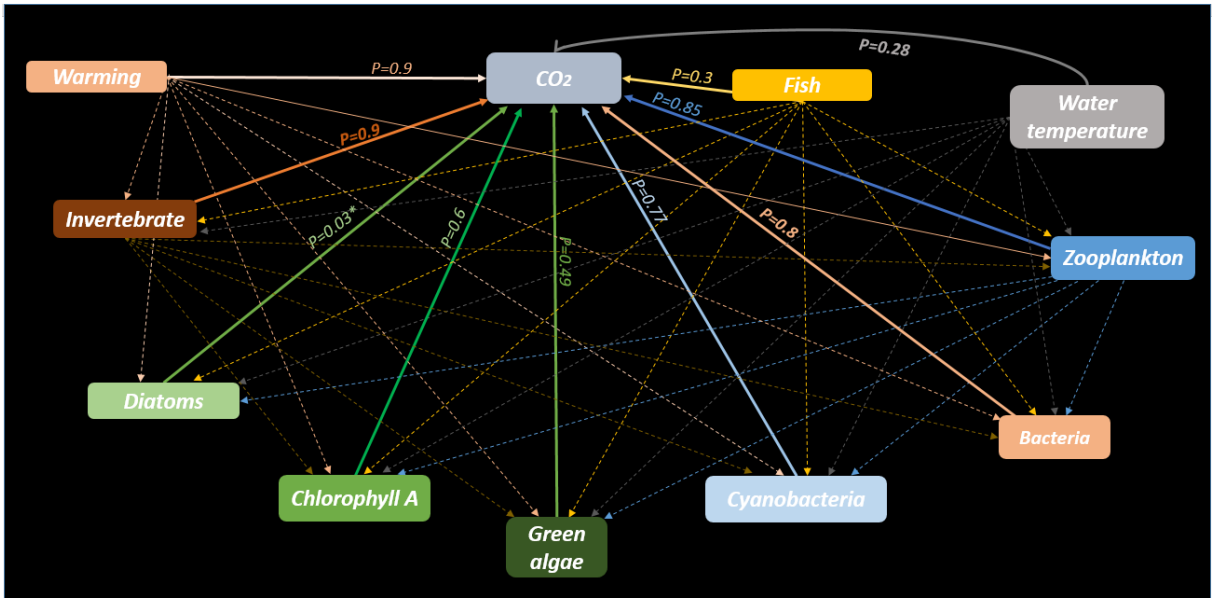


***Figure.12**: Illustration of links included in the SEM analyses between the different trophic compartments and the experimental test conditions for $CO_2$ concentration (three dates used). The solid lines correspond to the direct interactions with the ecosystem function studied, the dotted lines correspond to the relationships between the different trophic compartments. The values correspond to the p-values obtained.*

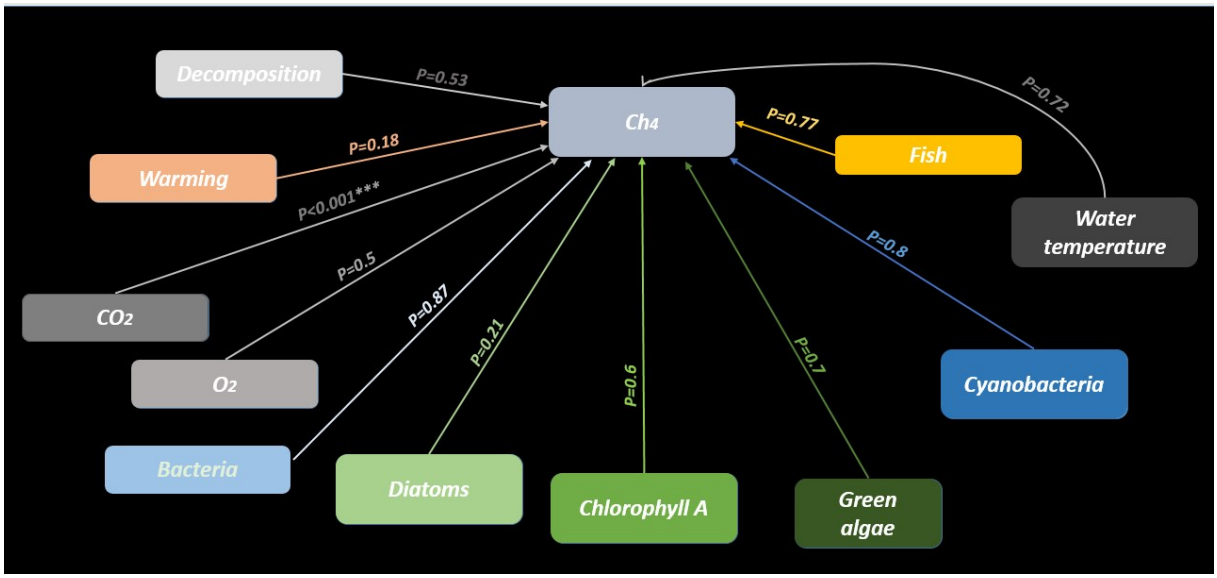


***Figure. 13:** Illustration of links included in the SEM analyses between the different trophic compartments and the experimental test conditions for $CH_4$ concentration (three dates used). The solid lines correspond to the direct interactions with the ecosystem function studied, the dotted lines correspond to the relationships between the different trophic compartments. The values correspond to the p-values obtained.*

# 4. Discussion

Changes in temperature in combination with body size modification are likely to have profound effects on individual, ecology and ecosystems (e.g. Brown et al., 2004). A few theoretical studies have explored these effects at the ecosystem level (Réveillon et al., 2022). Yet, there is still little empirical evidence on how global warming might alter ecosystems (Jeppesen et al., 2010; Kratina et al., 2012; Xie et al., 2024). This study aimed at better understanding the direct and indirect effects of temperature on ecosystem functions. We conducted a mesocosms experiment in which medakas fish exposed for several generations to 20°C (large fish) or 30°C (small fish) were introduced into mesocosms maintained at ambient temperature or heated (i.e. +4°C) in line with the predictions for 2100 from the RCP 8.5 scenario (IPCC 2023).

## 4.1 Organic Matter decomposition

The process of organic matter decomposition is an essential process of energy and nutrient cycling within and between ecosystem food web and contribute strongly to ecosystem respiration (Smith, 1982). Decomposition process is particularly sensitive to climate warming as decomposition depends strongly on temperature (Davidson and Janssens, 2006). The rate of enzymatic degradation of organic matter is driven by the kinetic of chemical reactions (Arrhenius, 1889) which leads to faster litter decomposition during warmer seasons and for lower altitudes and latitudes (Englert et al., 2015; Ferreira et al., 2015; Réveillon et al., 2022; Taylor and Chauvet, 2014; Tiegs et al., 2019). Thus warming can alter bottom-up control increasing decomposition rates (mostly rates of microbial decomposition) and the abundance of heterotrophic bacteria (Davidson and Janssens, 2006; Widden et al., 1989).

Nevertheless, in our study, we found no significant effect neither of the artificial warming nor of the thermal origin of fish. A few previous studies reported that overall litter decomposition may not differ between cold and warm streams (Boyero et al., 2011; Jinggut and Yule, 2015). Boyero et al., (2011) by using a latitudinal temperature gradient in a global experiment in streams, found that climate warming actually hasten microbial litter decomposition but produce an equivalent decline in invertebrate decomposition rates. As a result, overall decomposition rates remain unchanged.

Bazin et al (submitted), in a complementary study (data from the same mesocosms experiment) focusing on community structure, showed significantly lower invertebrate density in heated mesocosms than in unheated ones (Fig. S6) as in other studies (Allen et al., 2002; Yvon-Durocher et al., 2015) and found that increasing $T_{15}$ (water temperature) significantly increased the density of prokaryotic-like particles (PLP) ($p < 0.001$; Fig. S7). These results, in line with previous studies could suggest a compensation phenomenon and explain the absence of significant effects (Fig. S6, Fig. S7) although most taxa contributing to variation in macroinvertebrate density in our study were decomposer taxa (Diptera and Ephemeroptera; Fig. S8). Unfortunately, results obtained with SEMs on

the relationship between invertebrates and PLPs on the decomposition rate do not allow us to confirm this hypothesis. One potential limitation of the SEM for decomposition rate is that we could not take into account the water temperature ($T_{15}$) effect with duration because we only integrate one mean data by mesocosm for each function, while we see that the PLP increases with $T_{15}$ (fig. S7).

Actually, decomposition rate may depend on many other factors such as litter quality and the nature of the decomposers community. Fungi are considered to be the main decomposers of leaves through the release of extracellular enzymes (Gulis et al., 2019; Tennakoon, 2021) while bacteria are more important in the final stages of decomposition (Duarte et al, 2010; Komínková et al., 2000) because they mainly use the decomposition products released by the fungi (Baldy et al., 1995; Gonçalves et al., 2013; Hieber and Gessner, 2002; Johnston et al., 2016; Pascoal and Cássio, 2004; Soares et al., 2017; Weyers and Suberkropp, 1996). Thus, because we did not inoculate the mesocosms with fungi the fungal composition could be poor, which may explain the lack of a significant effect. Finally, if warming decreases oxygen solubility, litter decomposition may be inhibited (Medeiros et al., 2009) but the relatively high levels of oxygen in our study do not appear to explain the lack of decomposition.

## 4.2 Primary production

To ensure primary production process light energy is converted by autotrophic organisms into chemical energy to produce organic molecules from mineral substances ($CO_2$ and mineral salts, often themselves derived from the decomposition). The main general trends of our results was an increase in the concentration of cyanobacteria and diatoms in the presence of fish in heated mesocosms and as a function of water temperature ($T_{15}$). Moreover, SEMs confirms direct effects of artificial warming and fish treatments on cyanobacteria and diatoms concentration, respectively. An increase in temperature intensifies the primary production process by affecting metabolism and enzymes involved in photosynthesis (Davidson and Janssens, 2006; Dusenge et al., 2019). Gross primary productivity and respiration thus increase (exponentially) with temperature within the normal range (non-stressful) of natural environmental temperatures (Allen et al., 2005; Brylinsky and Mann, 1973; Davison et al., 1991; Lee et al., 2007). These results may also be linked to a zooplankton predation effect. In fact, zooplankton densities followed a hump-shaped pattern with $T_{15}$, with lower densities in heated mesocosms compared to unheated ones (Fig. S6). The fish predation of zooplankton and invertebrates leading to a lack of pressure on phytoplankton can increase primary production (Jeppesen et al., 1990). Moreover, access to essential nutrients for producers growth and photosynthesis, such as nitrogen, phosphorus, and potassium is another factor influencing the primary productivity of an ecosystem (Paerl and Barnard, 2020; Schindler et al., 2016). At the end of the experiment heated mesocosms appeared to be richer in nutrient which could explain the observed increase in primary production Fig. S9, Fig. S10).

In addition, we observe a general trend (mainly concerning green algae, diatoms and chlorophyll A) towards higher concentrations of primary producers with F30 fish (i.e. smaller fish) in heated mesocosms. Even if smaller-bodied fish from warm populations should have lower effects on populations of large zooplankton due to gap-size limitation (morphological), smaller organisms can be more energetically efficient, which may make them more competitive than larger ones (Edeline et al., 2013; Ohlberger, 2013). Thus, the higher energy efficiency expected from smaller individuals in warmer environments could lead to increased predation pressure on zooplankton, freeing phytoplankton from top-down control. Moreover, large cold fish raised at 20°C have a lower maximum thermal tolerance than smaller warm fish (Morla et al., In prep) which may decrease their activity in heated mesocosm.

## 4.3 Gaz concentrations

An increase in metabolic rate with temperature leads to greater energy requirements. As a result, organisms must consume more prey and oxygen, which alters interactions between consumers and resources (Bideault et al., 2019; Sentis et al., 2012). In this study, we found an interactive effect of the presence of fish and the water temperature ($T_{15}$) in heated mesocosms, although the role of fish in this pattern was not clear. Our results showed that mainly warming and water temperature ($T_{15}$) reduce oxygen concentration. SEMs analyses highlight a direct link of invertebrate and bacteria on oxygen concentrations which may suggest that these two trophic compartments contribute the most to the respiration of the ecosystem. However, lower levels of dissolved oxygen can mainly be due to the direct negative impact of temperature on oxygen solubility (Truesdale et al., 1955; Verberk et al., 2016). More generally, the amount of oxygen that dissolves in water can vary daily and seasonally. It decreases with higher temperature, salinity, and elevation (Wetzel, 2001). In addition, surface water, near the water-atmosphere interface and with sufficient light for photosynthesis, is generally saturated or even supersaturated in oxygen that could explain the general high oxygen concentration in our study, especially since mesocosms are shallow and stagnant habitats with a large exchange surface area for a small volume.

Lakes are an important source of atmospheric greenhouse gases such as methane ($CH_4$), and carbon dioxide ($CO_2$) and are an active component of the global carbon (C) cycle (Bastviken et al., 2011; Battin et al., 2009; Cole et al., 2007; Deemer et al., 2016; DelSontro et al., 2018; Holgerson and Raymond, 2016; Tranvik et al., 2009, 2009). In our study, we found a significant interaction between water temperature ($T_{15}$) and fish in heated mesocosms, through a decrease in $CO_2$ concentration with water temperature ($T_{15}$). We also found a significant effect of $T_{15}$ ($F = 8.5$, $df = 1$, $P = 0.004^{**}$) on $CO_2$ concentration for non-heated mesocosms. These results are difficult to interpret and the impact of fish and warming appear to be antagonistic. SEMs analyses revealed a direct effect of Diatoms on $CO_2$ concentration that could suggest a higher contribution of this trophic compartment. $CO_2$ emissions from river and lake surfaces are mainly controlled by the balance of respiration and primary production (Perga et al., 2016; Xiao et al., 2020). High primary

productivity may decrease $CO_2$ concentration and emissions from lakes (e.g., 86 Sepulveda-Jauregui et al., 2018; Vachon et al., 2020; West et al., 2016, 2012; Yang et al., 2019). Consistently with previous studies, increased primary producers concentration (likely due to increased nutrient loadings) was negatively correlated with $CO_2$ concentrations in lakes (e.g., Li et al., 2015; Pacheco et al., 2014).

Additionally, in our study, the $CH_4$ concentration decreases with $T_{15}$ and was higher in heated mesocosms. In contrast to $CO_2$ fluxes, atmospheric $CH_4$ fluxes exhibit a positive relationships with nutrients and chlorophyll a concentrations which could explain the higher concentration that we observed for heated mesocosms (Colas et al., 2021; Vachon et al., 2020; Yang et al., 2019). However, some studies provided correlations between the solubility of methane in water and the temperature of the water through a solubility decrease with temperature, that could explain the $CH_4$ concentration decrease with the water temperature ($T_{15}$) in our study (Liu et al., 2014; Yamamoto et al., 1976; Yvon-Durocher et al., 2014). Moreover, following MTE predictions, Yvon-Durocher et al (2010a) showed that a warming of 4°C decreases $CO_2$ sequestration by 13% and increases the fraction of ecosystem respiration methane efflux by 9%. This may explain the direct link obtain by SEMs between $CH_4$ concentration and $CO_2$ concentration in our mesocosms (Figure 13). Finally, the SEMs did not reveal any impact of bacteria on the $CH_4$concentration, which seems surprising because bacteria constitute an important methanization compartment (Yao et al., 2016). To conclude the relationship between decreasing greenhouses gazes solubility with temperature and increasing emission of $CH_4$ and $CO_2$ with warming suggest that global warming may have a large impact on the relative contributions of total ecosystems.

This study highlight the complexity of the impact of temperature on ecosystem function via direct and indirect effects. Although many responses to temperature are already known at the individual level, on trophic interactions and on ecosystem functions, few studies take into account the response to temperature at these different scales in an empirical context to understand the overall response of the ecosystem. Over and above the expected responses of temperature to ecosystem functions, our study demonstrates the importance of trophic relationships and complex effects on the ecosystem's response to temperature. Even if our results suggest that the presence and differences in fish biology had lower effects on ecosystem functions than the direct effects of water temperature (driven by seasonality) and artificial warming, our results highlights the different sensibility and thermal response of the ecosystem functions and draw our attention to the thermal vulnerability of greenhouse emissions in thermally constrained closed freshwater ecosystems.

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# 6. Supplementary material

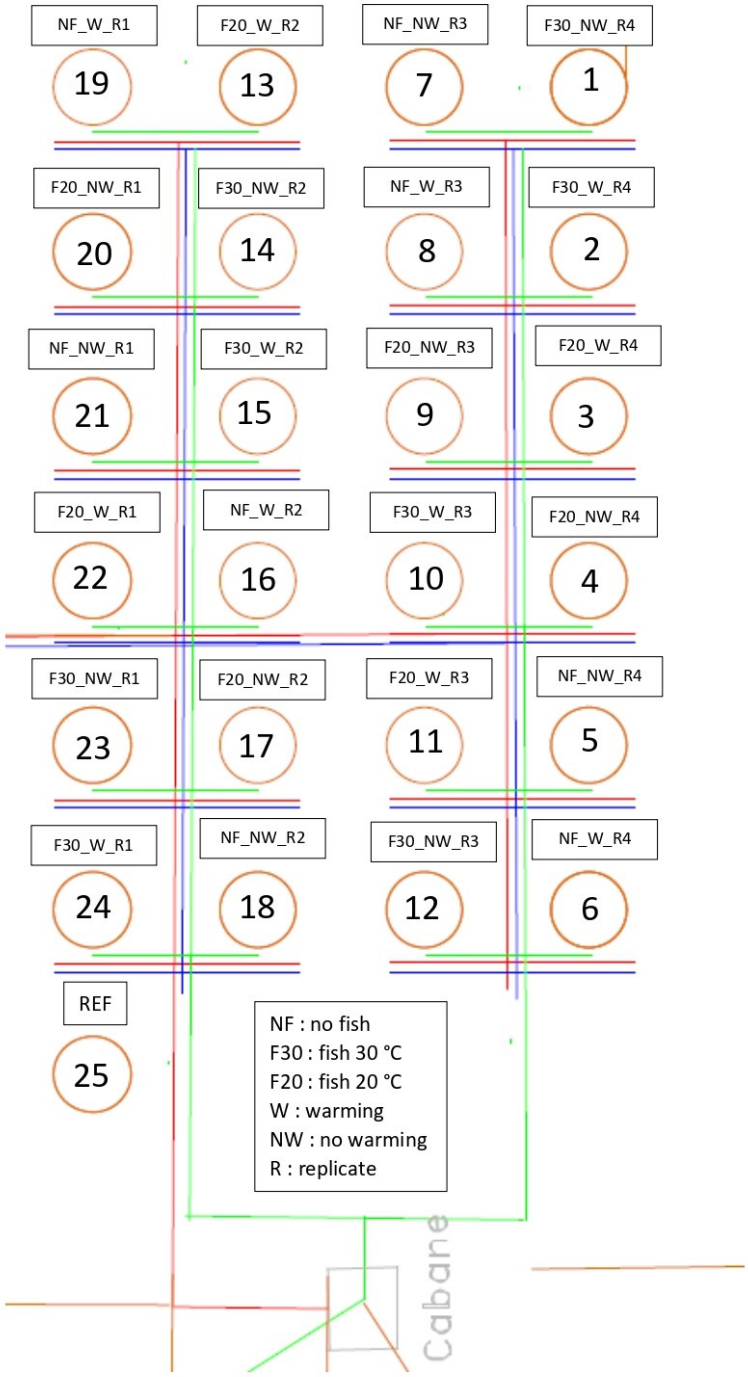



***Fig. S1:*** *Mesocosms design with treatment spatial repartition*

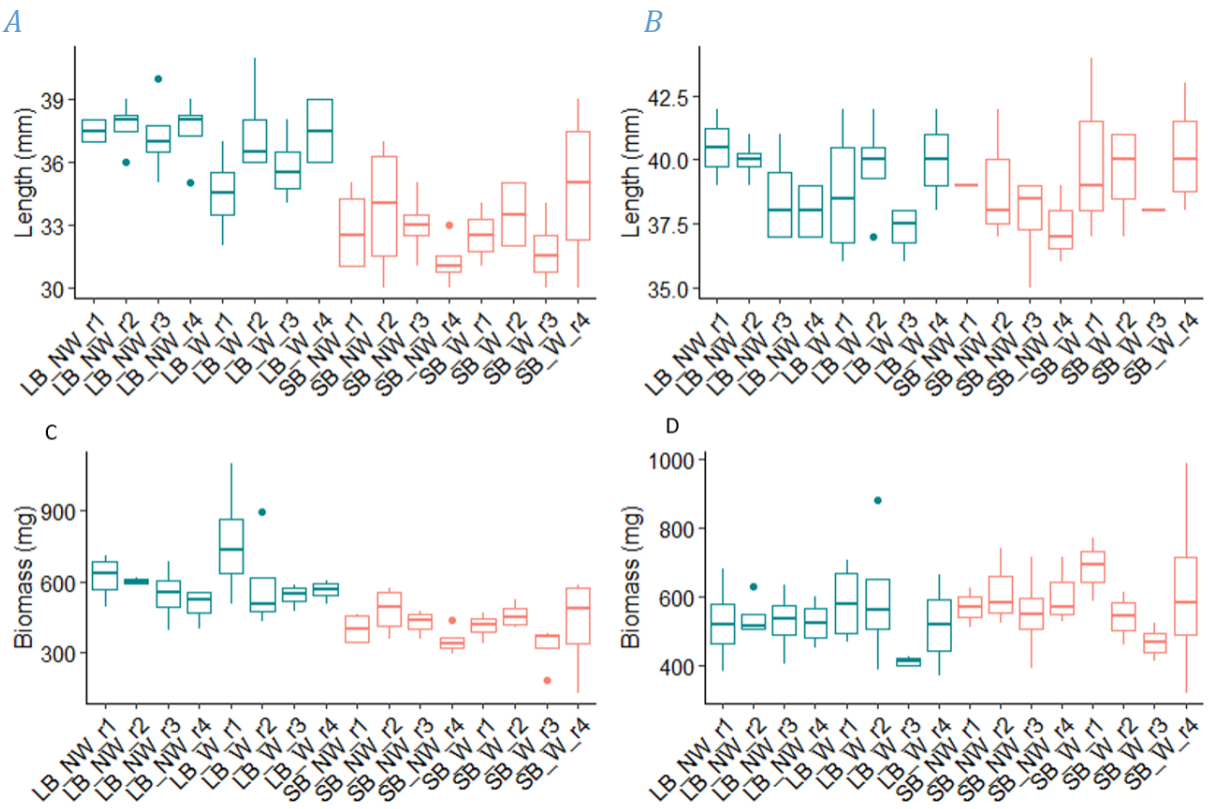



***Fig. S2:*** *boxplots of (A) Individual fish length at the beginning and (B) at the end of the experiment for each mesocosm (C) Individual fish body mass at the beginning and (D) at the end of the experiment for each mescosom. Blue and red colours represent fish reared at 20 and 30°C, respectively. SB for small body fish (F30) and LB for large body fish (F20). NW represent noon heated mesocosms and W represent heated mesocosms.*

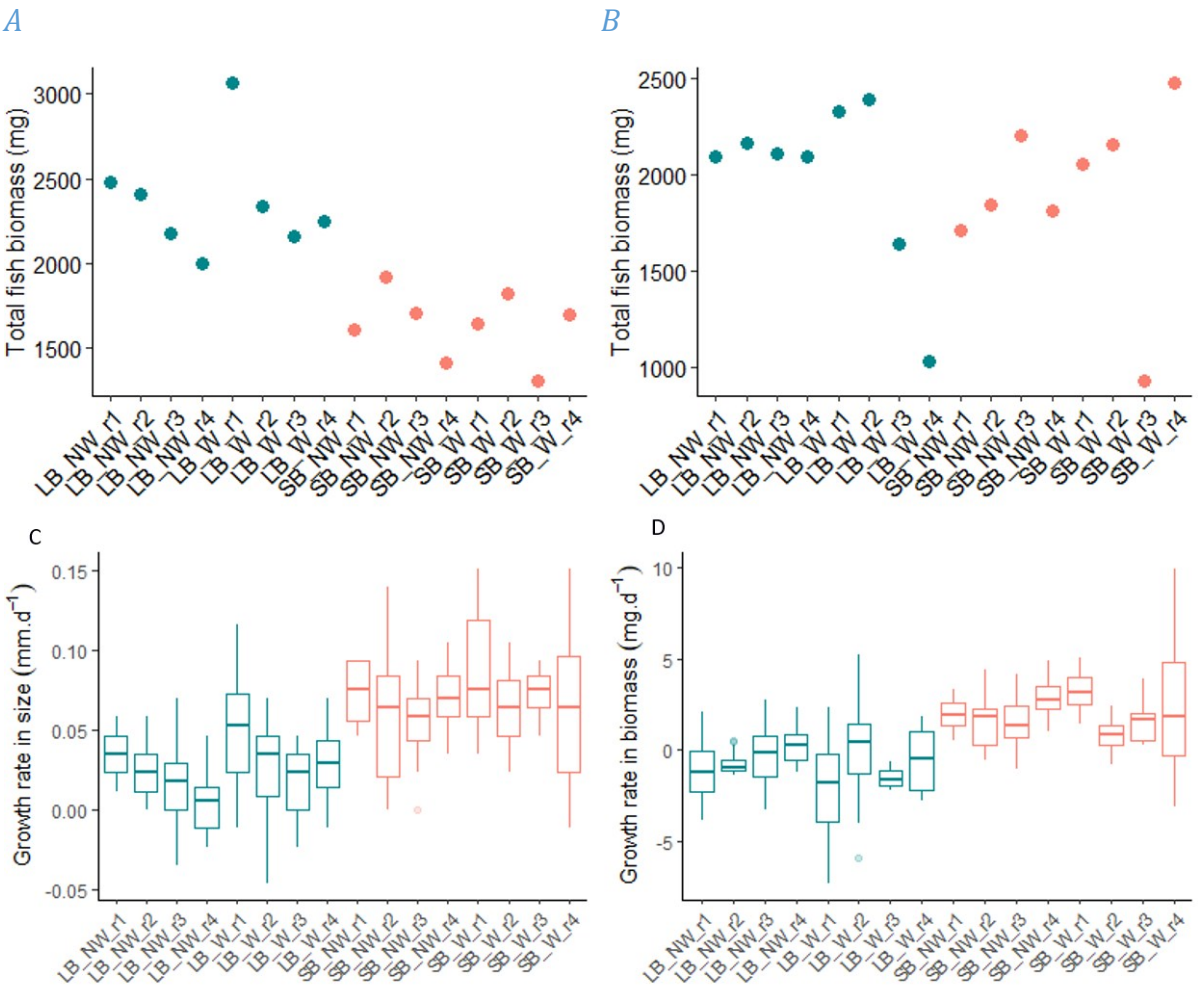



***Fig. S3:*** *(A) Total fish biomass at the beginning and (B) at the end of the experiment in each mesocosms. (C) Individual growth rate in size and (D) in biomass in each mesocosms at the end of the experiment. Blue and red colours represent fish reared at 20 and 30°C, respectively. SB for small body fish (F30) and LB for large body fish (F20). NW represent noon heated mesocosms and W represent heated mesocosms.*

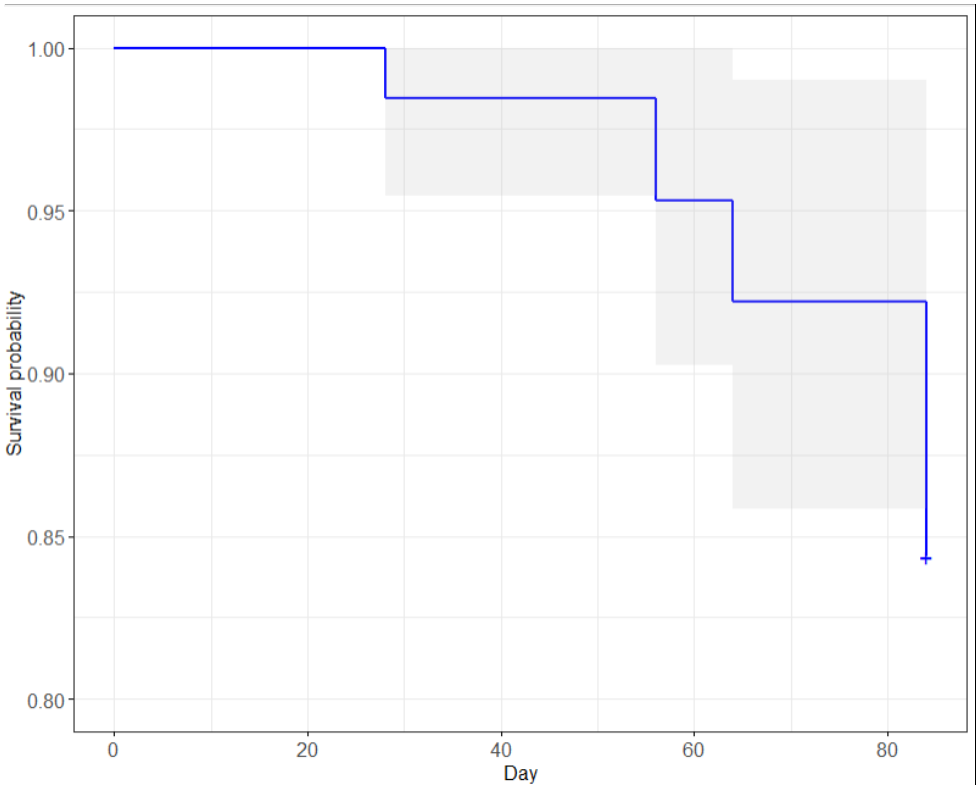



***Fig. S4**: Survival probability of fish from the start to the end of the mesocosms experiment.*

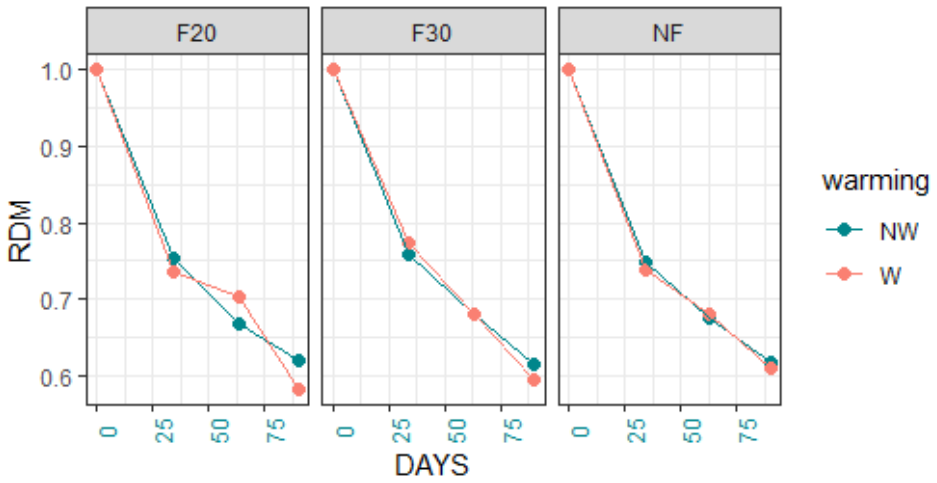



***Fig. S5:*** *RDM (Remaining Dry Mass) mean for each treatment, according to experimental time in days. Heated mesocosms are denoted (W) and unheated (NW). F20 indicates mesocosms with fish from exposure at 20°C, F30 fish from exposure at 30°C and NF mesocosms without fish.*

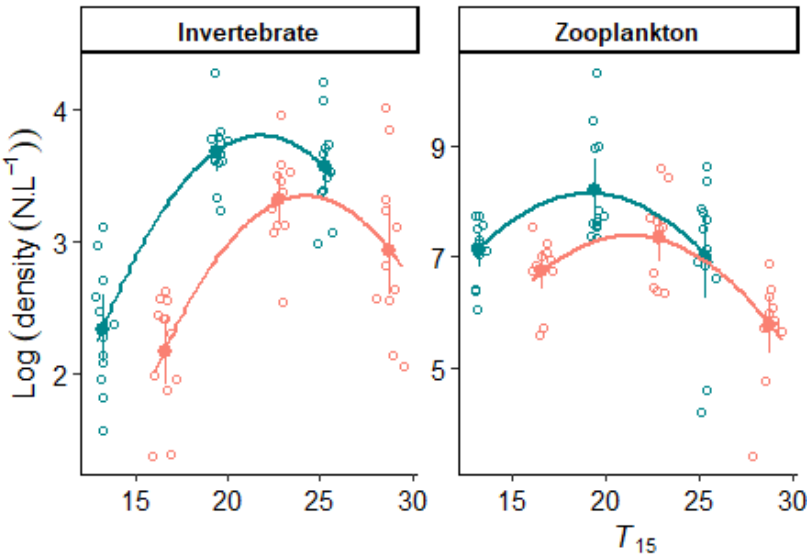



***Fig. S6**: $T_{15}$ and artificial warming effects on (a) macroinvertebrate and (b) zooplankton densities in log scale. Blue and red colours represent ambient and heated mesocosms, respectively. Empty circles represent experimental data. Filled circles represent the mean values at each sampling date and bars represent the 95 % confident intervals.*

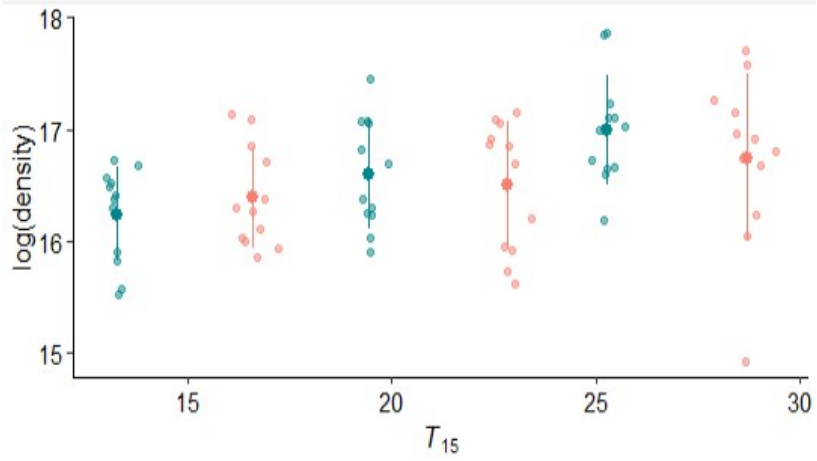



***Fig. S7:** PLP (prokaryote-like particles) Log density according to $T_{15}$. Blue and red colours represent ambient and heated mesocosms, respectively. Empty circles represent experimental data. Filled circles represent the mean values at each sampling date and bars represent the 95% confident intervals.*

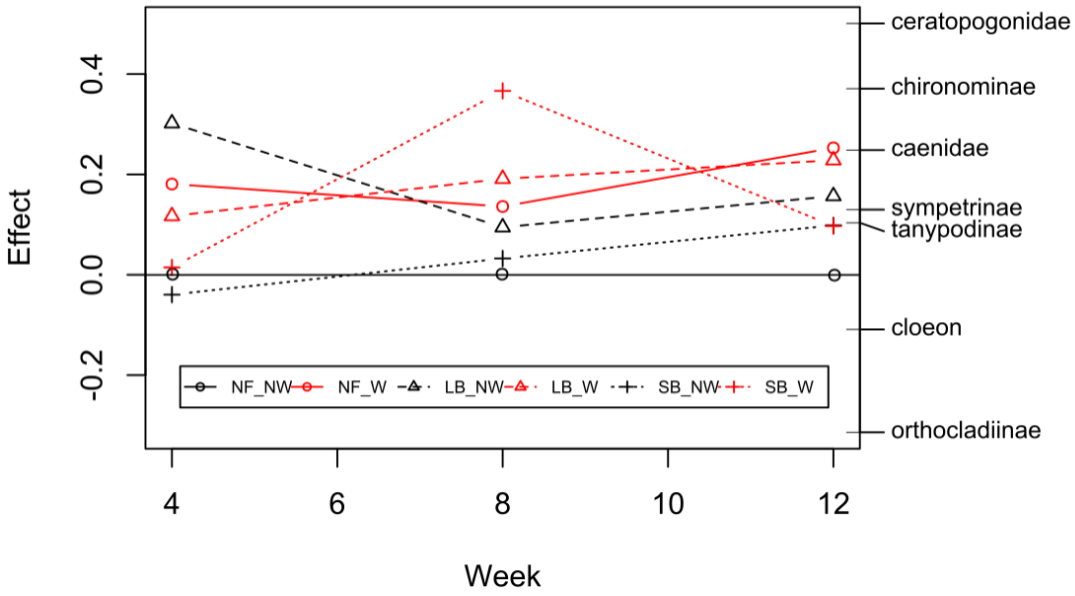



***Fig. S8**: Principal Response Curve of Macroinvertebrates community. Black and red colours represent unheated and heated mesocosms, respectively. Circles, triangles and crosses represent the NF (No fish), LB (Large body fish from F20) and SB (Small body fish from F30) fish treatments, respectively.*

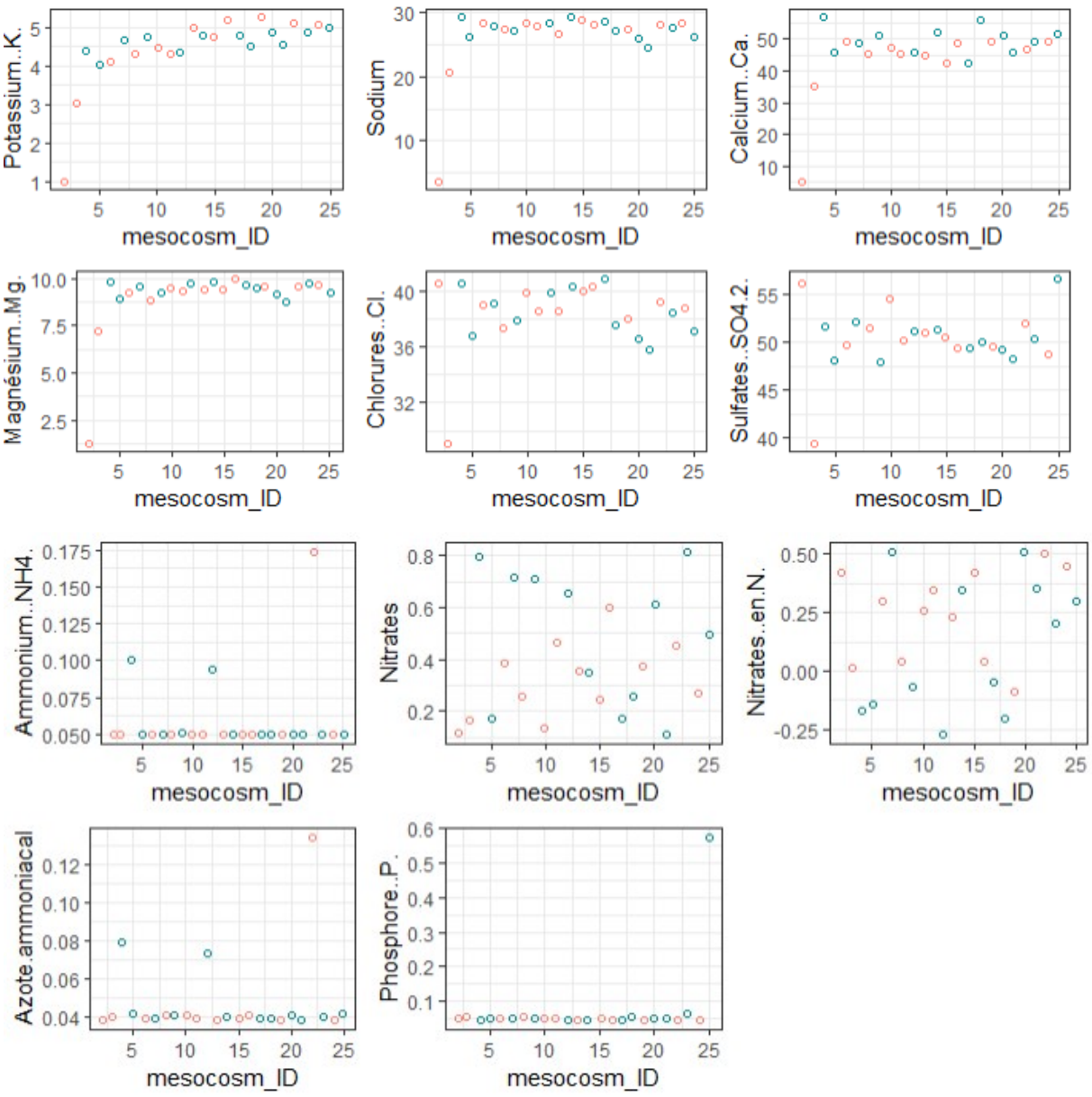



**Fig. S9:** Nutrient concentration in each mesocosm at the beginning of the experiment (03/23/2021). Blue and red colours represent ambient and heated mesocosms, respectively.

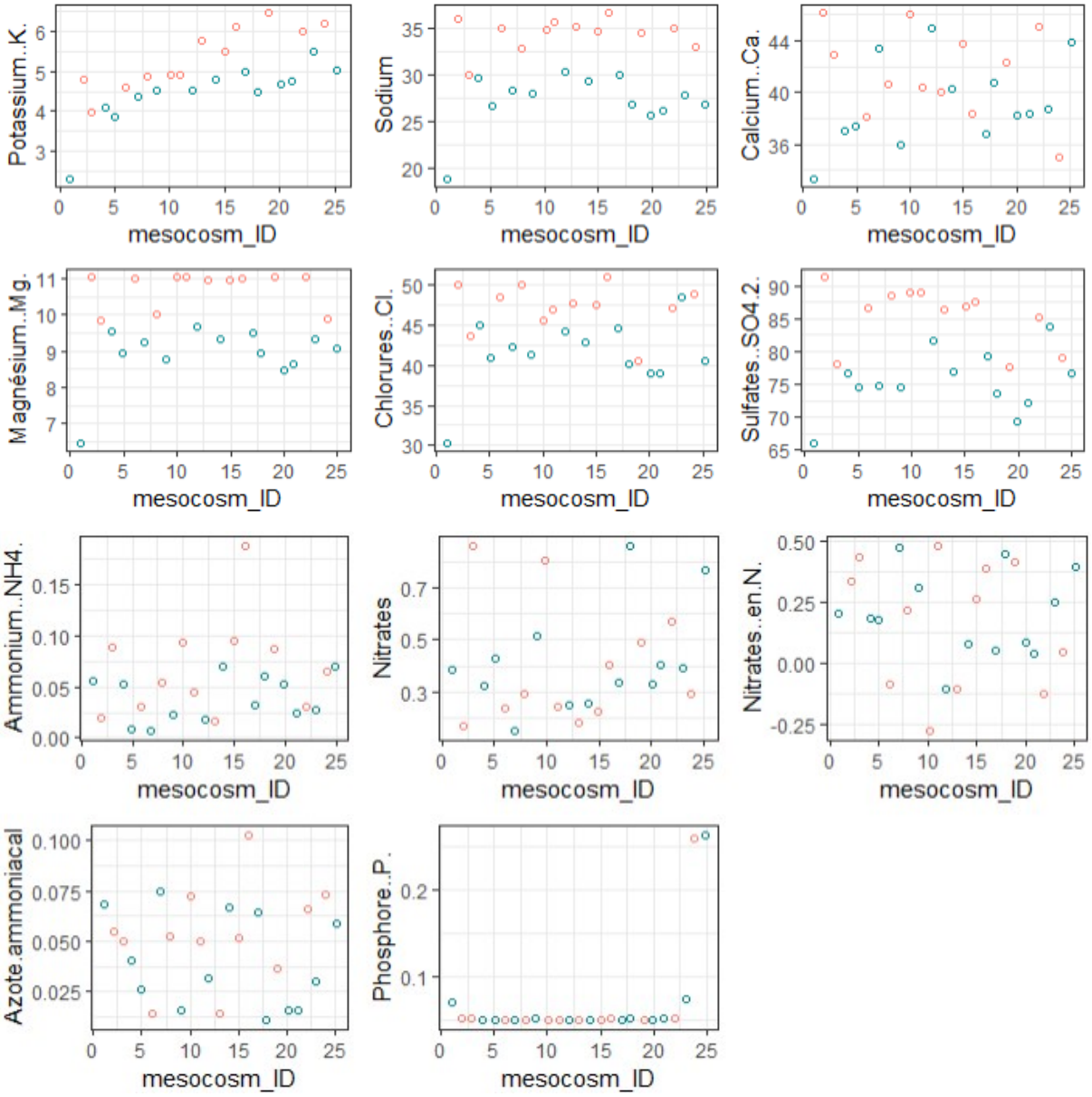



***Fig. S10:*** *Nutrient concentration in each mesocosm at the end of the experiment (on 06/17/2021). Blue and red colours represent ambient and heated mesocosms, respectively.*

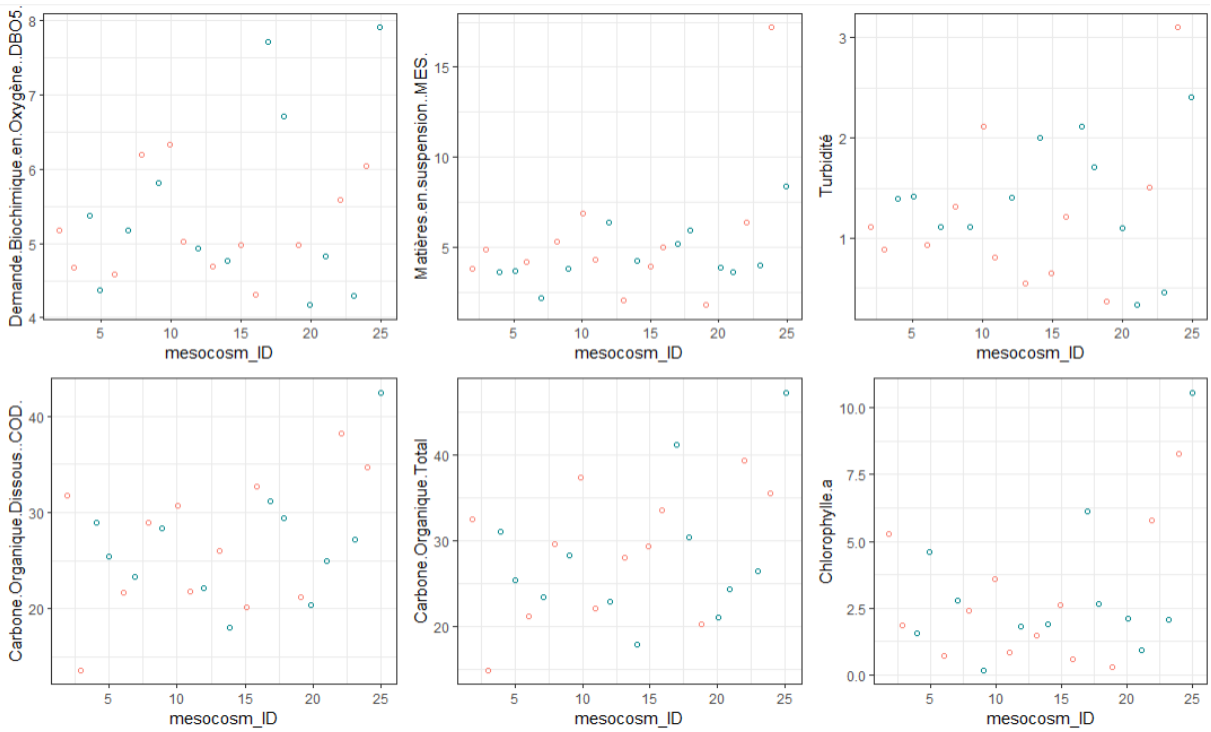



**Fig. S11:** Physical-chemical variables (les lister ici) in each mesocosm at the beginning of the experiment (03/23/2021). Blue and red colours represent ambient and heated mesocosms, respectively.

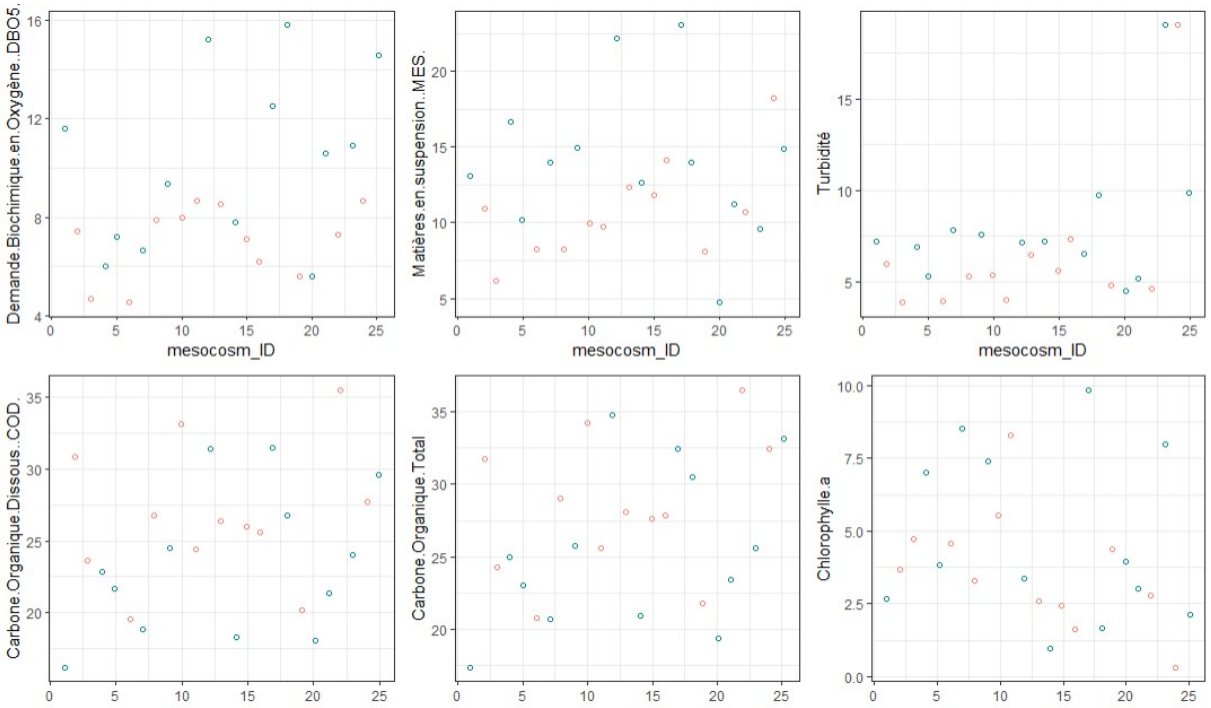



**Fig. S12:** Physical-chemical variables in each mesocosm at the end of the experiment (on 06/17/2021). Blue and red colours represent ambient and heated mesocosms, respectively.

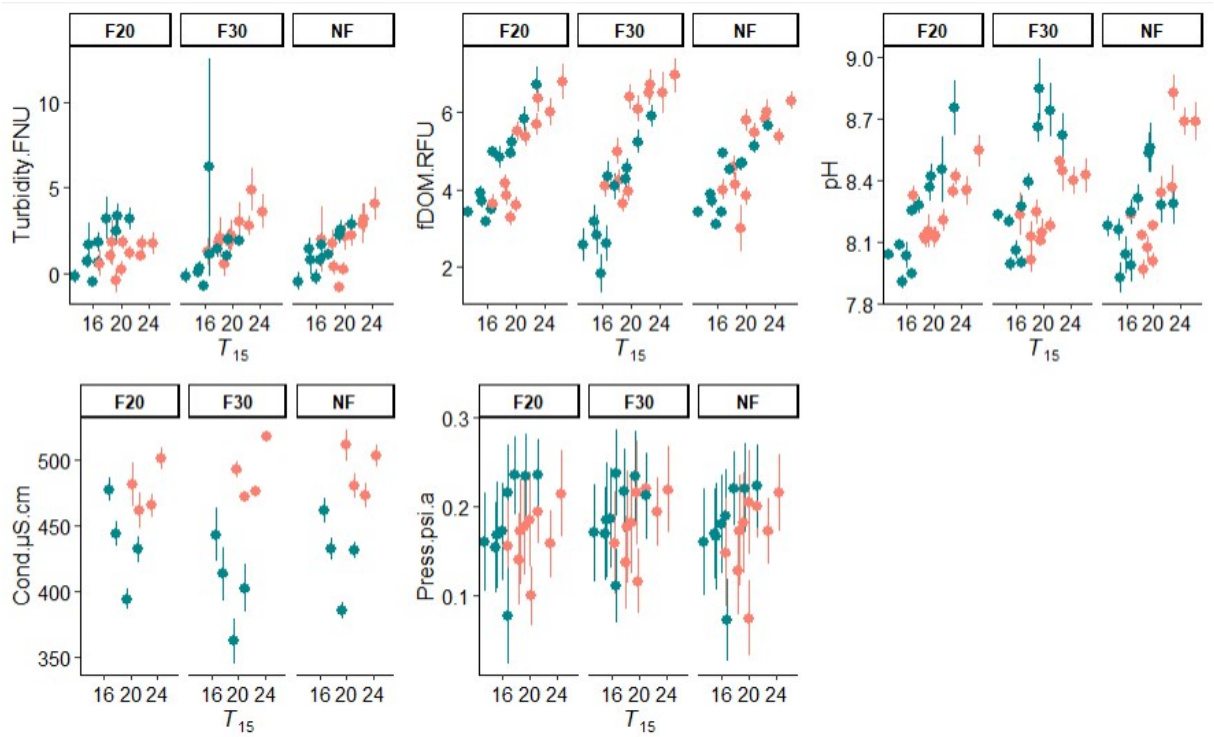



***Fig. S13:*** *Physical-chemical variables (turbidity (FNU), fDOM (RFU), pH, conductivity (µs.cm) and pressure (psi.a) during the experiment according to* $T_{15}$*. Blue and red colours represent ambient and heated mesocosms, respectively.*

# General Discussion

Qu'est-ce qu'un scientifique après tout?
C'est un homme curieux qui regarde à travers un trou de serrure, le trou de serrure de la nature, essayant de savoir ce qui se passe.

**Jacques-Yves Cousteau**
Océanographe, Scientifique (1910 - 1997)

Improving our understanding of temperature effects at different organizational levels is essential to explain patterns of biodiversity at multiple scales and to predict their evolution in the context of global warming. Freshwater ecosystems, especially slow-flowing and stagnant waters, are particularly vulnerable and threatened by climate change (Falfushynska et al., 2016; Sheldon et al., 2012; Woodward et al., 2010). Freshwater habitats support approximately 10% of all animal and plant species described and one third of vertebrate species (Dudgeon et al., 2006; Strayer and Dudgeon, 2010). Rates of freshwater biodiversity loss in recent decades are alarming and exceed those of terrestrial or marine species (Tickner et al., 2020). As an example, climate change is estimated to significantly threaten around 37% of freshwater fish species on the planet (Darwall and Freyhof, 2015).

Temperature is a key abiotic factor having a multi-scale impact from physiology to the functioning of ecosystems (Brown et al., 2004). The increase in average environmental temperature induces a considerable risk to many species, as temperatures alters physiological processes and organisms tolerance (Alfonso et al., 2021; Buckley and Huey, 2016). In this context, the thermal sensitivity and the long-term response through plasticity/or evolution of ectotherms is of particular interest to improve our understanding of the links between temperature and freshwater ecosystem trajectories. However, so far, few empirical studies have used a multi-scale perspective and accounted for the variations in responses of organisms and populations over the long-term, to predict the ecological consequences of temperature.

In this thesis, I explored mechanisms by which temperature influences the different ecological levels of organization. The main objective was to improve our understanding of the effect of a multigenerational exposure to temperature on ectothermic freshwater organisms by taking into account the different ecological scales, from the cellular metabolism, regulating the biological processes of organisms such as their thermal tolerance, to the functioning of the entire ecosystem. The physiological and ecological processes influenced by temperature were addressed using different experimental approaches ranging from intracellular physiology to ecosystem responses. First, I used a physiological and biological approach with the study of cellular respiration at high temperatures and intense heat stress. I focused on the mitochondria, the “powerhouse of cells" that plays a central role in the control of metabolism and life-history traits. Second, I studied ecosystem processes under thermal pressure and in the presence of fish from two thermally contrasted lineages, through an experiment lasting several months in mesocosms. This general discussion aims at summarizing the main results obtained and to place them into a larger perspective at multiple scales starting from the mitochondrial scale to the individual scale and then finishing with the ecosystem scale. A number of insights emerge from the results presented in this thesis, useful to complete our understanding of the impacts of environmental temperature on metabolic, biological and ecosystemic responses. To complement the results, I also present work carried out as part of this thesis but not presented in the main chapters. I also discuss the limits of this thesis and ways to take them into account for future studies.

# 1. Mitochondrial scale in global warming studies

## 1.1 Improving our understanding of the long-term mitochondrial thermal response through multigenerational exposure

The thermal sensitivity of metabolism is a key element in understanding the response of species to temperature. In ectotherms, it is already well established that many responses to short-term warming are determined by physiological processes at the metabolic level following the Arrhenius equation (i.e. rates of biological processes increase exponentially as the experimental temperature increases) (Abele et al., 2002; Arrhenius, 1896; Blanc et al., 2003; Brown et al., 2004; Harman, 1956; Hilton et al., 2010; Roussel and Voituron, 2020). However, to adjust to thermal changes after a long-term exposure, organisms can rely on physiological plasticity or genetic adaptation over generations.

Several studies mainly focusing on seasonality and/or thermally contrasting populations have mostly reported a decrease in mitochondrial activity and density and an increase in mitochondrial efficiency after long-term exposure to elevated temperature, contrasting with the short-term response of aerobic metabolism (Chung et al., 2017; Chung and Schulte, 2020, 2015; Fangue et al., 2009; Guderley and Johnston, 1996). However, the multigenerational consequences of temperature exposure on mitochondria response in laboratory remain largely unexplored (Shama et al., 2016). In the first chapter of this thesis, a multigenerational experiment (2 years) on a fish vertebrate species (*Oryzias Latipes*) provides evidence that several generations at 30°C can lead to lower mitochondrial activities (i.e. mitochondrial oxygen consumption rates (OCRs)) compared to fish maintained at 20°C, for the same assay temperature. These results must be interpreted cautiously because the mitochondrial response of warm fish exposed to 20°C could also be explained by a short-term effect of cooling on the mitochondrial scale, but the absence of a control line at 25°C does not allow to affirm or refute this hypothesis (Guderley, 2004; Schulte, 2015). Nevertheless, results in chapter 2 also indicate a trend towards lower fluxes for warm fish at their optimal temperature (i.e. 25°C assay temperature). This result complements existing studies on mitochondrial adjustments in response to temperature and can be attributed to the physiological plastic response during acclimation to thermal stress, a key strategy to cope with global warming. However, the acclimation capacity of an organism is limited (Alberto-Payet et al., 2022; Angilletta., and Dunham, 2003; Souissi et al., 2021) and the multigenerational aspect of our study suggests potential other mechanisms, resulting from adaptive phenomena.

## 1.2 Towards an adaptive compensatory phenomenon?

Environmental conditions encountered by previous generations can modify the performance of subsequent generations and the thermal response of species through genetic inherence, or epigenetic mechanisms such as adaptive plasticity (much quicker than adaptation by natural selection, see general introduction section 1.2) (Byrne et al., 2020; Chevin et al., 2010; Donelson et al., 2018; Kopp and Matuszewski, 2014; Le Roy et

al., 2017; Pörtner et al., 2006; Salinas et al., 2013; Yampolsky et al., 2014). These mechanisms complicate the observed response obtained after several generations under thermal exposure.

In my thesis, I also observed similarly mitochondrial oxygen consumption rate between cold and warm fish when their mitochondria were assessed at their (1) rearing temperature (chapter 1) and at their (2) mean CTmax temperature (Chapter 2), suggesting a convergence towards a similar mitochondrial respiration. This absence of difference could be explain by a compensation phenomenon and may suggest an optimal mitochondrial respiratory flux (as the fish did not present a decrease in respiratory efficiency (RCR) in our study) when organisms have time to acclimatise or adapt to local conditions. At the whole organism level, a previous study on medaka fish reared under the same conditions at the Aix-en-Provence INRAe laboratory (Alberto-Payet et al., 2022) showed that nine years of experimental warming did not influence the thermal sensitivity of metabolic rate which also tend towards a phenomenon of compensation between the thermal responses of warm and cold fish after several generations, contrasted with other studies (Pilakouta et al., 2020). Following several studies (Baumann and Conover, 2011; Fryxell et al., 2020), a phenomenon of counter-gradient over a long–term exposure (population thermally contrasted) to temperature has also been mentioned, modifying the life history strategies, physiological traits and behaviour of organisms. As a result, organisms from high latitudes/low temperatures would exhibit higher fecundity, shorter lifespans and faster development, growth and metabolic rates (unlike short term plastic responses). A recent study aimed at observing growth patterns and TSR strategies on ectothermic organisms from thermally contrasting populations revealed that populations of warmer origin expressed a lower juvenile growth rate and an increased reproductive effort at small size (Fryxell et al., 2020). These results are consistent with an adaptive basis of the temperature-size rule, and suggest that temperature itself may drive the evolution of counter-gradient variation counteracting plasticity and promotes similarity across environments. (Baumann and Conover, 2011; Conover and Schultz, 1997; Schultz et al., 1996). In addition, Loisel, Isla, and Daufresne (2019) (i.e. at the Aix-en-Provence INRAe laboratory) also demonstrated a reduction in plasticity of body size and reproduction in the medaka fish in response to temperature exposure over generations (i.e. 20°C and 30°C) suggesting that TSR responses could be attenuate between warm and cold fish after several generations by adaptive mechanisms (Loisel et al., 2019). Finally, the Atlantic killifish (*Fundulus heteroclitus*) was used to explore the role of mitochondrial properties as a mechanism underlying counter-gradient variation in a study by Chung et al in 2018 (Chung et al., 2018). Results of this study showed that the northern subspecies had faster development, faster growth, higher routine metabolic rate and higher activity than the southern subspecies when compared at a given temperature. The northern subspecies also has greater mitochondrial respiratory capacity in the liver tissue associated with increased activity of multiple electron transport complexes. There are also differences in the lipid composition of liver mitochondrial membranes. These data suggest that variation in mitochondrial properties could, at least in part, be responsible for variation in these counter-gradient strategies.

Observed modulation of the thermal response after several years exposure to temperature, makes us suggest that long-term exposure should also be taken into account to predict the ecological impacts of global warming. Several studies are using the metabolic theory of ecology based on Arrhenius law relationship between temperature and metabolic increases to predict thermal responses at different ecological scale but do not account directly for plastic or evolutionary responses to warming (Brown et al., 2004; Schulte et al., 2011; Yvon-Durocher et al., 2010a).

## 1.3 Are these long-term mitochondrial thermal responses maintained throughout the life cycle of the organism?

The acceleration of metabolism and oxygen demand induced by temperature can increase the formation of damaging chemical species at the cellular level (i.e. mainly Reactive Oxygen Species (ROS) production) (Abele et al., 2002; Clotfelter et al., 2013; Lushchak, 2011; Metcalfe and Alonso-Alvarez, 2010; Roussel and Voituron, 2020; Salin et al., 2019). Harman was one of the first (1956) to postulate that the use of oxygen at cellular level during mitochondrial respiration causes tissue damage through the production of free radicals (Harman, 1956). The long–term modulation of the mitochondrial response at elevated temperature observed in the first chapter could counteract the production of ROS in a warmer environment (see general introduction, section 2.2). Although ROS production was not measured directly in chapters 1 and 2 experiments, we know that ROS production plays a key role in life history traits that can potentially reduce the performance of organisms in terms of maintenance, growth, reproduction, longevity, and contribute to cellular senescence (i.e. loss of biological functions) (Blount et al., 2016; Costantini et al., 2010; Dowling and Simmons, 2009; Hill, 2015; Monaghan et al., 2009; Rubner, 1908; Salin et al., 2015; Speakman and Garratt, 2014).

When I performed the experiment presented in Chapter 1, I also wanted to determine whether the same response patterns (as described in chapter 1) are obtained in senescent organisms. I therefore carried out the exact same experimental protocol as in the first chapter but using senescent fish from the same initial fish pool (fish from this pool were maintained in the lab until they get older than 300 days which is well above their reproductive optimum) (see Appendix 1 for methods, text. S1).

Senescent fish presented a significant interaction between ancestral temperature and assay temperature for both OXPHOS (Chisq =9.6, df = 1, $p < 0.005$) and LEAK states (Chisq =6.5, df=1, $p < 0.05$) (Fig. 19). Then, as for adults fish, assay temperature (Chisq = 0.52, df = 1, p = 0.47) and ancestral temperature (Chisq =0.34, df = 1, p = 0.55) had no influence on RCR values (Fig. 19). It would therefore appear that mitochondrial responses are conserved throughout the life of the individual, in particular a respiratory underactivity in terms of oxygen consumption for warm fish at each assay temperature. This strategy would limit the cellular damage and early mortality associated with thermal warming. Nevertheless, warm exposed fish flux are relatively low that could also suggest mitochondrial dysfunction (interaction between the two experimental conditions and the age of the fish were significant, Chisq=5.5490, df=1, p= 0.02*). Indeed, other studies such as Ferguson et al., 2005 have also identified specific alterations in mitochondrial

respiratory functions during the aging process by measuring respiration rates and activities of electron transport chain complexes at different ages in the fruit fly, Drosophila melanogaster. They showed that State 3 respiration rates (stimulated by ADP), RCR as well as cytochrome c oxidase (complex IV) activity decreased significantly with age. Age-related changes in mitochondria are mainly associated with decline in mitochondrial function through a decrease in mitochondrial DNA volume, due to accumulation of mutations and oxidative damage induced by ROS (Chistiakov et al., 2014). These results support the hypothesis that impaired mitochondrial respiration could be a causal factor in the aging process.

However, is the lower oxygen fluxes of old fish really a dysfunction? We observed no more marked decrease in mitochondrial performance (i.e. oxygen consumption) between senescent and adult, for warm and cold fish at OXPHOS and LEAK state (Table. S2). These results could arise from a senescence process response only. A potential explanation of the particularly low flows could be that old fish raised in warm conditions for several generations no longer have to invest in reproduction. Therefore, their metabolism does not need to be as high as younger ones decreasing energy needs and energy intake in healthy older compared to younger adults (Giezenaar et al., 2016). This results suggest that old organisms have a lower metabolism due to the slowdown of their life cycle.

It would be interesting in future studies to measure the quantities of ROS in organisms in order to confirm or refute the accumulation of ROS with temperature in fish after several generations. It has been proposed that uncoupling of the oxidative phosphorylation activities could reduce free radical production, confirming the plastic aspect of mitochondria (Blanc et al., 2003; Brand, 2000; Nègre-Salvayre et al., 1997; Ricquier, 2005; Speakman and Garratt, 2014). This uncoupling can take place via particular proteins (called "UCP" for "UnCoupling Protein") or via a leakage of protons ($H^+$). However, this uncoupling is also responsible for heat production, particularly useful in mammals and birds but relatively poorly studied in ectothermic organisms (Seebacher et al., 2010) even if some proteins have been discovered in ectotherms, notably UCP2, which appears to be expressed differently depending on the acclimatisation temperature (Mark et al., 2006; Stuart et al., 1999). To deepen the understanding of our results, it would also be interesting to quantify UCPs (Gaudry and Jastroch, 2019; Nègre-Salvayre et al., 1997). According to some studies, thermo-dependent senescence factor would represent a key parameter explaining TSR patterns. Indeed, some studies have highlighted that an increase in temperature results in larger or smaller adults depending on the shape of the senescence function, emitting the hypothesis of a negative trade-off between mortality rate and growth rate; *the faster they grow, the sooner they die* (e.g. Dembski et al., 2006; Flouris and Piantoni, 2015; Keil et al., 2015; Kindlmann et al., 2001; Sibly & Calow, 1986; Stearns, 1992).

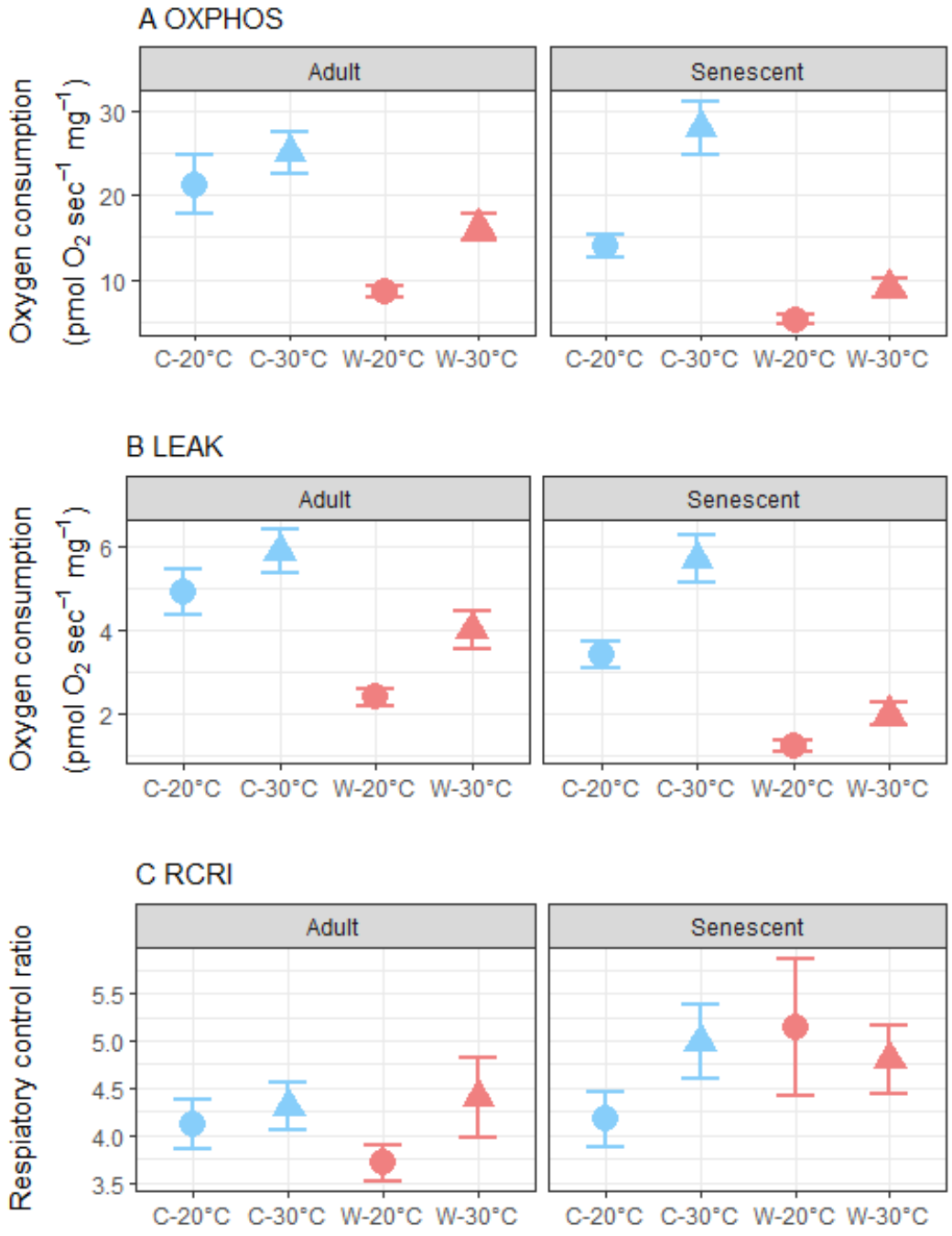



***Figure 19:*** Mean (± SE) of A. OXPHOS and B. LEAK oxygen consumption and for and C. RCR according to the ancestral temperature for juveniles and senescents WARM fish (in red) and COLD fish (in blue) and for assay temperature (dots; 20°C, triangles; 30°C).

# 2. Relationship between thermal tolerance of whole organism and mitochondrial functions

## 2.1. Multigenerational exposure modify upper thermal limits

Some recent studies have put forward the hypothesis that mitochondria are involved in thermal regulation delimiting the thermal range that ectotherms can tolerate. This is mainly due to their extreme sensitivity to temperature (e.g. catalytic capacities) dictating, at least in part, the temperature dependence of many biological functions (Brown et al., 2004; Gillooly, 2001; Guderley, 2004; Iftikar and Hickey, 2013; Le Lann et al., 2011; Pichaud et al., 2011; Savage et al., 2004).

In the first chapter, I highlighted that several generations exposure to non-stressful temperatures under controlled conditions can modify the mitochondrial response of organisms and this response appear to be conserved throughout organism's life. However, my studies were carried out at temperatures within the range tolerated by medakas. In its native range, the medaka generally inhabits small waterbodies such as ponds and rice fields where the water temperature falls to 0°C during the winter and can rise to almost 40 °C in summer, with marked temperature fluctuations (Hirshfield 1980; Kirchen & West 1976; Shima & Mitani, 2004). When temperature move away from the thermal ranges tolerated by organisms, their activity and metabolism decrease up to the thermal limits, which can lead to a total loss of function and death (i.e. the relationship between increased temperature and increased metabolism no longer applies). Before death, a loss of neuromuscular coordination is commonly observed, resulting from a breakdown of the neuromuscular junction (Cossins and Bowler, 1987; Hochachka and Somero, 2002; Pörtner et al., 2006).

Despite the well-established thermal sensitivity of metabolism, few studies have attempted to establish the link between mitochondrial failure and whole-organism thermal limits (Chung and Schulte, 2020; Ekström et al., 2016; Harada et al., 2019; Hraoui et al., 2020). Given that maximum critical temperature (CTmax) can be defined as the temperature at which loss of neuromuscular function occurs the assessment of brain mitochondrial function seemed to be particularly relevant in a context of thermal tolerance. In the second chapter, I investigated (i) the long-term (multigenerational) effects of elevated temperature on whole-organism CTmax in medaka fish (*Orizyas latipes*), and (ii) the link between whole-organism CTmax and mitochondrial dysfunction at elevated critical temperature in brain. Several studies suggested that CTmax varies with the exposure duration and may thus be enhanced after a period of acclimation to elevated temperature (Angilletta, 2001; Bilyk and DeVries, 2011; Morley et al., 2019). In my study, 5 years of multigenerational thermal acclimatisation to elevated temperature significantly increased (by 12.6%) the CTmax of the whole organism. This result is important, as to our knowledge no study has yet examined the maximum thermal tolerances of the medaka fish (*Oryzias Latipes)*. Results are in line with previous studies showing that adaptive evolution or phenotypic plasticity can modify the upper thermal

limits after long term exposure (Angilletta ., 2009; Angilletta and Dunham, 2003; Huey and Kingsolver, 1989).

Our results must however be nuanced because the CTmax method used (~ 1.5°C every 10 minutes) (Hutchison 1961, 1976), (slower than conventional methods: generally 0.3°C to 1°C/min, Lutterschmidt and Hutchison, 1997; Morash, 2024; Cowles et Bogert, 1944) probably overestimates or underestimates the true environmental tolerance of a fish (Beitinger et al., 2000; Desforges et al., 2023; Rezende et al., 2014; Gunter 1941). Thus, it would be interesting in our case to explore the link (correlation) between CTmax and the thermal tolerance/performance for life history traits such as growth or fecundity. Indeed, the mechanisms behind the decline in performance may differ depending on the considered trait (Clark et al., 2013). Clark et al. (2013) discussed the hypothesis that critical performances such as growth, locomotion, and reproduction are causally related to aerobic scope. Recent studies indicate that the brain and heart—two essential organs—are likely to be the last to lose their oxygen supply, resulting in trait dysfunction (loss of neural response to visual stimuli, locomotor-related neural activity) and to reach their upper thermal limit CTmax (Andreassen et al., 2022; Farrell, 2016). However, we still need future studies to better understand the relationship between cerebral dysfunction and CTmax.

## 2.2 Link between whole-organism CTmax and brain mitochondrial performance

It was suggested that temperature-induced dysfunction of the neuronal system could be at the origin of the whole organism thermal limits (Ern et al., 2015; Somero and DeVries, 1967). Thus, recent studies have suggested a link between brain mitochondrial dysfunction and whole-body thermal tolerance (Chung et al., 2017; Chung and Schulte, 2020). However, these very recent and few observations require further investigations. Results from Chapter 2 suggest a link between whole-organism CTmax and brain mitochondrial performance although brain mitochondria continue to function above the population-average CTmax (as suggested in other studies: Chung and Schulte, 2020; Pörtner, 2002; Somero, 2002; Somero et al., 1996). These results constitute new evidence supporting the hypothesis of cerebral dysfunction at upper thermal limits, but must be further explored in order to better understand the underlying mechanisms.

I also observed a high relative contribution of complex II substrate for warm fish at their CTmax compared to cold fish. Therefore, warm exposed fish can exhibit mitochondrial flexibility in complex II at temperatures reaching the thermal limit of the organism. The explanation of our results could be linked to the thermal tolerance of the first complex of the respiratory chain (Complex I). In the 1990s, initial studies questioned the thermal tolerance of respiratory chain complexes to explain the mitochondrial response to temperature. Thus El-Wadawi and Bowler (1996) studied the muscle of blow flies and observed that complex I (CI) (first complex of the respiratory chain) could be the main site of mitochondrial thermal damage following exposure to a sublethal high temperature (El-Wadawi and Bowler, 1996). Subsequently, other studies showed that complex I would

be the most affected complex by ROS and cellular aging within the respiratory chain (Genova et al., 2004). Recent studies have once again looked at the thermal tolerance of different mitochondrial complexes and their role in the long-term thermal response of ectotherms (Blier et al., 2014; Chung and Schulte, 2020). Today, several studies highlight that mitochondrial heat tolerance was correlated with the failure of mitochondrial oxygen consumption for complex I, suggesting that the response of complex I may determine the mitochondrial response during a critical exposure to heat (Jørgensen et al., 2021; Menail et al., 2022). Thus, the study of the different complexes would make it possible to explain the thermal limits of the mitochondria, or even those of the entire organism (see above), which is of capital importance in the context of current global warming.

Although the difference between males and females has not been addressed in this thesis (nevertheless taken into account in statistical analyses as a fixed or random effect), during the CTmax experiment, I observed differences between males and females regarding their behaviour at the end-point (Appendix 2, Fig. S2). Females stopped swimming but almost did not lose their equilibrium during the CTmax experiment which was the most frequent response for males. These observations can be explained by their morphological differences (females are round which can allow them to maintain their equilibrium) but taking into account differences in behaviour during thermal tolerance studies may be important in future studies because inter-sex differences at the metabolic level have been demonstrated in multiple taxa (not the case in my studies) (Costantini et al. 2009; Grilo et al. 2018; Loisel, 2019).

## 2.3 Physiological thermal tolerance can modulate population response

Some studies support the hypothesis that the temperature range of thermal performance curves can define the biogeographic range of populations, linking physiological and biological responses to the second universal response of species to global warming (i.e. the change in species distribution see section. 1.2) (Parmesan and Yohe, 2003; Angert et al. 2011; Dell et al. 2011; Huey and Kingsolver., 2011; Payne et al., 2021; Portner et al., 2021). In this sense Payne et al (2021) attempted to determine whether there are similar models of thermal tolerance of fish at the levels of individual physiology (i.e. CTmax) and the abundance of natural populations (Fig. 20)(Payne et al., 2021). They compared data on fish heating tolerances from laboratory experiments with abundance patterns in wild fish populations. They found that heating tolerances of individuals and population abundance decrease with increasing temperature at the same rate. These congruent relationships imply a tight coupling between the physiological and demographic processes that underlie macro-ecological models. Thus, the physiological responses at the mitochondrial scale of fish thermally contrasted over several generations could explain the patterns of abundance of wild populations in a changing global world. Chapter 2 of the thesis provides additional evidence that long-term exposure can increase the upper maximum tolerance of organisms. Thus, it can be expected that the difference between physiological tolerance and environmental temperature (i.e., thermal safety margin), a common measure for assessing the thermal vulnerability of organisms (Sunday et al.,

2014) can also be modulated after prolonged exposure to temperature. We can therefore suggest that the decrease in abundance should be less marked after long periods of exposure to high temperatures.

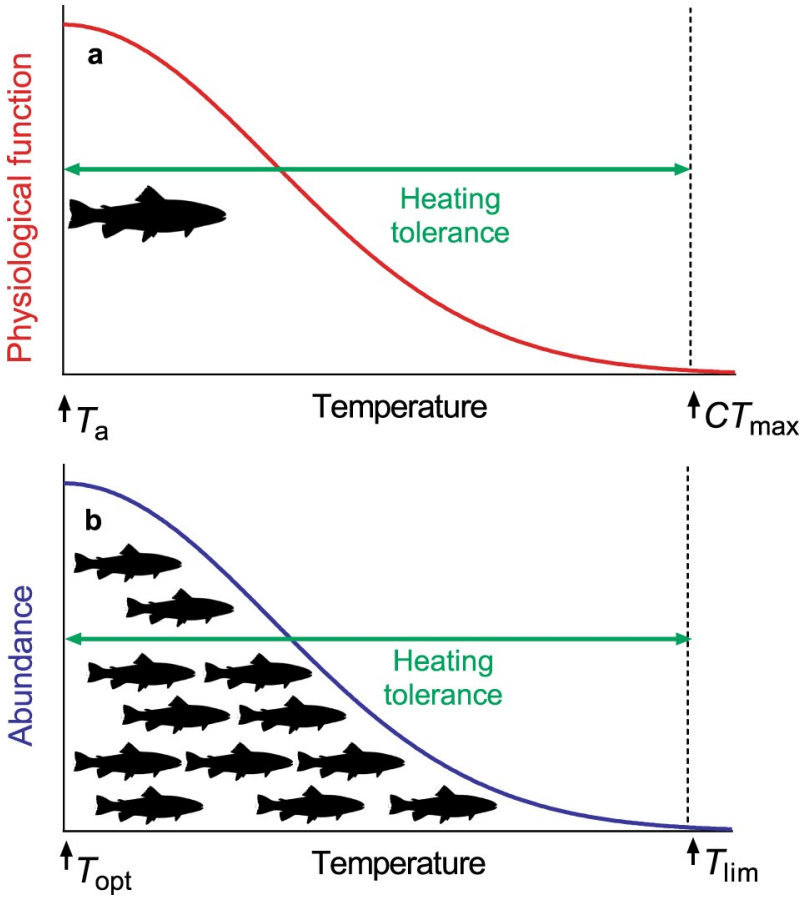



***Figure 20** : Conceptual illustration of fish heating tolerances at different biological scales. (a) At the individual level, Ta represents the temperature an individual is acclimated to in a laboratory experiment, and CTmax is the temperature coinciding with death or loss of critical functions. (b) At the population level, $T_{opt}$ is the temperature of highest abundance in the wild, and $T_{lim}$ is the 95$^{th}$ percentile of maximum temperatures encountered by that species in its natural range. (From: Payne et al., 2021).*

In addition to the direct effects of temperature on thermal tolerance, the decrease in individual body size under thermal pressure related by Daufresne et al. (2009) could modify the dispersal capacity generally positively correlated with body size (Cloyed et al., 2021; Daufresne et al., 2009; Jenkins et al., 2007; Sentis et al. Submitted). Finally, at the population scale, Daufresne et al. (2009) also proposed a higher proportion of juveniles relative to adults in the age structure of population under thermal pressure. Early life stages including juvenile organisms may exhibit different thermal tolerances compared to adult stages (Fig. 21). To complete our results it would therefore be interesting to determine the mitochondrial response and thermal tolerances of the first life stages under short and long-term temperature exposure (over several generations) for medakas fish raised at 20°C, 25°C and 30°C in order to extend our understanding of the relationship between temperature and the life cycle of organisms.

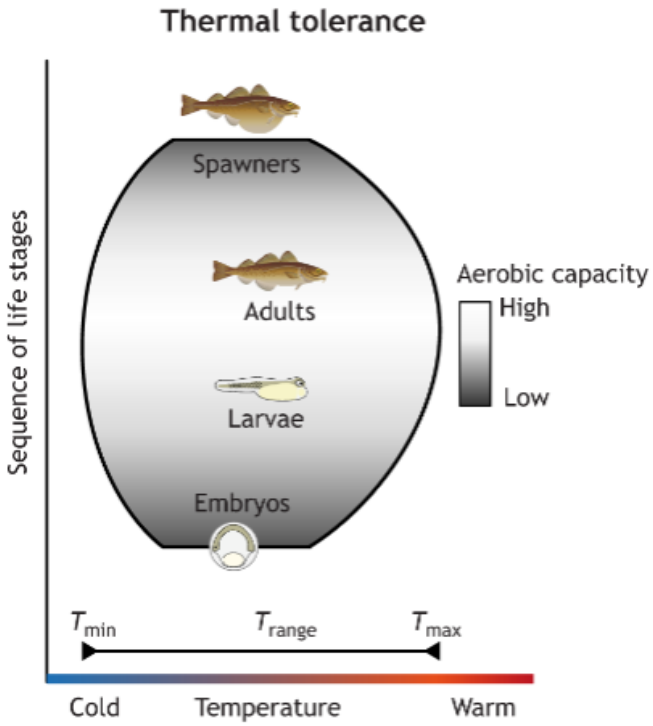



***Figure 21:*** *During ontogeny, the habitat selected by a species depends on the thermal range ($T_{range}$) of the respective life stage (where $T_{min}$ and $T_{max}$ are minimum and maximum temperature, respectively). This relates to relative aerobic scope across life stages (From Portner et al., 2021).*

# 3. Complex temperature effects on ecosystem

## 3.1 Limits of the MTE at the ecosystem scale

In this thesis, I also attempted to link impacts of an increase in temperature on the metabolic scale (chapter 1 and 2) and the size modifications induced by temperature, on ecosystem functions (chapter 3). Metabolism is linked to body size and species-specific functional traits and controls species feeding, respiration and excretion rates (Brown et al., 2004; Daufresne et al., 2009; Gillooly, 2001; Savage et al., 2004). The Metabolic theory of ecology (MTE) predicts how metabolism affect ecological processes at individuals, populations and ecosystems levels.

However, the MTE is based on numerous assumptions and on a limiting theoretical approach that requires more experimental/empirical researches. Indeed, the MTE is mainly based on the relationship between metabolism and temperature through the Arrhenius law which establishes the dependence of the speed of a chemical reaction on temperature. Several authors have thus taken into account the MTE and the relationship between temperature and aerobic metabolism to make predictions at the community and/or ecosystem scale (Allen et al., 2005; Tait and Schiel, 2013; Yvon-Durocher et al., 2010a). For example, the work of Allen, Gillooly and Brown in 2005 takes into account the thermal dependence of the mitochondrial respiratory chain and these different

complexes in their models in order to link the global carbon cycle with the metabolism of individuals. They thus determine that C fluxes from respiratory complexes increase predictably with temperature due to the kinetic effects of temperature on photosynthesis and respiration rates. The model predicts a 16-fold increase in rates controlled by respiration (e.g. decomposition, turnover of labile soil C and microbial biomass) over the temperature range 0–30°C due to the temperature dependence of ATP synthesis in respiratory complexes at mitochondrial level. In chapter 3, results at the ecosystem scale (i.e. mesocosms experiment) mainly showed an effect of temperature (and seasonality) on primary producers and gas solubility. Our results on green-house gas highlight a decreasing greenhouses gas solubility with warming potentially increasing emission of $CH_4$ and $CO_2$ suggesting that global warming may have a large impact on the relative contributions of total greenhouse gas emissions from aquatic ecosystems. According to a previous study, who tested theoretical models derived from the MTE, a warming of 4°C decreased $CO_2$ sequestration by 13%, increased the fraction of primary production efflux in the form of methane by 20% and the fraction of ecosystem respiration methane efflux by 9% (Yvon-Durocher et al., 2010a). Therefore, the relationship between photosynthesis, respiration and temperature is well established, but is only valid within the range of temperatures tolerated by organisms (Allen et al., 2005; Brylinsky and Mann, 1973; Davison, 1991; Lee et al., 2007).

The Arrhenius kinetics relationship between temperature and metabolism used by the MTE only apply over a narrow range of temperatures for ectotherms, complicating attempts to identify general patterns of temperature dependence (Knies and Kingsolver, 2010). In a community, there is variation in thermal performance with different species optimal temperatures. For some organisms, thermal ranges can be very wide, as for unicellular organisms (Storch et al., 2014) and some other species will decline or even disappear from geographical areas when environmental conditions change drastically (Mouthon and Daufresne, 2006). An increase (or decrease) in temperature can thus modify the composition of species in the community. In this sense, in my study, the presence of fish from 20°C in heated mesocosms, close to their thermal optimum, can modify the relationship between metabolism and elevated temperature, modulating predation pressure in heated mesocosms. Indeed, oxygen consumption, or the rate of ingestion of prey by a predator, does not follow a linear relationship with temperature and decreases at temperatures above the optimal temperature (Englund et al., 2011). This result illustrates the complexity of determining the effects of temperature at the ecosystem level.

## 3.2 Complex interactions

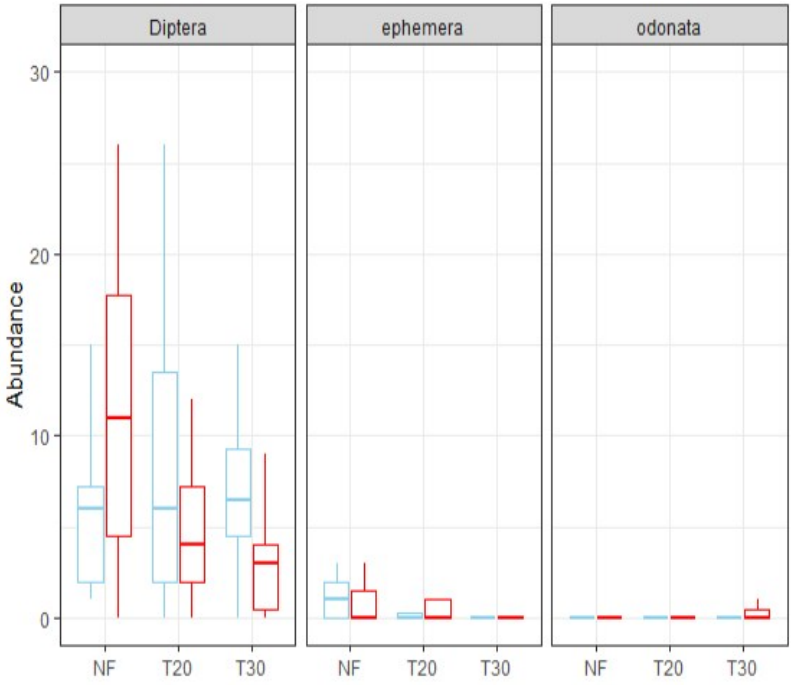
In some cases, the expected relationship between temperature and biological rate is not observed at all as antagonist thermal effects can cancel each other. For instance, the lack of effect on the rate of decomposition in our mesocosms experiment could be explained by antagonists warming effects that accelerates microbial decomposition of litter but produces an equivalent decrease in the density of macro-organisms under thermal

pressure, as reported in a previous study (Allen et al., 2002). As a result, the rate of decomposition can remain unchanged by a compensation phenomenon. These compensatory phenomena of temperature effects on ecosystems are still poorly taken into account in studies and deserve to be studied empirically in order to understand the theoretical response trajectories of temperature on ecosystem functioning.

It is also necessary in this type of ecosystem studies to take into account the presence of predators and the top down effects induce. The presence of fish in mesocosms increased the concentration of cyanobacteria and diatoms in heated mesocosms and as a function of water temperature in the Chapter 3. In fact, as explained in chapter 3, the fish predation of zooplankton and invertebrates can lead to a lack of pressure on phytoplankton increasing primary production. Although my results suggest that the presence and differences in fish size have smaller effects on ecosystem functions than direct temperature effects, our study demonstrates the importance of trophic relationships and complex antagonistic effects on ecosystem response to temperature. To better understand the food chain structure and to highlight variations within the different mesocosms, I firstly tried to reconstruct food webs from allometric relationships (i.e., relationships between the body size of predators and those of their prey) by using different metrics that characterise a food web, like the number of trophic levels, the length of the food chain, the trophic position and the connectance (Bramon Mora et al., 2018; Delmas et al., 2019; Laigle et al., 2018; Pellissier et al., 2018) but there was no significant variation in the binary structure of food webs in mesocosms.

Finally, freshwater aquatic environments also support terrestrial biodiversity. Some freshwater invertebrates present an emergence phenomenon allowing them to move from an aquatic environment to a terrestrial and therefore represents a transfer of biomass between these two environments (Jackson and Fisher, 1986; Paetzold and Tockner, 2005). Increasing temperatures modifies the phenology of organisms, accelerating their metabolism, their sexual maturity, and the emergence of aquatic invertebrates by several weeks (Hogg and Williams, 1996). In our mesocosms experiment, we also tried to determine the impact of treatments on macroinvertebrates emergences (Appendix 3 for methods). In our study, we found that emerging Ephemera had smaller sizes in heated mesocosms ($P<0.001^{***}$) (Fig. 22). In line with our results, previous studies have shown that the average body size of insects decreased under warming conditions, even if the degree of variation in organism size with temperature varied within and between taxa (Sardiña et al., 2017). We also found a significant interaction between artificial warming and fish conditions for diptera ($P<0.001^{***}$), leading to greater sizes and higher abundances in heated mesocosms without fish, and lower abundance in warmed mesocosms with fish (Fig. 22). The decreasing emergence in warmed mesocosms in presence of fish could be explained by predation pressure as in other studies (Feuchtmayr et al., 2007). However, this results are complex and need to be further explored (Hogg & Williams, 1996).

A

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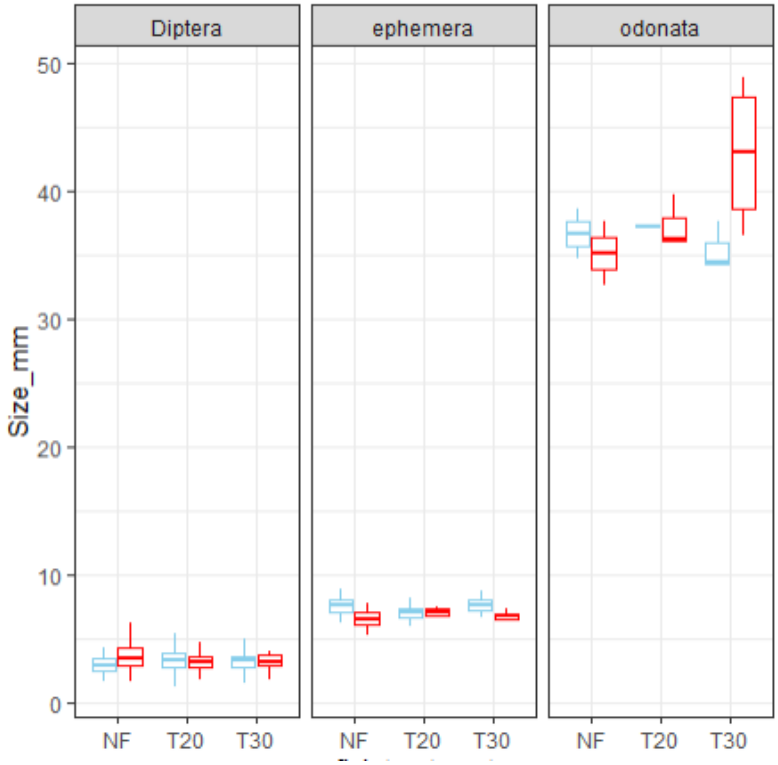


***Figure 22**: (A) Abundance and (B) Size in mm according to fish conditions (without fish NF, Fish at 20°C T20 and Fish at 30°C T30). Heated mesocosms are in red and non-heated mesocoms are in blue.*

To conclude this section, this mesocosms study demonstrates the key importance of empirical studies to better understand the effects of temperature at the ecosystem scale and indicates that the use of MTE alone to predict the responses of ecosystems to warming presents significant limits by, for instance, by not taking into account the maximum thermal tolerances of organisms, the predation phenomena and potential compensatory effects at the metabolic or ecological scale.

# 4. Limits and perspectives of the thesis

In this section I discuss limitations and constraints of this thesis and I also propose some research perspectives that could guide future studies.

## 4.1. A better discrimination of temperature exposure effects, for a better understanding of the thermal response of ectothermic organisms

As mentioned by one of the reviewers of the article from chapter 1, a limitation of our study lies in the absence of control fish lineage at the Medaka thermal optimum (i.e. 25°C). The initial objective when the breeding was set up (prior to this thesis) was to evaluate the multi-generational response of life history traits and physiology (oxidative balance) on thermally contrasted populations (Hemmer-brepton 2014; Loisel, 2019). However, this choice may have complicated the interpretation of our results and the understanding of the mitochondrial response. A better comparison would be to have the ancestral population raised at 25°C, to look at both long-term warming and cooling effects. Some effect(s) of long-term cooling may have altered mitochondria, but these cannot be reported without 25°C lineage control. As a result, I mainly discuss in terms of fish relative differences and not in terms of pure warming or cooling effects in my studies. The presence of lines reared for several generations at 25°C would allow a better discrimination of the effects of long-term warming, would add an extra dimension to our study, and could be considered for future multigenerational experiments. Moreover, in my studies only the mitochondria have been exposed to assay temperature and it may be interesting to also expose fish to acute temperature exposure in order to discriminate the effects of a long and short-term exposure.

## 4.2. Distinguish plastic and genetic modifications to better understand responses of organisms and populations to warming

The multigenerational aspect adds some complexity to the interpretation of results. Although it has been established that mitochondrial responses and TSR patterns are mainly plastic, it is difficult to determine if my results are driven by plasticity, by genetic

evolution, or by other processes over generations. The multigenerational aspect may make it possible selection phenomena, but these cannot be dissociated from the effects of plasticity in this thesis. Common-garden experiments and monitoring of the response of each generation as well as molecular DNA analyses could help to differentiate plastic effects from genetic responses and refine our results. A previous study on Medaka fish reared at INRAe Aix-en-Provence, revealed changes in TSR patterns as a function of ancestral and developmental temperatures. Developmental temperature mainly influenced age at maturity and asymptotic size, while size at maturity was determined more by the dissimilarity between developmental and ancestral temperatures (Loisel, 2019). This study also highlighting a loss of plasticity in temperature-size-reproduction patterns for fish reared in warm conditions over six generations. Finally, it would also be interesting to discriminate maternal effects from paternal effects, because mitochondria are transmitted by mothers and variation in the metabolic rate can be strongly influenced by maternal effects, which can have long-term consequences for life history strategies and fitness-related traits such as growth and maturation (Shama and Wegner, 2014).

## 4.3 Mesocosms: a complex interpretation of multiple factors

At mesocosms scale, interpreting results proved to be more difficult than expected due to a number of factors that had not been taken into account at the outset, such as the presence and accumulation of pollen and the significant colonisation of mesocosms by carnivorous aquatic Notonectidae. Although the number of fish introduced was chosen according to the bibliography (Diaz Pauli et al., 2020; Kratina et al., 2012), such colonisation may have induced significant predation, potentially overcoming the one from fish, and thus hindering our ability to interpret our results with accuracy.

A new study using male and female juvenile medaka fish in mesocosms has been carried out in order to study heat waves response of organisms and several methodological adjustments have been made thanks to this first experience in mesocosm at INRAE laboratory. Protective nets were installed on the mesocosms in order to prevent colonization by flying insects and oxygen probes measuring continuously were installed in order to discriminate respiration and net primary production.

## 4.4 Other stress factors

It would be interesting to study the relationship between biological scales thermal response and oxygen levels after multigenerational exposure because, oxygen availability appears to play a role (i) in the mitochondrial response, (ii) in the thermal tolerance response and (iii) is one of the hypotheses to explain the TSR (Hoefnagel and Verberk, 2015). This is of concern in aquatic environments, where warming reduces the solubility of oxygen, leading to a mismatch between demand in response to increased metabolic activity and the oxygen available (Atkinson and Sibly, 1996; Pörtner, 2002; Truesdale et al., 1955; Verberk et al., 2011). Furthermore, some hypotheses have been put forward to

explain metabolic limitations, including one in particular which postulates that animals are characterized by an oxygen capacity-limited thermal tolerance (OCLTT) hypothesis (Frederich and Pörtner, 2000; Pörtner, 2001, 2002; Pörtner et al., 2006). This hypothesis suggests that at cold and warm temperatures, aerobic metabolism would be impaired either by a mismatch between mitochondrial oxygen requirements and oxygen supply or by mitochondrial catalytic limitation. Finally, the resource, also constitutes a major factor that influences most life history traits such as growth, reproduction or survival and could be taken into account in future studies (Bazin et al., 2023; Elser et al., 2003;Sterner and Elser 2017).

# 5. Conclusion

Overall, this thesis contributes to improve the knowledge on how temperature modifies the physiology and thermal tolerance of a freshwater fish and provides valuable information on how the functioning of aquatic ecosystems can be affected by global warming. Our results (i) improve our understanding of the effects of a multigenerational temperature exposure on the mitochondrial metabolism of the studied species and its maximum thermal tolerances, (ii) provide evidence that multigenerational exposure to a high temperature can modulate mitochondrial activity over the entire lifespan and (iii) suggests that the (theoretical) relationship between temperature and metabolism has limits at cellular and ecosystem levels and requires additional experimental/empirical tests. The various studies carried out during this thesis and the combination of approaches allow us to conclude that exposure to a non-stressful temperature for several generations has consequences on physiology and ecological traits, in particular on the thermal tolerance of the whole organism. This thesis also confirms the interest of integrative ecophysiology studies. Following the example of the metabolic theory of ecology, which predicts how metabolism controls ecological processes on the scale of individuals, populations and ecosystems, it is necessary to take better account of the different scales of living organisms in quantitative terms, but also through empirical approaches that can give different or antagonistic response compared to predictive theoretical studies. Finally, as we have reported, acclimatisation to temperature over several generations can modify the predicted responses of thermal impacts. Future studies are needed to complete this thesis work in order to better predict the adaptive potential of fish to global warming.

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# Supplementary Material

# Appendix 1: Comparison of oxygen fluxes between cold and warm senescent fish

## ***Text S1****: Materials & Methods:*

For this complementary experiment, we use the same design as in chapter 1, only the age of the fish used was different (senescent fish). In other words, we used the fish from the same generation and same lineage but they were older when we measured their mitochondrial activity. We used 28 fish from the two different thermal origin (COLD fish reared at 20°C and WARM fish reared at 30°C). Specifically, we used 15 fish maintained at 20°C for three generations ($F_3$): 8 adult females (mean age in days ± se: 509.6 ± 3.8; i.e. 10 180 degree days) and 7 adult males (507.8 ± 3.9 i.e. 10 157 degree days). We also used 13 fish maintained at 30°C for fourth generation ($F_4$): 6 adult females (378.6 ± 2.6 days; i.e. 11 340 degree days) and 7 adult males (377.6 ± 2.4 days i.e. 10 157 degree days) (Tab. S1). Although they had different ages in days, their physiological ages in degree-days were relatively close. The fish used were more than 300 days and thus no longer at their reproductive optimum.

***Tab. S1****: Thermal origin, number of fish, sex, mass, and age of the senescent fish used in the experiment*

| Thermal origin | N | sex | M.Mass | ± SE | M.age | ± SE |
|---|---|---|---|---|---|---|
|  | 8 | F | 622.88 | 33.72 | 509.62 | 3.82 |
|  | 6 | F | 878.83 | 83.42 | 378.66 | 2.58 |
|  | 7 | M | 543.57 | 22.05 | 507.86 | 3.90 |
|  | 7 | M | 553.71 | 47.00 | 377.57 | 2.44 |

Fish were euthanized by percussion of the cranial cavity and then measured (mm), weighed (mg) and dissected to sample muscle from the two flanks of each fish. We then measured oxygen consumption from homogenate skeletal white muscle for OXPHOS (state 3) and LEAK (state 4o) using two Oxygraph-2k high-resolution respirometers (*Oroboros Instruments, Innsbruck Austria*). For each state we calculated RCR (i.e. Respiratory Control Ratio) and we used linear mixed models (LMMs) to test the effects of long-term multigenerational exposure at 20°C or 30°C (i.e. ancestral temperature), assay exposure at 20 °C or 30 °C (i.e. assay temperature) and their interaction, on the mitochondria oxygen consumption (in picomol.$s^{-1}$.$mg^{-1}$).

***Tab. S2:*** *Senescent and adult fish mean flux and standard error (se) in pmol $0_2$ $s^{-1}$ $mg^{-1}$ for OXPHOS (A) and LEAK (B) states for each conditions (i.e. C for cold fish, W for warm fish, 20 for 20°C assay temperature, 30 for 30°C assay temperature).*

**A.**

| *Conditions* | *Age* | *Mean flux* | *se* |
|---|---|---|---|
| *C-20* | *Adult* | *21.26* | *3.49* |
| *C-20* | *Senescent* | *14.02* | *1.35* |
| *C-30* | *Adult* | *25.06* | *2.54* |
| *C-30* | *Senescent* | *28.02* | *3.10* |
| *W-20* | *Adult* | *8.48* | *0.65* |
| *W-20* | *Senescent* | *5.23* | *0.53* |
| *W-30* | *Adult* | *16.17* | *1.60* |
| *W-30* | *Senescent* | *9.04* | *1.13* |

***B.***

| *Conditions* | *Age* | *Mean flux* | *se* |
|---|---|---|---|
| *C-20* | *Adult* | *4.93* | *0.56* |
| *C-20* | *Senescent* | *3.43* | *0.32* |
| *C-30* | *Adult* | *5.90* | *0.51* |
| *C-30* | *Senescent* | *5.72* | *0.59* |
| *W-20* | *Adult* | *2.39* | *0.20* |
| *W-20* | *Senescent* | *1.20* | *0.14* |
| *W-30* | *Adult* | *4.01* | *0.45* |
| *W-30* | *Senescent* | *1.98* | *0.26* |

## Appendix 2: CTmax Endpoint between sexes

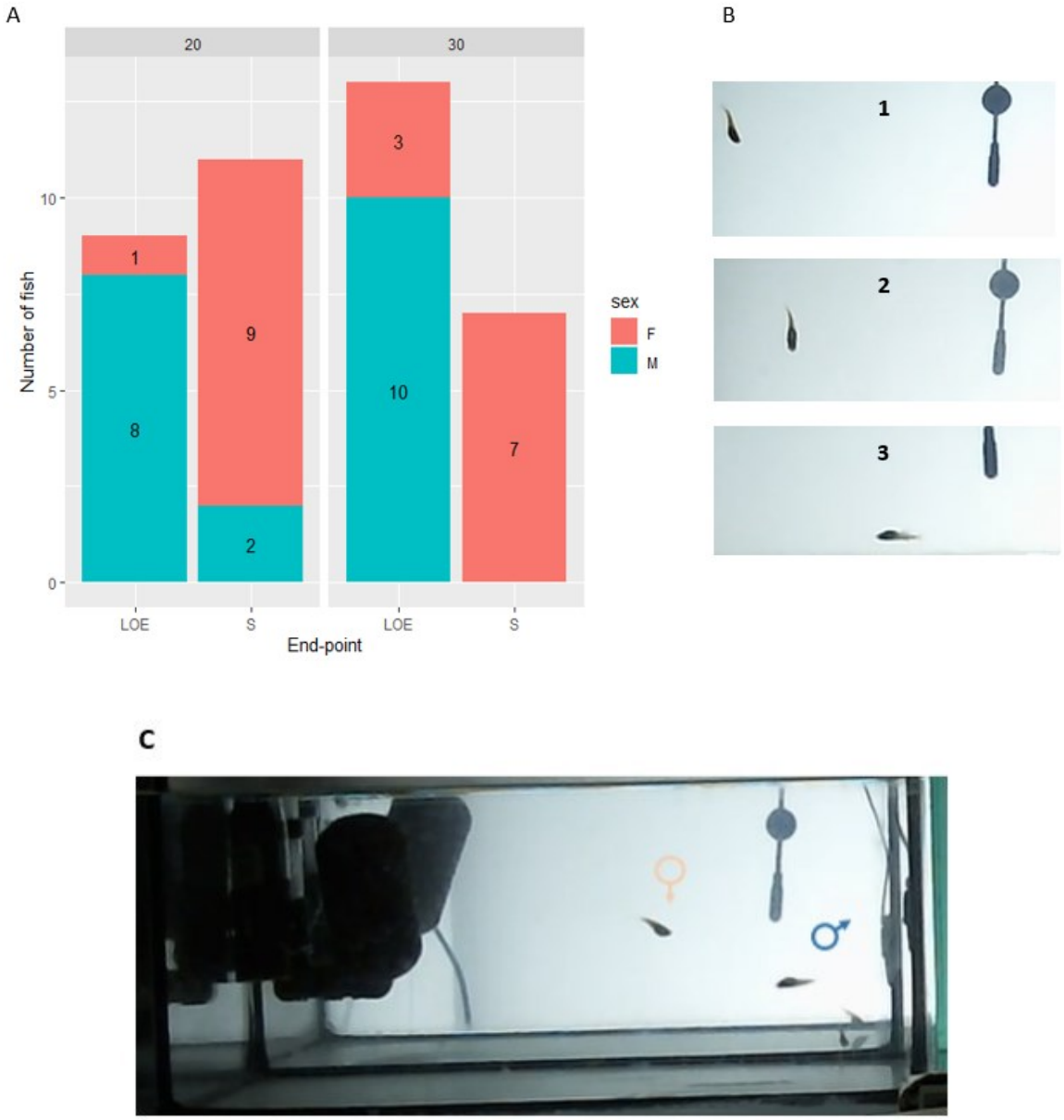


***Fig. S2**: (A) Number of individuals from 20°C and 30°C according to their CTmax End-point (LOE for Loss of Equilibrium or S for Stagnancy) and their Sex. (B) Camera observation of the loss of equilibrium moment for a male medaka. (C) Differences in the end-point between male and female.*

# Appendix 3: Emergence measurement

## ***Text S2****: Emergence traps in mesocosms*

We built emergence traps and placed them in the mesocosms to recover the body size and abundance of emerging animals. The traps were placed during the period of emergence of the organisms, April 13, 2021, May 6, 2021 and May 25, 2021 over 72-hour periods. Then, each trap were sampled and organisms were identified, counted and measured in the laboratory. This allowed us to measure the specific abundances and sizes of invertebrates.

These emergence traps consist of a float, a mosquito net and a collecting pot containing alcohol at 70° at the top of the bell (Fig. S3). They do not cover the entire water surface of each mesocosm but each trap is positioned in the same place in each mesocosm which allows equivalent sampling. When the aquatic larvae transit to the terrestrial adult life stage, they find themselves trapped under the mosquito net bell. While trying to climb to the top of the trap to escape, they fall into the alcohol pot. The individuals are then collected, sorted in order (Diptera, Odonata or Ephemera) using a binocular magnifying glass, photographed and measured from the tip of the head to the end of the abdomen using the Piximeter software. Measurements were taken with an accuracy of around a tenth of a millimeter.

The size of individuals was then used to estimate the dry biomass (Fig. S4) represented by the emergence of invertebrates based on a formula and constants specific to each order of insect. These constants were all taken from (Sabo et al., 2002).

The formula for estimating the dry mass of an invertebrate based on its length was (Sabo et al., 2002):

$$M = a L^{b}$$

For each order, we used linear mixed models to determine whether artificial warming (ambient vs. +4°C), Fish treatments (No fish. fish from 20°C vs. fish from 30°C) and their interaction significantly influenced the abundance of emergences. The conditions for applying the statistical tests were verified, in particular the homoscedasticity of the variances and the normality of our data set.



***Fig. S3:*** *Emergence traps floating at the surface of the mesocosms*

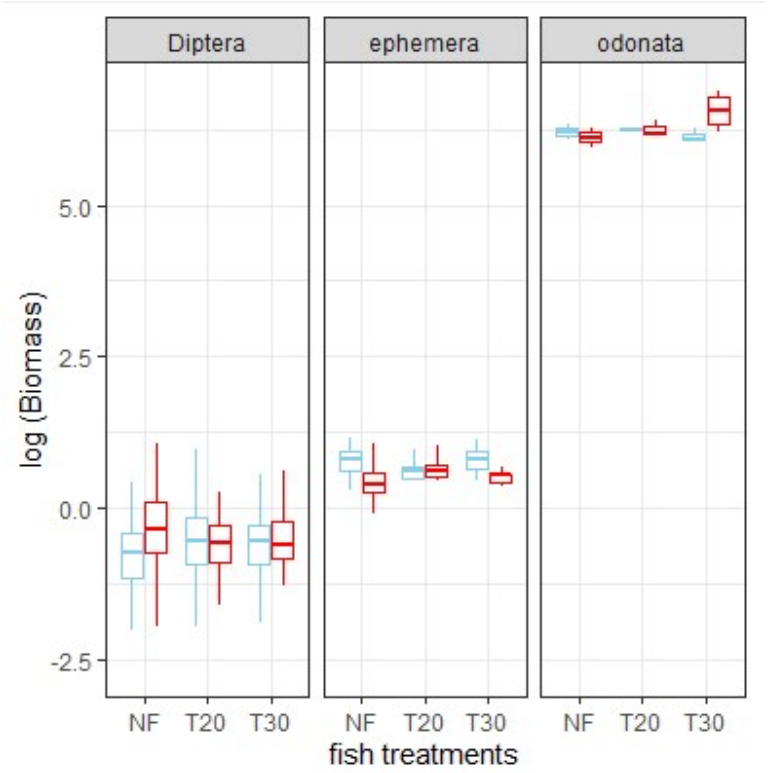


***Fig. S4****: Log biomass according to fish conditions (without fish NF, Fish at 20°C T20 and Fish at 30°C T30.*

# Résumé complet de la thèse en français

La température joue un rôle essentiel pour les organismes vivants car elle régule une grande partie de leurs processus physiologiques et biologiques. Dans le contexte actuel de réchauffement climatique, une meilleure compréhension des conséquences écologiques d'une augmentation de la température est cruciale. Le constat d'une augmentation d'environ 0,85°C de la température moyenne de la planète sur la période 1880-2012 et de 1,1°C sur la période 2011-2020 (par rapport à 1850-1900) nous alerte sur les risques futurs liés au réchauffement à l'échelle globale. Les activités humaines, principalement par le biais des émissions de gaz à effet de serre, ont sans équivoque provoqué le réchauffement climatique. Selon le dernier rapport AR6 du GIEC (Groupe d'experts intergouvernemental sur l'évolution du climat) (2023), les températures mondiales augmenteront de 1,5°C en 2030, dix ans plus tôt que les prévisions précédentes. Toujours selon le GIEC, « le réchauffement climatique est désormais une réalité, avec des conséquences irréversibles sur des siècles ou des millénaires ».

Les écosystèmes d'eau douce représentent environ 3 % de la surface terrestre et sont particulièrement sensibles à l'augmentation de la température de l'eau et aux changements du cycle hydrologique car il s'agit d'habitats peu profonds, avec de faibles possibilités de migration et de dispersion. Ils se réchauffent également plus rapidement que de nombreux habitats terrestres ou marins et comptent parmi les écosystèmes les plus perturbés et les plus vulnérables. La hausse des températures affecte particulièrement les ectothermes d'eau douce dont la température corporelle dépend de la température ambiante.

Selon Brown et al (2004), « le métabolisme fournit une base pour utiliser les premiers principes de la physique, de la chimie et de la biologie pour relier la biologie des organismes individuels à l'écologie des populations, des communautés et des écosystèmes ». La théorie métabolique de l'écologie est une théorie quantitative qui permet d'expliquer le lien entre le métabolisme, la taille des individus et la température. Le métabolisme des organismes correspond à l'ensemble des processus de transformation biochimique qui se produisent dans les tissus des organismes vivants. A la base du métabolisme cellulaire, les mitochondries sont des organelles souvent définies comme « la centrale électrique de la cellule » car elles déterminent la quantité d'énergie disponible pour maintenir les fonctions essentielles du corps et sont particulièrement sensibles à la température. Des études récentes suggèrent que pour de nombreuses espèces, il existerait un lien entre la défaillance mitochondriale et les limites thermiques de l'organisme entier dont la réponse à la température peut également dépendre de la durée de l'exposition (phénomènes d'acclimatation, d'adaptation). Pour mieux anticiper la réponse des organismes au changement climatique actuel et futur, il est important de prendre en compte les conséquences de l'exposition à la température aux différentes échelles du vivant en fonction de la durée d'exposition.

De nombreuses études se sont concentrées sur les échelles individuelles ou populationnelles plutôt que sur les niveaux supérieurs d'organisation (c'est-à-dire les communautés et les écosystèmes) pour déterminer les impacts de la température. La complexité de l'impact de la température réside dans le caractère multi-échelle allant du métabolisme des individus en passant par la biologie des organismes jusqu'au fonctionnement des écosystèmes. De plus, il existe une variation intra-spécifique et interspécifique des réponses à la température qui complexifient la réponse globale. Les

théories telles que la théorie métabolique de l'écologique se concentrent depuis les années 2000 sur les aspects multi-échelles des systèmes écologiques, mais peu d'études ont encore tenté de prendre en compte expérimentalement ces aspects. Cependant, pour mieux anticiper la réponse des organismes au changement climatique, et proposer des outils de protection des écosystèmes et de la biodiversité aquatique, il est nécessaire de prendre en compte empiriquement les différentes échelles temporelles et biologiques auxquelles la température agit.

## Les objectifs et la structure de la thèse

L'objectif principal de cette thèse est d'améliorer notre compréhension de l'effet d'une exposition multigénérationnelle à la température sur les ectothermes d'eau douce en tenant compte des différentes échelles écologiques. Les processus biologiques influencés par la température sont abordés à l'aide de différentes approches expérimentales. Tout d'abord, j'ai utilisé une approche physiologique, axée sur la respiration cellulaire à des températures élevées, en se concentrant sur les mitochondries, qui jouent un rôle central dans le contrôle du métabolisme et des caractéristiques de l'histoire de la vie. Ensuite j'ai combiné respiration cellulaire et avec des mesures de la limite thermique supérieure des organismes (CTmax) pour déterminer si une exposition multigénérationnelle peut moduler ces deux traits. Finalement j'ai utilisé une approche en mésocosmes visant à étudier les processus de l'écosystème sous pression thermique en présence de poissons issus de deux lignées thermiquement contrastées.

Le premier chapitre de ma thèse s'intéresse au métabolisme cellulaire d'individus poissons Medaka (*Orizyas Latipes*) élevés depuis plusieurs générations (deux ans) à 20°C ou 30°C. L'objectif de ce chapitre est de mieux comprendre la réponse des ectothermes à la température après une exposition multigénérationnelle et de mettre en évidence la modulation de la réponse des mitochondries.

Le deuxième chapitre de ma thèse se concentre sur la tolérance thermique maximale supérieure (CTmax) des poissons médaka maintenus sur plusieurs générations (cinq ans) à des températures froides (20°C) et chaudes (30°C) et vise à mettre en évidence un lien entre le dysfonctionnement des mitochondries du cerveau des poissons et la tolérance thermique de l'organisme entier (CTmax).

Un dernier chapitre s'articule autour d'une expérience de trois mois en mésocosmes où j'ai manipulé la température (+4°C ; GIEC, 2023) et l'origine thermique des poissons médaka. L'objectif est de déterminer si les effets induits par la température sur la taille corporelle des poissons et leurs métabolismes se répercutent sur les fonctions des écosystèmes (production primaire, décomposition, respiration des écosystèmes, production de gaz à effet de serre) et d'évaluer le poids relatif de ces effets par rapport aux effets directs de la température sur les fonctions écosystémiques.

## Modèle biologique et dispositifs expérimentaux :

Le poisson medaka japonais *(Oryzias Latipes)* est un petit poisson d'eau douce amphidrome (20-40 mm) originaire d'Asie du Sud-Est. Cette espèce est eurytherme avec une plage thermique allant de zéro à 40°C et un optimum thermique d'environ 25°C. Dans son aire d'origine, le médaka vit généralement dans de petits plans d'eau comme les rizières, dans la partie supérieure de la colonne d'eau ou dans des habitats peu profonds, souvent sensibles à des variations de température importantes. C'est un bon modèle biologique pour l'expérimentation thermique multigénérationnelle car il a un temps de génération court et peut atteindre la maturité sexuelle en 10 semaines environ. Les mâles ont une nageoire dorsale plus développée que les femelles et ont généralement une apparence plus élancée permettant de les différencier visuellement sans manipulation. Il a une durée de vie moyenne de 1 an dans la nature et jusqu'à 5 ans en captivité. Le medaka est une espèce omnivore qui se nourrit principalement de zooplancton mais aussi de diatomées, d'algues et d'insectes aquatiques.

Les Medaka utilisés dans cette thèse ont été élevés à 20°C (poisson FROID) ou 30°C (poisson CHAUD) (températures non stressantes) sur plusieurs générations dans des conditions expérimentales contrôlées dans des chambres climatiques. Pour chaque traitement thermique, deux lignées ont été obtenues à partir d'une population $F_0$ de départ de 320 Medakas (160 femelles & 160 mâles) appartenant à la souche CAB de Carolina Biological Supply Company (USA) et fournie par AMAGEN© (France). Les poissons de la génération F0 ont été maintenus à 25°C dans des aquariums de 20 L puis progressivement acclimatés à 20 ou 30°C à raison d'un degré tous les 2 jours. Pour démarrer une nouvelle génération de poissons, environ 300 œufs sont collectés pendant la période de fécondité optimale. Les œufs sont ensuite placés en couvoirs à la température parentale (20°C ou 30°C). Les nouveau-nés sont maintenus dans des chambres climatiques à la même température que leurs parents, puis ils sont placés en aquariums jusqu'à maturité, avec une densité d'environ 30 poissons adultes par aquarium. Les poissons sont nourris "ad libitum" trois fois par jour. Après 2 ans d'expérimentation, des poissons des générations F3 à 20°C et F4 à 30°C ont été utilisés dans le chapitre 1 et des poissons des générations F3-à 20°C et F5 à 30°C ont été utilisés dans le chapitre 3. Enfin, après 5 ans d'exposition multigénérationnelle, des poissons des générations F6 à 20°C et F10 à 30°C ont été utilisés dans le chapitre 2.

## Chapitre 1: L'exposition multigénérationnelle à la température influence les flux d'oxygène mitochondriaux chez le poisson Medaka (*Orizias Latipes*)

La sensibilité thermique du métabolisme cellulaire est cruciale pour la physiologie animale et la survie face au changement climatique. Malgré les efforts récents, les effets de l'exposition multigénérationnelle à la température sur le fonctionnement métabolique restent mal compris. Mon objectif était de déterminer si l'exposition multigénérationnelle à la température module la réponse respiratoire mitochondriale du poisson Medaka.

Pour ce faire, la consommation d'oxygène mitochondrial d'homogénat de muscle entier des flancs de poissons Medaka issus d'une exposition multigénérationnelle à 20 et 30°C (poissons FROID et CHAUD, respectivement) a été mesurée à deux températures de test (20°C et 30°C). La fonction mitochondriale a été déterminée comme la respiration soutenant la synthèse d'ATP (OXPHOS) et la respiration nécessaire pour compenser la fuite de protons (LEAK) dans un plan factoriel complet (FROID-20°C ; FROID-30°C ; CHAUD-20°C ; CHAUD-30°C). Le RCR (Ratio de Contrôle Respiratoire) qui reflète le couplage de la phosphorylation oxydative (efficacité respiratoire mitochondriale) a également été calculé comme étant le ratio de la mesure de respiration favorisant la synthèse d'ATP (OXPHOS), divisé par la mesure de respiration mitochondriale en absence d'ADP permettant la formation d'ATP (LEAK). Enfin Les flux mitochondriaux reflétant l'état d'activité COX sont également mesurés pour obtenir la respiration de l'enzyme cytochrome C oxydase (COX) utilisé comme proxy de la densité mitochondriale des tissus. L'ensemble des données obtenues ont été analysées en utilisant des modèles mixtes linéaires (LMMs).

Les résultats démontrent des flux d'OXPHOS et de LEAK plus élevés à 30°C par rapport à une température de test de 20°C. À chaque température d'analyse, les poissons CHAUDS présentaient des flux d'oxygène mitochondriaux inférieurs à ceux des poissons FROID. Il est intéressant de noter que les résultats obtenus ne présentent pas de différences significatives dans le flux respiratoire lorsque les mitochondries ont été testées à la température d'élevage du poisson (c'est-à-dire FROID-20°C vs CHAUD-30°C). Nous avons également observé une absence de différence significative concernant les RCRs et la densité mitochondriale estimée des poissons du chaud et du froid.

Les capacités inférieures d'OXPHOS et de FUITE chez les poissons chauds sont probablement le résultat de l'exposition multigénérationnelle à des températures chaudes. Ceci est cohérent avec une réponse modulatrice de la capacité mitochondriale pour compenser l'effet néfaste potentiel du réchauffement sur le métabolisme. Enfin, l'absence de différences significatives dans les flux respiratoires entre les poissons FROID-20°C et CHAUD-30°C reflète probablement un flux respiratoire optimal lorsque les organismes s'adaptent à leurs conditions thermiques. Notre étude met en évidence les effets opposés d'une exposition à des températures courtes et longues sur le poisson medaka et indique que la prise en compte des deux types de réponses est importante dans le contexte du changement climatique. Il est difficile ici de comprendre comment les mitochondries régulent la consommation d'oxygène car de nombreux mécanismes sont impliqués. Une meilleure compréhension de la réponse à long terme pourrait également conduire à une meilleure compréhension du fonctionnement des mitochondries et des répercutions aux différentes échelles supérieures du vivant.

## Chapitre 2: Effets multigénérationnels de l'exposition à la température sur la tolérance critique maximale supérieure (CTmax) et le fonctionnement mitochondrial dans le cerveau du poisson Medaka (*Orizias Latipes*).

La température est un facteur clé qui affecte les processus physiologiques et détermine en partie les limites du vivant. Malgré la sensibilité thermique bien établie du

métabolisme, très peu d'études ont tenté d'établir le lien entre le métabolisme des mitochondries et les limites thermiques de l'organisme entier. Puisque le maximum thermique critique supérieur (CTmax) correspond à la température à laquelle les fonctions neuromusculaires sont perdues, l'évaluation des fonctions mitochondriales cérébrales semble particulièrement pertinente pour comprendre les mécanismes physiologiques qui sous-tendent la tolérance thermique. Dans cette étude, je tente de déterminer les effets d'une exposition multigénérationnelle à la température sur le CTmax du poisson medaka (*Orizyas Latipes*) et le lien potentiel entre le CTmax et le dysfonctionnement mitochondrial à une température critique élevée dans le cerveau.

Dans un premier temps le CTmax de poissons médakas élevés sur plusieurs générations pendant 5 ans à 20°C (FROID) et 30°C (CHAUD) a été mesuré. Les organismes ont été exposés à une augmentation rapide de température avec un taux de réchauffement constant (~ 1,5°C toutes les 10 minutes) jusqu'à ce qu'un point final non létal soit atteint (perte d'équilibre). Après 20 jours de récupération suite à l'expérience CTmax, les taux de consommation d'oxygène mitochondriaux (OCR) du cerveau des poissons à différents stades de la chaine respiratoire ont également été mesurés à l'aide d'un oxygraphe aux températures de test de 25°C et à leurs températures moyennes de CTmax (établie précédemment).

L'exposition multigénérationnelle à des températures élevées a augmenté le $\overline{CTmax}$ moyen des médakas d'environ 13%. Les poissons élevés au chaud avaient un $\overline{CTmax}$ (43,8 °C ± 0,27) significativement plus élevé que les poissons élevés au froid (38,9 °C ± 0,32), ce qui représente une augmentation de 12,6%. Il a également été observé une diminution générale des OCRs des différents complexes mitochondriaux et de l'efficacité de couplage du complexe I entre des températures de test de 25°C et le $\overline{CTmax}$ moyen pour les poissons CHAUDS et FROID. Ceci suggère un lien entre le dysfonctionnement de l'organisme entier et la performance mitochondriale cérébrale. Enfin, nous avons observé une contribution relative élevée au substrat (SCR, c'est-à-dire la contribution relative à l'augmentation de l'OCR lors de l'ajout d'un nouveau substrat) des complexe I+II de la chaine respiratoire pour les poissons du CHAUD à leur $\overline{CTmax}$, suggérant une augmentation de la contribution relative du complexe II dans les mitochondries fonctionnant à des températures plus élevées.

Notre étude met en évidence la complexité du lien entre la sensibilité thermique des mitochondries et la tolérance thermique de l'organisme entier, ainsi que le fait que l'exposition multigénérationnelle peut modifier les performances mitochondriales et de tolérance thermique. Ces capacités doivent être prises en compte lors de la prévision des impacts écologiques du réchauffement climatique.

## Chapitre 3 : Impacts de la température et de la présence de poissons d'environnements thermiquements contrastés sur le fonctionnement des écosystèmes.

Les écosystèmes d'eau douce sont particulièrement sensibles à l'augmentation de la température de l'eau et aux facteurs de stress associés tels que la disponibilité de

l'oxygène. Pourtant, il existe encore peu d'évaluations empiriques de la manière dont le réchauffement climatique pourrait affecter les fonctions des écosystèmes d'eau douce. La température modifie la vitesse des réactions métaboliques biochimiques, la demande énergétique et les taux de consommation des ectothermes modulant ainsi les flux de matière et d'énergie. De plus, le réchauffement climatique affecte les phénotypes individuels et conduit souvent à une réduction de la taille corporelle individuelle, cette dernière structurant les interactions dans les réseaux trophiques aquatiques. Les changements de température combinés à la réduction de la taille du corps sont donc susceptibles d'avoir de profonds impacts écologiques qui pourraient se propager à l'ensemble de l'écosystème. Dans cette étude, l'objectif était de distinguer ces effets sur les fonctions des écosystèmes.

Une expérience en mésocosmes a été menée en manipulant (1) la réduction de la taille corporelle de poissons (*Oryzias Latipes*) en utilisant deux populations élevées pendant plusieurs générations à des températures contrastées et (2) la température de l'eau (réchauffement artificiel de +4 °C) pour tester leurs effets indépendants et interactifs sur la décomposition de la matière organique, la production primaire, la respiration et les concentrations de gaz à effet de serre.

Les résultats ne révèlent aucun effet significatif du réchauffement et des modalités poissons sur le taux de décomposition, ce qui pourrait s'expliquer par le fait que le réchauffement accélère la décomposition microbienne des litières mais réduit également la décomposition des détritivores; par conséquent le taux de décomposition peut rester inchangé par un phénomène de compensation. Il a également été constaté une augmentation de la concentration de la production primaire en présence de poissons et en fonction de la température de l'eau (c'est-à-dire la température moyenne des 15 derniers jours) dans les mésocosmes chauffés. Conformément aux études précédentes, la productivité primaire brute augmente avec la température et en présence de poissons grâce à une cascade trophique positive pour le phytoplancton. Enfin, dans notre étude, c'est principalement la température de l'eau qui diminue les concentrations de gaz et on observe également des concentrations de $CH_4$ plus élevées dans les mésocosmes réchauffés. La relation bien connue entre l'augmentation de la température de l'eau et la diminution de la solubilité des gaz peut expliquer en grande partie les concentrations plus faible des gazs.

Ces travaux démontrent que la présence et les différences d'origine thermique et de taille chez les poissons ont des effets moindres sur les fonctions des écosystèmes par rapport aux effets directs de la température de l'eau et du réchauffement artificiel. Ils mettent également en évidence la complexité du lien entre l'augmentation de la température et les fonctions des écosystèmes d'eau douce pouvant induire des phénomènes compensatoires, non pris en compte dans de nombreux modèles théoriques basés sur les prédictions de la théorie métabolique de l'écologie énoncée par Brown et al en 2004.

## Discussion générale

La température est un facteur abiotique clé, étudié depuis des décennies, et faisant l'objet d'une attention particulière depuis plusieurs années par la prise en compte de son aspect multi-échelle popularisé par la théorie métabolique de l'écologie (Brown et al. 2004). Dans ce contexte, la sensibilité thermique et la réponse à long terme des ectothermes sont particulièrement intéressantes pour améliorer notre compréhension des liens entre la température et les trajectoires des écosystèmes d'eau douce. Cependant, jusqu'à présent, peu d'études ont utilisé la prise en compte empirique d'une perspective multi-échelles et des variations de réponses des organismes et des populations observées sur le long terme pour prédire les conséquences écologiques de la température à l'échelle globale. Dans cette thèse, j'ai exploré les mécanismes par lesquels la température influence les différents niveaux écologiques d'organisation. L'objectif principal était d'améliorer notre compréhension de l'effet d'une exposition multigénérationnelle à la température en prenant en compte les différentes échelles écologiques, depuis le métabolisme cellulaire, jusqu'au fonctionnement de l'ensemble de l'écosystème.

Dans le premier chapitre de cette thèse, une expérience multigénérationnelle (2 ans) sur une espèce de poisson vertébré (*Oryzias Latipes*) montre que plusieurs générations à 30°C peuvent conduire à des activités mitochondriales plus faibles par rapport aux poissons maintenu à 20°C pour une même température de test. Les résultats du chapitre 2 indiquent également une tendance vers des flux plus faibles pour les poissons chauds à leur température optimale (température d'essai de 25°C) venant soutenir la pertinence des résultats du premier chapitre. Ces résultats complètent les études déjà existantes sur les ajustements mitochondriaux en réponse à la température à long-terme pour faire face au réchauffement climatique. Cependant, la capacité d'acclimatation d'un organisme et l'aspect multigénérationnel de notre étude suggèrent de potentiels mécanismes compensatoires.

Il a également observé un taux de consommation d'oxygène mitochondrial similaire entre les poissons froids et chauds lorsque leurs mitochondries ont été mesurées à leur (1) température d'élevage (chapitre 1) et à leur (2) température moyenne de CTmax (chapitre 2), suggérant une convergence de la respiration mitochondriale des poissons des deux origines thermiques. Cette absence de différence pourrait s'expliquer par un phénomène de compensation. Une étude antérieure sur des poissons médaka élevés dans les mêmes conditions a montré que neuf années de réchauffement expérimental n'influencent pas les taux métaboliques individuels. Ces résultats peuvent suggérer l'existence d'un flux respiratoire mitochondrial optimal lorsque les organismes ont le temps de s'acclimate/s'adapter aux conditions locales. Une étude récente visant à observer les modèles de croissance sur des organismes ectothermes de populations thermiquement contrastées a révélé que les populations d'origine plus chaude exprimaient un taux de croissance juvénile plus faible et un effort de reproduction accru à petite taille. Ces résultats sont cohérents avec une base adaptative et suggèrent que la température elle-même peut conduire à l'évolution d'une variation à contre-gradient, neutralisant la plasticité et favorisant la similarité entre les environnements.

Enfin, l'accélération du métabolisme et de la demande en oxygène induite par la température peuvent augmenter la formation d'espèces chimiques réactives avec l'oxygène. Ces dernières sont nocives au niveau cellulaire en accélérant notamment la sénescence. Ainsi la réponse mitochondriale à température élevée observée dans le premier chapitre pourrait servir à contrecarrer la production d'espèces réactives à l'oxygène (ERO) dans un environnement plus chaud. Afin de tester cette hypothèse J'ai réalisé le même protocole expérimental que pour le chapitre 1 sur des poissons

sénescents issus du même pool initial. Il apparaît que les réponses mitochondriales sont conservées tout au long de la vie de l'individu. Cependant, les flux de poissons sénescents étaient inférieurs à ceux des poissons adultes pouvant suggérer un dysfonctionnement mitochondrial, soutenu par d'autres études démontrant une diminution des taux respiratoires mitochondriaux avec l'âge. Une autre explication pourrait être un effet de senescence pure, les poissons sénescents ayant moins besoin d'investir dans la reproduction, par conséquent leur métabolisme n'aurait pas besoin d'être aussi élevé que celui des plus jeunes. Il serait intéressant dans les études futures de mesurer les quantités d'ERO dans les organismes afin de confirmer ou d'infirmer cette hypothèse.

Certaines études récentes ont émis l'hypothèse que les mitochondries seraient impliquées dans la régulation thermique, en raison de leur extrême sensibilité à la température. Dans le premier chapitre, j'ai souligné que l'exposition sur plusieurs générations à des températures non stressantes dans des conditions contrôlées peut modifier la réponse mitochondriale des organismes. Cependant, lorsque la température s'éloigne des plages thermiques tolérées par les organismes, leur activité et leur métabolisme diminuent jusqu'aux limites thermiques supérieures, ce qui peut entraîner une perte totale de fonction et la mort. J'ai constaté que 5 années d'acclimatation thermique multigénérationnelle à des températures élevées augmentaient considérablement les limites supérieures (CTmax) de l'organisme entier. Plusieurs études suggèrent également que le CTmax varie en fonction de la durée d'exposition et peut donc être amélioré après une période d'acclimatation à une température. Ces résultats doivent cependant être nuancés car la méthode CTmax utilisée surestime la véritable tolérance environnementale d'un poisson dans son milieu naturel. Ainsi, il serait intéressant dans notre cas d'explorer le lien entre CTmax et performance thermique pour des traits d'histoire de vie tels que la croissance ou la fécondité car ces liens demeurent peu étudiés.

Les résultats du chapitre 2 suggèrent également un lien entre le CTmax de l'organisme entier et la performance mitochondriale du cerveau. J'ai observé une contribution relative élevée du complexe II pour les poissons chauds à leur CTmax par rapport aux poissons froids. Par conséquent, les poissons exposés à la chaleur peuvent présenter une flexibilité mitochondriale dans le complexe II à des températures atteignant la limite thermique de l'organisme, une stratégie destinée à compenser la plus faible tolérance thermique du complexe I. Des études antérieures ont rapporté que les différents complexes mitochondriaux ont des tolérances thermiques différentes et jouent un rôle important dans l'adaptation thermique des ectothermes. Ainsi, l'étude de ces complexes peut aider à expliquer les limites thermiques des ectothermes.

Enfin, certaines études ont découvert que les tolérances au réchauffement en termes de physiologies individuelles en laboratoire et d'abondance de la population dans la nature diminuent avec l'augmentation de la température au même rythme. Ces relations congruentes impliquent un couplage étroit entre les processus physiologiques et populationnels qui sous-tendent les modèles macro-écologiques. Ainsi, les réponses physiologiques à l'échelle mitochondriale des poissons thermiquement contrastées sur plusieurs générations, pourraient expliquer les schémas d'abondance des populations sauvages.

Dans cette thèse, j'ai également tenté de relier les impacts d'une augmentation de température à l'échelle métabolique (chapitre 1 et 2) et les modifications de taille induites par la température, sur les fonctions des écosystèmes (chapitre 3). La théorie métabolique

de l'écologie (MTE) prédit comment le métabolisme affecte les processus écologiques aux niveaux des individus, des populations et des écosystèmes. Cependant, elle repose sur de nombreuses hypothèses et sur une approche théorique qui nécessite davantage de recherches expérimentales/empiriques. Elle repose principalement sur la relation entre métabolisme et température à travers la loi d'Arrhenius qui établit la dépendance de la vitesse d'une réaction chimique à la température. Dans le chapitre 3, les résultats de l'expérience en mésocosmes ont principalement montré un effet de la température (et de la saisonnalité) sur les producteurs primaires et la solubilité du gaz. Nos résultats sur les gaz à effet de serre mettent en évidence une diminution de la solubilité des gaz à effet de serre avec un réchauffement. Selon une étude précédente, qui testait des modèles théoriques dérivés de la théorie métabolique de l'écologie, un réchauffement de 4°C réduirait la séquestration du $CO_2$ et augmenterait la fraction des efflux de méthane par la production primaire et la respiration de l'écosystème. Par conséquent, la relation entre la photosynthèse, la respiration et la température est bien établie, mais n'est valable que sur une plage étroite de températures pour les ectothermes, ce qui complique les tentatives d'identification de modèles généraux de dépendance à la température.

Au sein d'une communauté, il existe une variation de performances thermiques selon l'espèce pouvant modifier la composition des espèces. En ce sens, dans mon étude en mésocosme, la présence de poissons élevés à 20°C dans des mésocosmes chauffés, proches de leur optimum thermique, peut modifier la relation entre métabolisme et température élevée, modulant la pression de prédation dans les mésocosmes chauffés. En effet, la consommation d'oxygène, ou le taux d'ingestion d'une proie par un prédateur, ne suit pas une relation linéaire avec la température comme indiqué dans le MTE, et diminue aux températures supérieures maximales tolérées. Ce résultat illustre la complexité de déterminer les effets de la température au niveau de l'écosystème.

De plus, dans certains cas, la relation attendue entre la température et la vitesse biologique n'est pas du tout observée du fait d'effets thermiques antagonistes. Par exemple, l'absence d'effet sur le taux de décomposition dans nos résultats en mésocosmes pourrait s'expliquer par les effets antagonistes entre une accélération de la décomposition microbienne et une diminution équivalente de la densité des macroorganismes sous pression thermique. Ces phénomènes compensatoires des effets de la température sur les écosystèmes sont encore peu pris en compte dans les études et méritent d'être étudiés empiriquement afin de comprendre les trajectoires théoriques de réponse à la température sur le fonctionnement des écosystèmes. Enfin, la présence de poissons dans les mésocosmes augmente la concentration de cyanobactéries et de diatomées dans les mésocosmes chauffés et en fonction de la température de l'eau (chapitre 3) par un potentiel effet de prédation des poissons sur le zooplancton entrainant un manque de pression sur le phytoplancton. Bien que mes résultats suggèrent que la présence et les différences de taille des poissons ont des effets moindres sur les fonctions de l'écosystème que les effets directs de la température, notre étude démontre l'importance des relations trophiques et des effets antagonistes complexes sur la réponse de l'écosystème à la température.

# Conclusion

Globalement, cette thèse contribue à améliorer les connaissances sur la manière dont la température modifie la physiologie et la tolérance thermique d'un poisson d'eau douce et fournit des informations précieuses sur la manière dont le fonctionnement des écosystèmes aquatiques peut être affecté par le réchauffement climatique. Nos résultats (1) améliorent notre compréhension des effets d'une exposition multigénérationnelle à une température sur la demande métabolique mitochondriale et la tolérance thermique maximale, (2) montrent qu'une exposition multigénérationnelle à une température élevée peut moduler l'activité mitochondriale tout au long de la durée de vie et (3) suggère que la relation (théorique) entre la température et le métabolisme a des limites quand elle est appliquée aux niveaux cellulaire et écosystémique. Il est donc nécessaire de mieux prendre en compte les différentes échelles du vivant en termes quantitatifs, mais aussi au travers d'approches empiriques pouvant entrainer une réponse différente de celle des études théoriques prédictives. De futures études sont nécessaires pour compléter ce travail de thèse afin de mieux prédire les potentiels d'adaptation des poissons au réchauffement climatique.