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Response of a cold water adapted species to environmental changes : phenotypic plasticity and divergence in early life-history traits of arctic charr (Salvelinus alpinus) Southernmost populations

Lisandrina Mari

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Communauté
UNIVERSITÉ Grenoble Alpes

THÈSE

Pour obtenir le grade de

DOCTEUR DE LA COMMUNAUTE UNIVERSITE GRENOBLE ALPES

Spécialité : **Biodiversité, Ecologie, Environnement**

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Présentée par

Lisandrina MARI

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préparée au sein du **Laboratoire CARRTEL (INRA, Université Savoie-Mont Blanc)**
dans **l'École Doctorale SISEO**

Réponse d'une espèce d'eau froide aux changements environnementaux: plasticité phénotypique et divergence des traits d'histoire de vie précoce chez les populations d'omble chevalier (*Salvelinus alpinus*) en limite Sud de distribution

Thèse soutenue publiquement le **7 mai 2019**,
devant le jury composé de :

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Response of a cold water species to environmental changes: phenotypic plasticity and divergence in early life-history traits of arctic charr (*Salvelinus alpinus*) Southernmost populations

A thesis submitted in partial fulfillment of the requirements for the degree of

Docteur de la Communauté Université Grenoble Alpes

Specialty: Biodiversity, Ecology, Environment

by Lisandrina MARI

To be defended on May 7th 2019

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"One task of naturalists, whether literary naturalists or research ecologists, is to "see the unseen". What species is no longer here? What did this place look like in the Pleistocene? What will it look like next month? What could it look like if people lived to their potential? (…) We naturalists – scientists and activists, professionals and amateurs – undertake the practice of natural history in tribute to the world, as a token of esteem for the world, in gratitude for the gift of living in a world that is inestimably more diverse and gorgeous than it might have been. (…) But to be a naturalist you needn't have fancy letters after your name. In fact, hope for the future of the world will increase in direct proportion to the percentage of regular folks who practice natural history – the oldest form of human attentiveness, requiring the skill and humility to examine something larger than ourselves."

Thomas L. Fleischner, Natural History and the Spiral of Offering (2001)

Abstract

In a warming climate, the capacity of species to adopt effective strategies to cope with temperature increase will be key to their persistence in hostile environments. Furthermore, elevated temperatures are likely to modulate the effects of other environmental pressures on biota.

The Arctic charr (*Salvelinus alpinus*) is a cold water adapted stenotherm, found essentially below the Arctic circle. In Europe, the Southernmost edge of its native distribution range is located in the Alps where the species remained following the glacier retreat after the last glaciation. As alpine and peri-alpine lakes are deemed particularly vulnerable to warming, these isolated populations can be considered as sentinels of climate change and constitute an interesting study system to investigate i) local adaptation to thermal habitat and ii) population response to multiple environmental stressors.

Using a common garden experimental design, we first reared embryos originating from four lakes – located along an altitudinal gradient – to an optimum temperature (5°C) or to a temperature close to the thermal tolerance limit of arctic charr embryos (8.5°C). We measured fitness-related traits in hatched larvae and at near emergence and investigated the evolutionary processes underlying population divergence using a QST-FST approach. Results revealed contrasted thermal reaction norms with effects of temperature affecting survival, body size and timing of hatching, that reflected genetic divergence among populations. However, pairwise population comparisons showed that quantitative trait divergence could often be explained by drift and homogenous selection. Results also indicated that neutral genetic diversity was relatively high, but adaptive potential to warming was limited in populations managed by supportive breeding, suggesting a negative effect of local management practices on populations.

A second study investigated the combined effects of temperature and fine sediment, a stressor that affects strongly oxygen availability and habitat quality of salmonids. Results revealed that temperature can decrease the tolerance of arctic charr early life stages to fine sediment. Exposure to a gradient of fine sediment

under stressful thermal conditions during egg incubation had dramatic effects on embryonic life-history traits, with temperatures strongly exacerbating the impacts of sediment loads that did not exert any detrimental effects at cold.

Finally, a third study showed that co-exposure to temperature and fine sediment during embryo development can lead to different trade-offs between size at hatching, yolk conversion efficiency and timing of hatching among wild populations. As a confirmation of the previous study, temperature and sediment did affect early life traits interactively, although these effects were essentially sublethal and temperature was dominant. This indicated that wild populations were more resistant than the previously studied captive stock, certainly due to natural exposure to sediment, but the observed reduction in size and increased energy expenditure might have critical consequences in later life stages.

Overall, this work showed that wild arctic charr populations exhibit adaptive divergence in response to temperature to a certain extent, but the history of anthropogenic disturbances in alpine lakes (inappropriate stocking practices, allochthonous fish introductions, overfishing) is likely to hinder their capacity to respond and adapt to future temperature elevations. This work also highlighted that the reevaluation of common stressors in freshwaters (*e.g.* fine sediment deposition) under different thermal scenarios while taking into account population specificities is necessary.

Résumé

Dans le contexte actuel de réchauffement climatique, la capacité des espèces à adopter des stratégies de mitigation, face aux effets d'une augmentation des températures est cruciale à leur persistance dans des environnements non favorables. De plus, la température est susceptible de moduler les impacts d'autres facteurs environnementaux.

L'omble chevalier (*Salvelinus alpinus)* est un poisson sténotherme d'eau froide que l'on retrouve essentiellement sous le cercle arctique. En Europe sa limite Sud de distribution se situe dans la région alpine où l'espèce s'est retrouvée enclavée suite à la dernière glaciation. Les lacs alpins et péri-alpins sont particulièrement vulnérables au réchauffement. Ces populations peuvent donc être considérées comme des sentinelles du changement climatique et constitue un système d'étude intéressant pour l'étude de i) l'adaptation locale à la température, et ii) la réponse des populations à des stress multiples.

Suivant un schéma expérimental de jardin commun, des œufs originaires de quatre lacs situés le long d'un gradient altitudinal ont été exposés à une température optimale (5°C) ou proche de leur limite de tolérance thermique (8.5°C). Nous avons mesuré des traits liés à la fitness chez les individus à l'éclosion et proches de l'émergence et étudié les processus qui sous-tendent les divergences entre ces traits par une approche QST-FST. Les résultats ont montré des normes de réaction contrastées, la température affectant la survie, la taille et le timing de l'éclosion, reflétant des différences entre populations dont la base est génétique. Des comparaisons entre paires de population ont montré que la divergence entre traits quantitatifs peut être attribuable en grande partie à la de la sélection homogénéisante ou de la dérive. Les résultats ont également montré que la diversité génétique neutre est relativement élevée, mais que le potentiel adaptatif des populations gérées par des pratiques de repeuplement est limité, suggérant un impact négatif des pratiques de gestion locales.

Une deuxième expérience s'est focalisée sur les effets combinés de la température et des sédiments fins, une pression qui affecte fortement la disponibilité en

oxygène et la qualité de l'habitat des salmonidés. Les résultats ont révélé que la température diminue la tolérance aux sédiments fins des stades précoces d'omble. L'exposition à un gradient de sédiments sous des conditions thermiques défavorables ont eu des effets dramatiques sur les traits de vie embryonnaires, montrant que la température peut exacerber les effets des sédiments.

Une troisième étude a montré qu'une exposition à la température et aux sédiments fins durant le développement embryonnaire peut amener à des compromis entre la taille à l'éclosion, l'efficacité de conversion de la vésicule vitelline et la date d'éclosion, qui diffèrent selon les populations. Confirmant ainsi que la température et les sédiments fins peuvent interagir, cette étude montre néanmoins qu'aux charges sédimentaires testées les effets de cette interaction sont essentiellement sub-léthaux et que l'effet de la température est dominant. Elle indique également que les populations sauvages sont plus résistantes que la population captive précédemment étudiée, certainement dû à une exposition aux sédiments en milieu naturel, mais la réduction de taille observée et la consommation de réserves vitellines peuvent avoir des conséquences lourdes sur les stades de vie suivants.

Globalement, ce travail a montré que les populations sauvages d'omble chevalier divergent en partie adaptativement en réponse à a température, mais l'historique de perturbations d'origine anthropique affectant ces populations dans les lacs alpins (pratiques de gestion et repeuplement non adaptées, introduction allochtones, surpêche) peuvent réduire leur capacité à répondre et s'adapter aux futures augmentations de température. Ce travail a également mis en avant la nécessité de ré-examiner les effets de pressions classiques dans les écosystèmes aquatiques (notamment l'accumulation de sédiments fins) sous différents scénarii thermiques tout en prenant en compte les particularités de chaque population.

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Et enfin.. Puisque l'adaptation locale à la température n'existe pas que chez les ombles, mais surtout chez les humains : merci à ma famille ! A Juju et Tomi qui en font partie malgré eux, mais surtout à mes parents et ma sœur qui m'ont supportée dans tous les sens du terme, du bout de la Méditerranée jusqu'au bout du monde.

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1. Introduction and context

Biodiversity in a changing environment

Human populations have exploited the earth natural resources for millennia. It is only in the 19th century that the development of industry and population growth led to the acceleration of carbone dioxide release into the atmosphere. With an average increase of 0.05°C per decade, global climate change constitutes one if the main threats on ecosystems and will have serious implications on both terrestrial and aquatic biodiversity (IPCC 2018). A major concern is the timescale at which global change occurs compared to what would be considered average temperature fluctuations: taking place at an extremely rapid pace, its detrimental effects have now been documented across every ecosystem at the planetary level (Scheffers *et al.*, 2016). The reported impacts on biodiversity include, but are not limited to, changes in the phenology, morphology, abundance and distribution of species, inducing in turn new population dynamics and interactions (Walther *et al.*, 2002; Parmesan and Yohe, 2003; Parmesan, 2006; Crozier and Hutchings, 2014; Scheffers *et al.*, 2016).

The consequences of global climate change are pervasive but will vary across systems, species and ontogeny, depending on three interlinked determinants of vulnerability: i) exposure, that characterizes the changes that will occur within a species thermal habitat, ii) sensitivity, that includes physiological limits and ontogenetic stage, and iii) species capacity to avoid or mitigate the impacts of temperature increases by dispersal, plasticity or microevolutionary changes (Foden *et al.*, 2013; Comte, Murienne and Grenouillet, 2014; Comte and Olden, 2016).

As such, species with narrow thermal tolerance ranges, and inhabiting vulnerable regions with limited or absent opportunities to relocate to more favorable environments, will be most at risk. The effects of global climate change also relate to ontogeny and it is well acknowledged that the early-life stages of many taxa are extremely sensitive (Pankhurst and King, 2010; Przeslawski, Byrne and Mellin, 2015; Gunderson, Armstrong and Stillman, 2016). Global warming is projected to increase by 1.5°C to 4°C during the 21st century (IPCC 2018), and future

temperature elevations will thus have far-reaching consequences if organisms are not plastic enough to cope with these changes, or if they precede the capacity of vulnerable life stages to adapt.

Environmental changes in freshwater systems

Freshwater ecosystems host 9% of the planet's described biodiversity while accounting for less than 1% of its surface (Balian *et al.*, 2007), and are the source of a large number of ecosystem services such as water resource, transport, fisheries, land use, or tourism. These systems are however highly threatened as the impacts of climate change are likely to affect continental systems more intensely through temperature increases and changes in the variability of precipitation regimes (Heino, Virkkala and Toivonen, 2009; Anneville *et al.*, 2013; Salmaso, Anneville and Straile, 2018). The decline of freshwater species over the past decades is alarming (Dudgeon *et al.*, 2006; Reid *et al.*, 2018), with an average of 83% since 1970, a far steeper drop for freshwater species than terrestrial or marine species, and worryingly higher extinction rates (Harrison *et al.*, 2018).

A vast majority of freshwater organisms are ectotherms, *i.e.* organisms that have little to no capacity to regulate their body temperature, and can only cope with above-average temperature increases by behavioral regulation to local refugia and/or physiological adjustments (Bellard *et al.*, 2012; Comte, Murienne and Grenouillet, 2014). Over 50% of fish species are found in freshwater environments (Balian 2007). Fish are an important component of trophic networks and constitute an extremely valuable resource through fisheries. However, fish populations are on the decline worldwide as they are persistently threatened by a multitude of pressures (Hering *et al.*, 2015; Gordon *et al.*, 2018; Reid *et al.*, 2018)

Multiple stressor interactions

Concurrently with climate change-induced temperature increases, all systems are faced with local pressures that depend on local levels of anthropogenic activities and natural regional variability. In freshwater ecosystems, such pressures include

eutrophication, pollutants, hypoxia, overharvesting or invasive species (Heino, Virkkala and Toivonen, 2009). Global and local pressures co-occur in the environment, which indubitably leads to the possibility of interactive effects on organisms. The major types of interactions are determined according to the magnitude and direction of the biological response the combined stressors elicit, compared to the effects of single stressors (Piggott *et al.*, 2015; Piggott, Townsend and Matthaei, 2015b; Côté, Darling and Brown, 2016). At such, if we consider two stressors having an effect going in the same direction when experienced separately, a combined effect can be:

- equal to one of the two stressors (dominance of one stressor over the other);
- additive, when equal to the sum of the two separate stressors;
- antagonistic, when going in the opposite direction than that of the additive effect;
- synergistic, when stronger than the additive effect.

However, two separate stressors can have opposite biological effects, which complicates the definition of synergism when they are experienced simultaneously and renders interactions labeling difficult (Przeslawski, Byrne and Mellin, 2015; Côté, Darling and Brown, 2016; Jackson *et al.*, 2016). Several frameworks have been proposed but require a thorough quantification of effects to classify interactions properly (Brook, Sodhi and Bradshaw, 2008; Piggott, Townsend and Matthaei, 2015b; Côté, Darling and Brown, 2016).

Thus, the complex nature of interactions increases the urgency to better understand the impacts of both anthropogenic and natural stressors on freshwater ecosystems. The biological responses are complex and good characterization of both separate and joint effects of stressors constitute essential knowledge to aid proper ecosystem management (Hering *et al.*, 2015; Schinegger *et al.*, 2016).

Evolutionary concepts: responses of populations to environmental variations

Relationship between phenotype and environment

In order to persist when environmental conditions become hostile, species must set up adjustment strategies to environmental changes to avoid local extinction. We distinguish three categories of responses (Chevin, Lande and Mace, 2010): dispersal, that allows escaping current conditions to track a more suitable environment; phenotypic plasticity, that allows a short-term response to environmental changes; and adaptation, that is, an evolutionary response that would allow longer-term adjustment to conditions because it is genetically based. These responses exist along a continuum of acclimation-adaptation-speciation (Fitzpatrick, 2012) and are not mutually exclusive (Chevin, Lande and Mace, 2010). Plasticity is a short term response that occurs at the individual scale, whereas adaptation occurs at the population scale and over the course of several generations. The capacity of species to respond by phenotypic changes and how this response can evolve is fundamental to predict which organisms will thrive under non-optimal conditions and which will flounder.

Phenotypic values can be seen as a function of a genotype across a range of environments, a representation called the reaction norm (Hutchings, 2011, Oomen & Hutchings 2015). Reaction norms can vary in mean trait value or in slope, (Figure 1). As example, within environment 1, a difference in the trait value (A vs. B) indicates differences that are linked to the genotype, whereas the difference in slope between environments for a given genotype (A1 and A2) indicates plasticity in response to the influence of the environment. Comparing the reaction norm slopes among several genotypes is informative (A2 – A1 vs. B2 – B1) of a more complex phenomenon: when they are parallel, all the phenotypes respond in a similar manner. However, observing reaction norms that diverge in one direction or the other (non-parallel reaction norms, whether they are crossed or not) underscores the existence of a gene by environment interaction (G x E) that indicates genotypes vary differently across environments (Figure 1D).

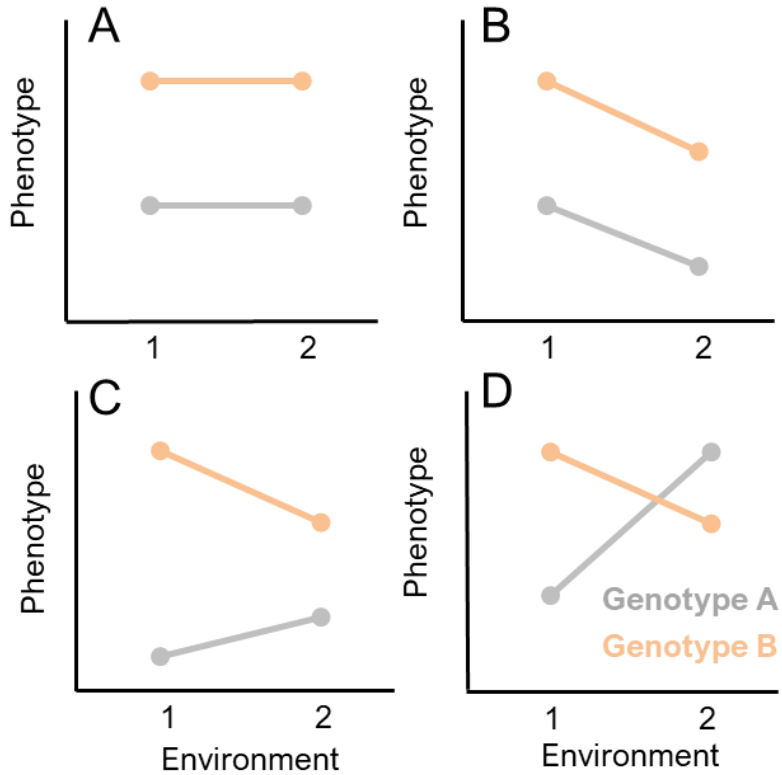

Figure 1-1 | Reaction norms of two genotypes across two environments. (A) no phenotypic plasticity, (B) phenotypic plasticity but the two genotypes respond similarly, (C) and (D) GxE interactions.

Adaptive phenotypic plasticity describes such a situation when different genotypes have distinct reaction norms within a population (Ghalambor *et al.*, 2007). Overall, a phenotype (P) results from the influence of a genotype (G), an environment (E) and the interaction between both, following this equation: P = G + E + G x E.

Local adaptation

Four distinct processes can influence the evolution of genetic diversity (i.e. allelic frequencies) within populations: i) mutation, that allows the creation of new alleles, ii) migration that consists in the dispersal of individuals, propagules or gametes and creates gene flow among populations, iii) drift, a stochastic process that leads to random changes in allelic frequencies and notably a loss of diversity in finite populations, and iv) selection, that can take various forms (*e.g.* directional, homogeneous, balancing) and have different effects on allele frequencies.

Adaptation is a genetic and phenotypic change that affects fitness at the population level. For adaptation to occur, there must be genetic variance for heritable fitness related traits in the population (Hoffmann and Sgrò, 2011; Franks and Hoffmann, 2012).

Under limited gene flow, spatial heterogeneity among habitats can successfully act as a differential selective pressure on genotypes. Adaptation can occur at the local scale, resulting in the maximization of individual fitness in a given local environment. Thus, resident genotypes should have higher fitness in their habitat than any non-resident genotypes in the same environment ('local vs. foreign' criterion, [Kawecki and Ebert, 2004]).

The expression of phenotypic plasticity, gene by environment interactions and local adaptation are interlinked, and appropriate tools are required to disentangle these processes. The study of local adaptation relies on experimental designs that allow controlling for environmental factors to observe genetically-based differences among the phenotypes measured (Kawecki and Ebert, 2004; Blanquart *et al.*, 2013). Two types of experiments offer the opportunity to examine reaction norms and the processes that can affect their variation in this sense, both consisting in rearing individuals from distinct genetic groups in the same environmental conditions:

- reciprocal transplants that consist in translocating individuals of distinct genetic origins in their respective natural habitats. While transplants allow encompassing the entire spectrum of pressures that might occur in one habitat, they are impractical for most mobile organisms.
- common garden experiments that consist in rearing individuals of distinct genetic origins under a same set of controlled environmental conditions. Although they seem more limited than reciprocal transplants, they allow targeting the specific impacts of one or several environmental factors in laboratory, and remain the only viable solution to study local adaptation in e.g. vertebrates.

Study model and research questions

Alpine populations of a cold-water specialist, the arctic charr *Salvelinus alpinus* in a warming context

Due to global climate change, salmonid fishes are experiencing regional and global population declines (Beer and Anderson, 2011, 2013). Among the most temperature sensitive salmonids is the arctic charr (*Salvelinus alpinus L.*). Essentially circumpolar and distributed up to 80°N, the arctic charr (Figure 2) is the Northernmost freshwater fish on Earth (Klemetsen *et al.*, 2003) and is a cold-water adapted stenotherm (Power, 2002). As such the species has a significantly lower thermal tolerance range than other salmonids for both the early and adult stages of its life cycle, tolerating water temperatures < 3°C in winter (Baroudy and Elliott, 1994; Amundsen and Knudsen, 2009; Elliott and Elliott, 2010; Sæther, Siikavuopio and Jobling, 2016). Much of the information relative to the environmental requirements of arctic charr is in relation to hatchery rearing and aquaculture practices, due to the species important economic value in Northern America and Northern Europe (Klemetsen *et al.*, 2003) . As such, arctic charr reproductive capacity is optimal under 8°C (Gillet, 1991) and optimal egg incubation temperatures range from 4 to 7°C (Janhunen, Piironen and Peuhkuri, 2010; Henrik Jeuthe, Brännäs and Nilsson, 2015; Nilsson *et al.*, 2016), with reported reproductive function impairment and increase of egg and embryo mortality when temperature rises above 8°C (Jungwirth and Winkler, 1984; Humpesch, 1985). In the wild, Northern arctic charr populations are also well-studied because of the extremely high morphological plasticity of the species (Figure 2), that often leads to the co-existence of several more or less differentiated morphotypes that occupy distinct trophic niches (Adams, Wilson and Ferguson, 2008; Klemetsen, 2010a, 2013; Parsons, Skúlason and Ferguson, 2010).

A rather broad body of literature exists on arctic charr plasticity in regards to development and diet (Knudsen *et al.*, 2006, 2016; Klemetsen, 2010b), but the knowledge of the adaptive potential of wild arctic charr populations in face of

climate change, and multiple stressors, is currently limited. In this thesis, I focus on five populations of the alpine and peri-alpine region (Figure 3).

Figure 1-2 | (A) Distribution map of *Salvelinus alpinus*, with blue: sedentary populations and red: migratory populations, from Klemetsen 2010a. Note that the alpine region constitutes the Southernmost range at which the arctic charr is found. (B) Arctic charr on a typical spawning ground, constituted of large gravel and stones. (C) Example of the extreme morphological plasticity of the species, with three morphotypes found in Iceland each specialized in a different diet and lifestyle. SB: small benthic, LB: large benthic, PL: pelagic. From Gudbrandsson et al. 2016, adapted from Sandlund 1992.

Temperature increases as modelled by future projections are expected to affect lakes more rapidly (Adrian *et al.*, 2009), and particularly alpine and peri-alpine lakes due to local climate and alpine region geomorphology (Bogataj, 2007).

Alpine arctic charr populations, located at the Southernmost edge of the species range in Europe, constitute an interesting model to investigate population response to environmental stressors. Whereas recent reports at much higher latitudes tend to provide mixed opinions on the emergency of addressing the effects of climate change on arctic charr (see Lehnherr *et al.*, 2018 and the response by Moore *et al.*, 2018), these Southernmost alpine populations could be considered as sentinels

of climate change. We could also expect that they are locally adapted to their thermal habitat compared to their Northern counterparts. Functional spawning grounds are found in contrasted thermal environments (Figure 4), indicating that early life stages are exposed to temperatures that are above the thermal limits that can be found in literature (Jungwirth and Winkler, 1984; Sæther, Siikavuopio and Jobling, 2016). Furthermore, it is worth mentioning that the arctic charr is only native to a few lakes in this region (*e.g.* Geneva and Constance) where it remained trapped following the last glacier retreat 13.000 years ago, but has been introduced over the past centuries for fishery purpose to a great number of isolated lakes, representing a wide variety of habitats along altitudinal gradients, which could further strengthen the existence of local adaptation occurring over short timescales among these populations.

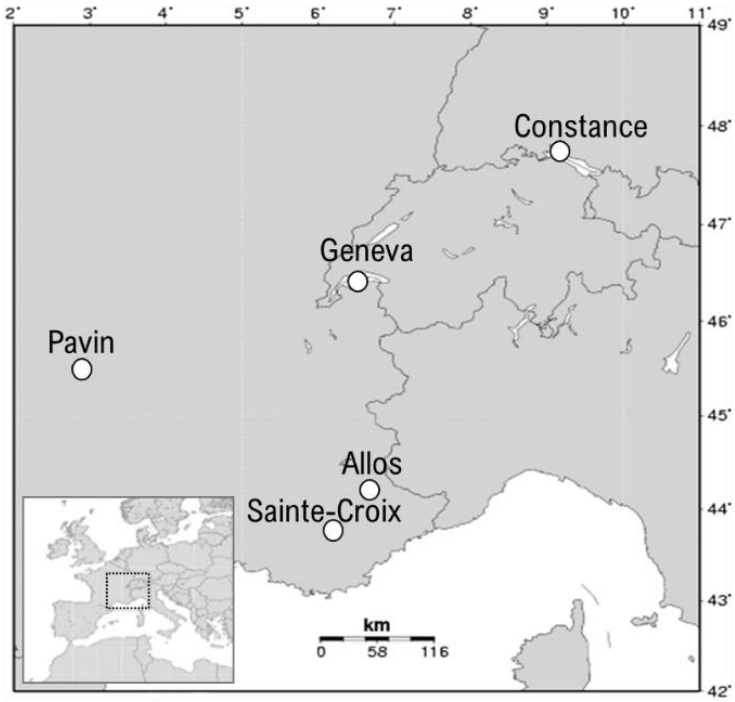

Figure 1-3 | Location of the five populations studied.

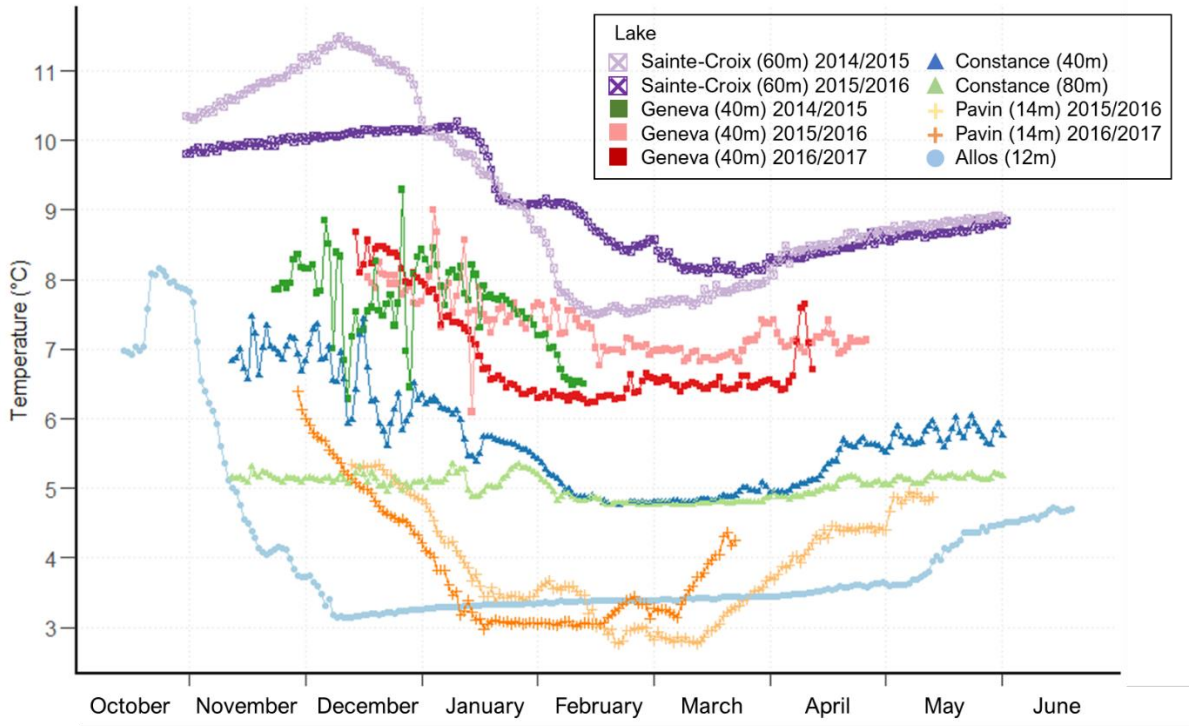

Figure 1-4 | Temperatures recorded in the five study lakes, measured close to known arctic charr spawning grounds (Geneva, Pavin, Allos), at depths where charr spawning grounds are found (Constance), or at maximum depth when the depth of such areas was unknown so as to measure the coldest temperature layer of the lake (Sainte-Croix).

Local threats: fine sediment impacts on salmonids and potential for interactions with temperature

In freshwater ecosystems, many local pressures are to be considered, such as eutrophication, pollutants, hypoxia, overharvesting or invasive species (Heino, Virkkala and Toivonen, 2009). Among them, sediment accumulation constitutes an important part in the normal functioning of freshwater systems but fine sediment – particles of less than 2mm of diameter – can be, when in excess, particularly destructive, and has been acknowledged to exert a wide range of physical or chemical effects (Kemp *et al.*, 2011).

Most importantly, the impacts of sediment are tightly linked to species life and reproductive cycle. Sediment accumulation at the bottom of streams and lake beds will primarily affect the habitat of benthic species and spawning habitat of benthic spawners (Sly and Evans, 1996; Kondolf *et al.*, 2008). In salmonids in particular, spawning habitat quality is determinant for survival of early life stages (Greig, Sear and Carling, 2005; Greig, Sear and Carling, 2007; Cocchiglia, 2012; Chapman *et al.*, 2014; Sear *et al.*, 2016) and most salmonid species require well-oxygenated water and sediment-devoid substrate, constituted of gravel and stones, where eggs will be laid. Fine sediment also affects benthic invertebrate community structure and functionality (Townsend, Uhlmann and Matthaei, 2008; Piggott, Townsend and Matthaei, 2015a; Magbanua *et al.*, 2016; Louhi, Richardson and Muotka, 2017) thus modifying food resource for fish, with possible consequences on trophic structure.

Fine sediment has a negative effect on gravel permeability, thus preventing proper hyporheic water circulation (Soulsby *et al.*, 2001; Heywood and Walling, 2007). The smothering of interstitial zones in streams and lakes beds is particularly concerning for the survival and proper development of early life stages that remain within the spawning ground gravel for weeks to months. With such strong implications for salmonids, the impacts of spawning habitat degradation due to sediment have been quite intensively studied (Chapman *et al.*, 2014) and it is clear that the major impact of fine sediment on early life stages of salmonids is akin to prolonged hypoxia (Greig, Sear and Carling, 2007). In fact, sediment has been identified as a major cause of reproduction failure in lake salmonids, in the second part of the 20th century (Müller, 1992). Eutrophication of alpine lakes at this time, accompanied by increased lake productivity, led to intense sediment deposition that induced massive embryo mortality within spawning grounds. In the current global change context, temperature elevations – that, from a physiological point of view, induce an increase in organism oxygen demand – will coincide with sediment exposure. As temperature and hypoxia can interact (McBryan *et al.*, 2013), we can similarly expect temperature elevations to affect the impacts of sediment, and both stressors to affect the early life stages of salmonids in an interactive manner, although this has never been tested (Mari *et al.*, 2016).

Research aims

Populations of cold-water species found at the Southernmost range edges are especially vulnerable to temperature changes and multiple stressor interactions. Through this work, I try to bring insights to how arctic charr populations that are likely to be vulnerable to climate change can respond – adaptively or not – to environmental perturbations. Since the arctic charr is a cold water stenotherm, I argue that temperature remains a stressor of primary importance, but fine sediment can jointly affect early life traits and both stressors will act interactively.

Thus, the aims of this research were:

I. To analyze the response of Southernmost charr populations early life stages (embryos and larvae, until emergence) to thermal constraints (Chapter 2 & 4)
II. To investigate the potential of wild populations for an adaptive response to temperature – including an examination of patterns of local adaptation in a warming climate context (Chapter 2).
III. To investigate whether temperature affects sensitivity to sediments depending on sediment load (Chapter 3) and geographic origin (Chapter 4).

The core of this thesis is constituted of three chapters of results (Chapters 2 – 4) that can each be read independently. They are preceded by a short introduction and written in the format of journal articles, comprising an abstract, introduction to the study, material and methods section, results section, and discussion, although at various stages of completion (one published, two in preparation). A subsequent general discussion chapter (Chapter 5), puts the main results of this work in a larger context and presents future research directions.

2. Temperature as a selective pressure – investigating local adaptation patterns in native and introduced populations

Foreword

Spatio-temporal heterogeneity of the environment can create differential selective pressures among habitats and, depending on other processes such as gene flow or other demographic events, lead to population differentiation across locations. In the context of climate change, it remains essential to investigate whether vulnerable populations of cold water ectotherms can respond adaptively to temperature increases.

The following chapter presents a common garden study on four populations of arctic charr of the alpine region that investigated local adaptation and adaptive potential of these populations in respect to temperature. This experiment focuses on the responses of the early life stages of the species by measuring traits at hatching and near emergence in individuals reared at an optimal or at a stressful temperature. As several evolutionary processes (divergent selection, homogeneous selection, drift) can influence divergence among allopatric populations, this study relies on a QST-FST approach that compares quantitative trait variation to neutral genetic differentiation, to attempt disentangling the causes of population differentiation. Two populations are native from the Rhone and Rhine basin, whereas the two others have been introduced from individuals of the two native catchments, between the late 19th century and the 1920s. Microsatellite genotyping brings further insight into the patterns of neutral genetic diversity and admixture among these populations, whereas the use of a half-sib crossing design allows looking into adaptive diversity.

Overall, this study provides interesting insight to the capacity of native and introduced, managed and non-managed charr populations to respond to temperature changes and on the potential issues linked to supportive breeding practices in alpine lakes.

Article information

Status: manuscript in preparation.

Authors contribution: conception and design: EL, GE and LM. Experimental data acquisition: LM. Genotyping: LF. Experimental and genotyping data analysis: LM with input from GE and EL. Interpretation of data: LM, EL and GE. Drafting of manuscript: LM with input from GE and EL. Writing of manuscript: LM, GE, EL, MD.

Evidence for adaptive thermal reactions norms but also uniform selection among Arctic charr southern populations

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Abstract

The arctic charr (*Salvelinus alpinus*) is a cold water stenotherm with early life stages (embryos and alevins) that are particularly vulnerable to temperature increases. The persistence of Southernmost populations will partly depend upon the adaptive potential of these critical life stages to environmental changes. In this study, we used a common garden approach to test the hypothesis of local adaptation of charr populations from geographically isolated lakes to temperature. We reared eggs originating from four thermally contrasted lakes at an either optimal (5°C) or stressful but realistic (8.5°C) temperature throughout the entire larval development, and examined fitness-related traits at hatching and near emergence. We report evidence of detrimental effects of temperature on body size, survival and timing of hatching. Thermal reaction norms were contrasted and revealed some adaptive divergences among populations. However, pairwise population comparisons indicated that quantitative trait divergence could often be explained by drift alone and by homogeneous selection. Our results showed relatively high neutral genetic diversity but limited adaptive potential to warming in populations managed by supportive breeding, suggesting local management practices might hinder these populations capacity to adapt to environmental changes. While this study does not entirely validate temperature as a strong selective pressure in Southernmost arctic charr populations, it highlights that other drivers linked to the population history (allochthonous introduction events, reductions in population size, stocking practices) might play an important role.

Introduction

Several processes can allow populations to persist in response to environmental changes (Chevin, Lande and Mace, 2010). First, phenotypic plasticity, *i.e.*, the propensity of a given genotype to express various phenotypes under different environmental conditions, facilitates adaptation to novel environments (Pigliucci, 2005; Fitzpatrick, 2012). Second, genetic adaptation or microevolution, can occur at longer timescales when fitness traits are selected for distinct optima in different habitats (Stearns, 1992; Gienapp *et al.*, 2008). Phenotypic plasticity can also be adaptive when genotypes have distinct reaction norms, hence significant genotype-by-environment (GxE) interactions (Saltz *et al.*, 2018). When observed among populations, genotype-by-environment interactions suggest local adaptation that occurs when populations exhibit higher fitness in their local habitat than in another habitat (Kawecki and Ebert, 2004; Hutchings *et al.*, 2007; Blanquart *et al.*, 2013).

Understanding the mechanisms that drive divergence among populations is crucial to predict how vulnerable, isolated populations may respond to fast environmental changes. Different approaches can be used to test for genotype-by-environment and adaptive divergence (Hutchings, 2011; Oomen and Hutchings, 2015). Among them, reciprocal transplants are the most powerful designs to detect local adaptation, but are difficult to carry out in large organisms like vertebrates (Kawecki and Ebert, 2004). Conversely, common garden experiments offer an alternative solution to address such hypotheses. Furthermore, the use of a QST-FST approach allows distinguishing whether population differentiation for quantitative traits has been shaped by selection or drift (Leinonen *et al.*, 2013). This approach relies on the comparison of QST, an index of among-population differentiation for quantitative traits (Spitze, 1993), with FST, an analogous index of among-population differentiation measured with neutral molecular markers (Weir and Cockerham, 1984). By comparing QST to FST, this approach provides a mean to disentangle causes of population differentiation, with three possible outcomes: (i) QST > FST, when phenotypes are selected for different optima, thus evidencing divergent selection among populations, (ii) QST < FST, when phenotypes are

selected for a same optima, evidencing homogeneous selection, and (iii) QST = FST, when population divergence can be explained by drift alone. QST-FST approaches have been efficiently used across a wide range of taxa including fish (Jensen *et al.*, 2008; Janhunen, Piironen and Peuhkuri, 2010; Tibblin *et al.*, 2015; Côte *et al.*, 2016), amphibians (Knopp *et al.*, 2007; Miaud and Ple, 2015), birds (Dubuc-Messier et al. 2018), and plants (Lamy *et al.*, 2012; Milano, Kenney and Juenger, 2016).

In the context of climate change, ectotherms are particularly relevant models to study the mechanisms of microevolution/adaptation to fast environmental changes. The majority of aquatic organisms are obligate ectotherms/poikilotherms that must cope with thermal environment that influences their body temperature and all subsequent biological processes. In many fish species, boosted temperatures can alter fitness-related traits significantly, including survival, development, growth, and metabolism (Larsson and Berglund, 2005; Hemmer-Brepson *et al.*, 2014), and reproductive capacity and output of adults (King, Pankhurst and Watts, 2007; Pankhurst and King, 2010; Pankhurst and Munday, 2011).

Climate change globally threatens the maintaining of populations in the wild, but continental and freshwater systems are deemed particularly exposed (Heino, Virkkala and Toivonen, 2009). Due to its specific climate and topography, the alpine region is severely threatened by climate change-induced temperature increases (Bogataj, 2007). Global warming poses a serious threat to alpine and peri-alpine lakes (Adrian *et al.*, 2009) and knowledge on the evolutionary potential of lake species is critical to evaluate and predict the biogeographical implications of global change. Salmonids form a diverse and species-rich family that holds economic and heritage value, and are particularly relevant to the study of adaptation to thermal changes in ectotherms (Whoriskey, 2005). In particular, their embryos are energetically demanding life stages that are highly sensitive to environmental changes (Rombough, 2007), and undergo high mortality in the wild. Thermal constraints thus act as a strong selective pressure on these stages with potential critical outcomes to population growth.

Here, we investigate the plasticity and divergence among populations of a cold-adapted salmonid, the arctic charr *Salvelinus alpinus*. The arctic charr is the Northernmost freshwater fish in the world (Klemetsen, 2013) and, in Europe, the peri-alpine lakes region constitutes the natural southern edge of its range, albeit the species has been introduced at lower latitudes over the past two centuries mainly in high elevation lakes (Machino, 1995). Previous studies indicate very narrow thermal tolerance ranges for the early life stages of the species (< 6°C [Elliott and Elliott, 2010; Sæther, Siikavuopio and Jobling, 2016]) but investigations were essentially carried out on hatchery stocks (De March, 1995; Bebak, Hankins and Summerfelt, 2000; Jeuthe, Brännäs and Nilsson, 2015a; Jeuthe, Brännäs and Nilsson, 2015b), and information on wild populations is scarce (Swift, 1965; Jungwirth and Winkler, 1984). In alpine lakes, temperatures on charr spawning grounds exceed 8 °C during embryo development (Mari *et al.*, 2016). Given the occurrence of higher temperatures during embryonic development period in peri-alpine lakes compared to arctic charr habitat at higher latitudes, local adaptation of *S. alpinus* to temperature at the Southern limit of its range is likely but has never been tested. In addition, the adaptive potential of native *versus* introduced populations has never been investigated, although this potential may be lower in introduced populations often founded by a reduced number of individuals.

The goal of this study was thus to test the existence of genetically based differences in life history traits among wild arctic charr populations in response to thermal pressure. Specifically, we investigated the occurrence of i) phenotypic plasticity and GxE interactions in thermal reaction norms among populations from thermally contrasted habitats, ii) differences in molecular and quantitative genetic diversity among those populations, and iii) adaptive divergence on early-life history traits using a QST-FST approach.

Material and Methods

Experimental design

Study populations

This study was performed over winter 2015/2016 using four wild arctic charr populations located in different lakes of the alpine or sub-alpine region (Figure 1, Table 1). The arctic charr is native to lakes Geneva and Constance, where the populations remained landlocked following the glaciers retreat around 13.000 years ago, now representing the Southernmost native range limit of the species in Europe. As of today, these populations are still stocked yearly through supportive breeding, *i.e.* with 0+ individuals that are produced and reared in hatcheries from local wild-caught spawners, and released in the wild after having reached the first-feeding stage (Caudron *et al.*, 2014). In the past, both Geneva and Constance have further been stocked with occasional allochthonous fish input, but previous research showed these punctual introductions have had little to no impact on the genetic diversity (Brunner *et al.*, 2001) or genetic structure of these lakes (see Savary *et al.* [2017] for detailed study of Lake Geneva).

In lake Pavin, the species has been initially introduced in 1859 using 8000 alevins from the Huningue hatchery (Alsace, France) (Desmolles, 2016, Machino 1991). This hatchery translocated wild fish originating from the Rhine basin (Switzerland, Wurtemberg, and Bavaria, see Coumes, 1862), followed by a second introduction event between 1900 and 1906 using 2500 eyed eggs originating from lake Geneva (Reynouard 1909, Dussard 1955, Desmolles, 2016, Machino 1991). The Pavin population has been managed by supportive breeding since its introduction with no further allochthonous introduction documented. In lake Allos, the arctic charr was introduced in 1922 with 2500 alevins originating from lake Geneva, with the second and last introduction event documented in 1924 (Machino and Rivier, 2002). The Allos population is self-sustaining and has never been subject to management practices since 1924.

The four lakes follow an elevation gradient, constituting as such naturally different thermal conditions (Figure 1B). On charr spawning grounds, the temperature from

December to March averages 3-4°C in high elevation lakes Allos and Pavin that are ice-covered during that period. In lake Geneva, winter temperature on spawning grounds was known to be vary between 5.2°C and 7.1°C in the 90s' (Rubin and Buttiker, 1992), but recent observations showed that it could be even higher (>8°C, Mari *et al.*, [2016]). In Lake Constance, literature indicates temperatures of 5-6°C at depths corresponding to spawning grounds (80m deep, [Lieberherr and Wunderle, 2018]).

Sampling and crossings

At the beginning of spawning season (between mid-November and early December), spawners were sampled on spawning grounds using gill nets. For each population, individuals were crossed using 2 females x 3 males or 3 females x 4 males crossing designs (producing all possible sibgroup combinations), depending on the availability of mature individuals and female fecundity. We produced a total of 132 families (Table 1). All fish were measured, weighed, and pectoral fin clips were collected for genotyping (see below). We collected and dry-weighed 30 ova per female to calculate average egg size per family.

Rearing conditions

For the Geneva, Constance and Pavin populations, ova were fertilized in hatchery facilities near the lakes. Temperature was kept between 1 and 5° C during both fertilization and eggs transport to the INRA facilities in Thonon-les-Bains. For Allos population, spawners were transported for practical reasons to the INRA station where fertilization were performed. Following fertilization, eggs (n total = 8628) were distributed into 24-wells cell culture plates (Nunc MicroWell™), using three replicates of 12 eggs per family per treatment (one family was split across three plates, with 12 individuals per plate).

Eggs were exposed in thermal chambers to a temperature of either 5 °C (4.996 +/- 0.049 °C), referred to as cold or control treatment, or 8.5 °C (8.493 +/- 0.038 °C), referred to as warm treatment. Temperature was recorded every 30 minutes using HOBO loggers (HOBO Onset, #UX100-001) in both chambers.

Unfertilized eggs were recorded at eyed stage. Embryos were then monitored on a daily basis for mortality or hatching. Hatched fish could not be kept until yolk sac resorption in 24-wells plates. Thus, after hatching (five to nine days after recording the first hatched individual within a population) and until the end of the experiment, all live embryos from the same origin were pooled by families, split into two replicates and transferred in 50x50 mm incubators in a recirculating water circuit. They were monitored daily for mortality and dead individuals were removed. The experiment ended following near yolk sac absorption, at approximately 148 days post-fertilization (195 – 205 degree days) at cold and 100 days post-fertilization (215 – 230 degree days) at warm.

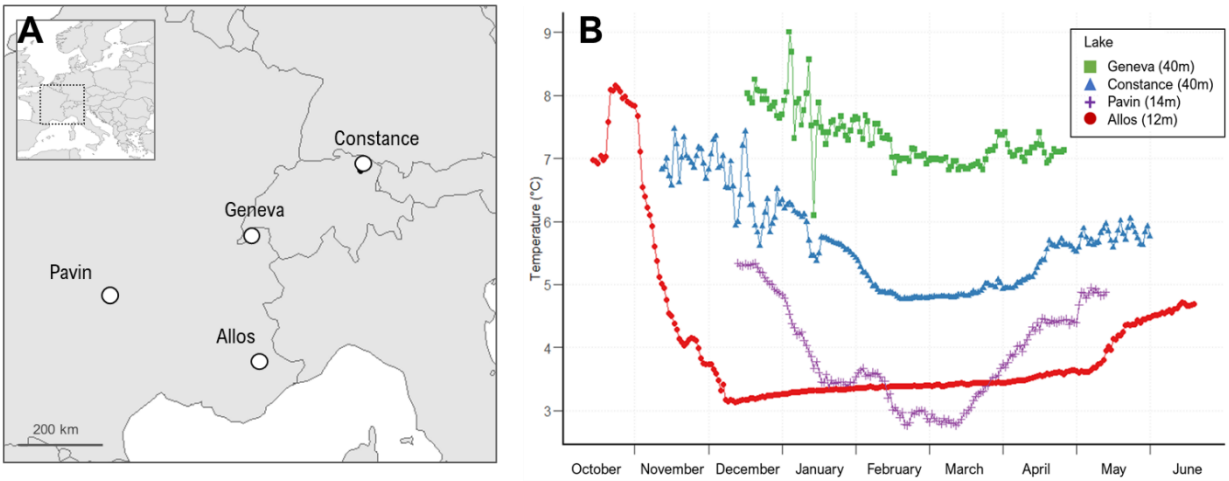

Figure 2-1 | (A) Location of the four study locations and (B) temperatures observed at depths corresponding to arctic charr spawning grounds in each lake, measured during winter 2014/2015.

Life history traits measurements

Survival at hatching was estimated as the number of successfully hatched individuals among those that reached the eyed stage. Overall survival was estimated as the number of living individuals from eyed stage to the end of the experiment. Incubation period was calculated in accumulated degree days (ADD) as the number of days from fertilization to hatching x average daily temperature.

Body size measurements were performed at two timepoints: (i) T1: At hatching, after being transferred into the recirculating water circuit so as to minimize the bias linked to strong differences in the length of incubation period among and within populations (aged between 468-488 ADD at cold, 512-564 ADD at warm) (ii) T2: at the end of the experiment, close to yolk resorption stage (740-752 ADD at cold, 862-866 ADD at warm). At both timepoints, all individuals were pooled per family and photographed (Nikon D5300, Nikon DX 18–105mm lens). Measurements were taken using IMAGEJ software (http://rsbweb.nih.gov/ ij/download.html). Length was measured as the total length (snout-tail length) in mm, and yolk sac volume was calculated after Kamler (2008) considering yolk sac shape as an ellipsoid, using the formula: $YSV = pi/6 \times L \times H^2$, with YSV: yolk sac volume, L: yolk sac length (longest axis), H: yolk sac height/width (shorter axis, perpendicular to L axis).

Because hatching was asynchronous but body size measurements of individuals of a same origin were taken at the same date, we standardized both length and yolk sac volume by the age at the respective timepoint (in ADD). Unbiased length at T1 was calculated as $length_{T1}$/ age at hatching, and at T2 as $Length_{T2}$ / age_{T2}, while unbiased yolk sac volume at T1 was calculated as YSV_{T1}/ age at hatching, and at T2 as YSV_{T2}/ age_{T2} (in ADD). For clarity, we will refer from now on to these two unbiased measurements as “length” and “yolk sac volume” only.

Data analysis

Phenotypic data

<u>Temperature effect on life history traits and differences among populations.</u> We first fitted a global model per trait using linear mixed models (LMMs). Individual size at hatching (T1) and near yolk resorption (T2), individual yolk sac volume at T1 and T2, and incubation period in ADD models were all fitted with normally distributed random errors using log-transformed data. Survival from eyed stage to T1 and overall survival were analyzed using generalized linear mixed models (GLMMs) fitted with a binomial error distribution. In all models, we included temperature, population and the interaction between the two as fixed factors, whereas sire

identity, dam identity, sire x dam interaction and plate or incubator were added as random factors. Because egg size is an important proxy for maternal effects, average egg size per female was added as a covariate in length, yolk sac, and incubation models (Kamler, 2005). We also fitted one model per population with temperature, sire, dam, sire x dam included as fixed factors, and plate identity as a random factor to investigate within population effects.

The choice of variables to be included in final models was based on likelihood ratio tests between models including the variable of interest or not. To investigate population differences within treatments, we performed post-hoc pairwise comparisons on selected models using the estimated marginal means method with a Benjamini-Hochberg correction for false discovery rate as implemented in the *emmeans* package (Lenth et al 2018). Traits were analyzed at T1 for all populations in both treatments, at T2 for all populations at warm and for three populations only at cold, due to the loss of Allos individuals following a technical issue in the cold treatment.

Adaptive variation. Within each temperature treatment, we calculated divergence in quantitative traits (QST) for all traits as follows: QST = $\sigma_{GB}^2/(\sigma_{GB}^2 + 2\sigma_{GW}^2)$, with σ^2_{GB} and σ^2_{GW} representing additive genetic variance components among and within-population, respectively (Merilä and Crnokrak, 2001). We calculated QST overall populations, but also pairwise populations QST to investigate differences among pairs of populations with different origins (native or introduced) or habitat (low or high elevation). We computed QST values and associated 95% confidence intervals on untransformed data after 10,000 simulation repeats using the *QstFstComp* package (Gilbert and Whitlock, 2015). Finally, we computed point estimates of narrow-sense heritability (h^2) for all traits at T1 and T2 for all population x temperature combinations by extracting variance components from LMMs and GLMMs using the *lme4* (Bates *et al.*, 2015) and *QGglmm* packages (de Villemereuil *et al.*, 2016) respectively. The models were fitted without any fixed effects so as not to overestimate the heritability values (Wilson *et al.*, 2009; Garcia-gonzalez *et al.*, 2012). Estimates were obtained as $h^2 = \sigma^2a/\sigma^2p$ where σ^2a is the additive genetic

variance, calculated as 4*σ^2sire, and σ^2p is the total phenotypic variance, calculated as the sum of residual and additive variances (Lynch and Walsh, 1998).

All analyses were performed in R-3.5.1 (R Core Team, 2016), with critical thresholds of significance set at α=0.05.

Neutral genetic variation

We genotyped 20-26 field-caught individuals per population using seven microsatellite markers known to be polymorphic in arctic charr (Brunner, Douglas and Bernatchez, 1998; Savary *et al.*, 2017). DNA was extracted from fin clips stored in 80° ethanol with a Qiagen BioSprint 96 extraction robot. The PCR cycles and multiplex genotyping were performed as indicated in (Savary *et al.*, 2017). Genotypes were scored with GENEMAPPER and analyzed with Micro-checker (Van Oosterhout *et al.*, 2003). No allelic dropouts or scoring errors were detected. Population genetics indices of expected (He) and observed heterozygosity (Ho), inbreeding coefficient (FIS), and pairwise FST (Weir & Cockerham, 1984) were calculated using GENEPOP. Allelic richness (Ar) was calculated with the *diveRsity* package in R (Keenan *et al.*, 2013).

Results

Thermal reaction norms

Overall, models indicated a significant temperature effect for all traits at hatching. A higher temperature induced up to a 15% decrease in survival at hatching and up to a 40% decrease in overall survival, and a significant increase in the incubation period in ADD. Populations varied markedly with respect to the incubation period: Allos individuals hatched earlier (436.14 +/- 10.4ADD at cold, 488.53 +/- 21.14ADD at warm), and Pavin individuals hatched generally later (489.83 +/- 27.65 ADD at cold, 548.14 +/- 22.75 ADD at warm), than offspring of other origins across both temperature treatments (Figure 2).

Body size at hatching was reduced at warm temperature, whereas individuals had bigger yolk sacs than in the cold treatment (Figure 2). Temperature still had a negative effect on size at near yolk resorption, but population-specific responses were weaker as no temperature x population interaction was detected (Figure 2). The Allos population differed from the other populations at T2 under warm conditions, as individuals exhibited higher survival and bigger body size but reduced yolk sac volumes. We did not systematically detect significant population differences but temperature x population effects were kept in all size models except length at T2 (Table 3, also evidenced by reaction norms on Figure 2).

Within all populations and at both life stages, dam effects were predominant. We detected significant dam x temperature and sire x temperature interactions in all populations (Table S2), but not all traits. Dam, sire, and interaction of both terms with treatment influenced survival, incubation and length at hatching. Dam and sire identity influenced yolk reserves at the near-emergence stage, but not at hatching.

Neutral genetic diversity

The overall FST was 0.095 (95% CI: 0.667 – 0.1255) and pairwise FST values ranged from 0.011 (+/- 0.07) to 0.16 (+/- 0.44) (Table 2). Pairwise estimates were lowest for Allos-Geneva and Constance-Pavin pairs and highest for Pavin-Geneva and Pavin-Constance comparisons, respectively. Heterozygosity and allelic richness were higher in the Constance and Pavin populations than in the other two populations (Table 1, Table S1). Inbreeding coefficients were significant and ranged from 0.10 to 0.17 across populations (Table 1).

Quantitative trait variation

Overall QST ranged from 0 to 0.81 depending on traits and treatments (Figure 3 and 4). QST was higher than FST for length at T1 for both treatments and for incubation and yolk sac volume at hatching under cold conditions. Pairwise QST estimates greatly varied across traits and populations pairs (range: 0 – 0.705 at cold; -0.059 – 0.81 at warm, Figure 4) but did not exceed FST, except for length at

T1 in the warm treatment, between the Allos and Pavin populations. However, we detected QST < FST in many pairwise comparisons and for both temperature treatments (Figure 4). For YSV at T1, QST was lower than FST for 8 out of 12 comparisons. Between the Allos and Geneva populations, only QST for YSV at hatching in the warm treatment was significantly lower than FST. For the Constance-Pavin pair, QST < FST were detected for survival at T1 and length at T2 in the cold treatment, and for incubation period in the warm treatment. For the Constance and Geneva QST < FST were observed for YSV at T1 & T2 at cold, and for survival at hatching, YSV at T1 and length at T2 at warm.

Heritability estimates were low to moderate and variable across populations and traits, ranging from 0 to 0.446, and were generally higher at cold than warm. The lowest estimates were found for survival, then YSV and higher values were detected for length at T1 and T2 and incubation period. For incubation and length at T1, lower heritability values in the warm treatment were mostly attributable to lower VA in this treatment. Interestingly for these two traits, we detected lower VA estimates for Allos and Geneva populations compared to Pavin and Constance (Table 4).

Table 2-1 | Descriptive characteristics of study populations with: source lake; altitude; ancestry; population genetic indices of inbreeding (FIS) with associated confidence intervals (bolded: significant values, p-value <0.05), expected (He) and observed (Ho) heterozygosity, allelic richness (Ar); and breeding design (number of blocks x number of females x number of males). Further populations genetic indices are presented in table S1.

Population	Alt. (m)	Species ancestry	Historical stocking	FIS (low – high)	Ho ; He	Ar	Breeding design
Allos	2225	Introduced	No	**0.15** (0.11 – 0.19)	0.63 ; 0.74	6.12	4 x 2x3
Pavin	1197	Introduced	Yes	**0.10** (0.03 – 0.18)	0.74 ; 0.82	7.5	3 x 3x4
Constance	395	Native	Yes	**0.16** (0.09 – 0.22)	0.72 ; 0.86	8.52	3 x 3x4
Geneva	372	Native	Yes	**0.17** (0.05 – 0.28)	0.59 ; 0.71	5.59	3 x 3x4

Table 2-2 | Pairwise FST among the four populations (all estimates were significant at 0.05 level).

Overall		Allos	Constance	Geneva
	Constance	**0.1127**		
	Geneva	**0.0110**	**0.0998**	
	Pavin	**0.1601**	**0.0255**	**0.1528**

Discussion

Using a common garden experimental design, we investigated divergence in early life history traits in response to elevated temperatures among native and introduced arctic charr populations. We detected an effect of temperature on reaction norms for all traits both shortly after hatching (T1), and at near yolk resorption (T2).

Temperature was associated with a reduction of individual size at both timepoints. In the warm treatment, individuals hatched generally earlier and were smaller with bigger yolk sac volumes, indicating they hatched at an earlier developmental stage compared to conspecifics in the cold treatment (Kamler 2008). Larval development rates increase with temperature has been shown in numerous aquatic taxa (Pankhurst and Munday, 2011).

We report much higher survival rates than observed in other studies on the effects of temperature on early life stages of arctic charr (Janhunen, Piironen and Peuhkuri, 2010; Jeuthe, Brännäs and Nilsson, 2015). Temperature can affect survival prior to eyed stage but we could not measure the proportion of dead *vs.* unfertilized eggs at this stage, and thus considered survival from eyed stage only. However, no differential mortality was observed between fertilization and eyed stage between thermal treatments, indicating temperature had no lethal impact on the earliest stages of development.

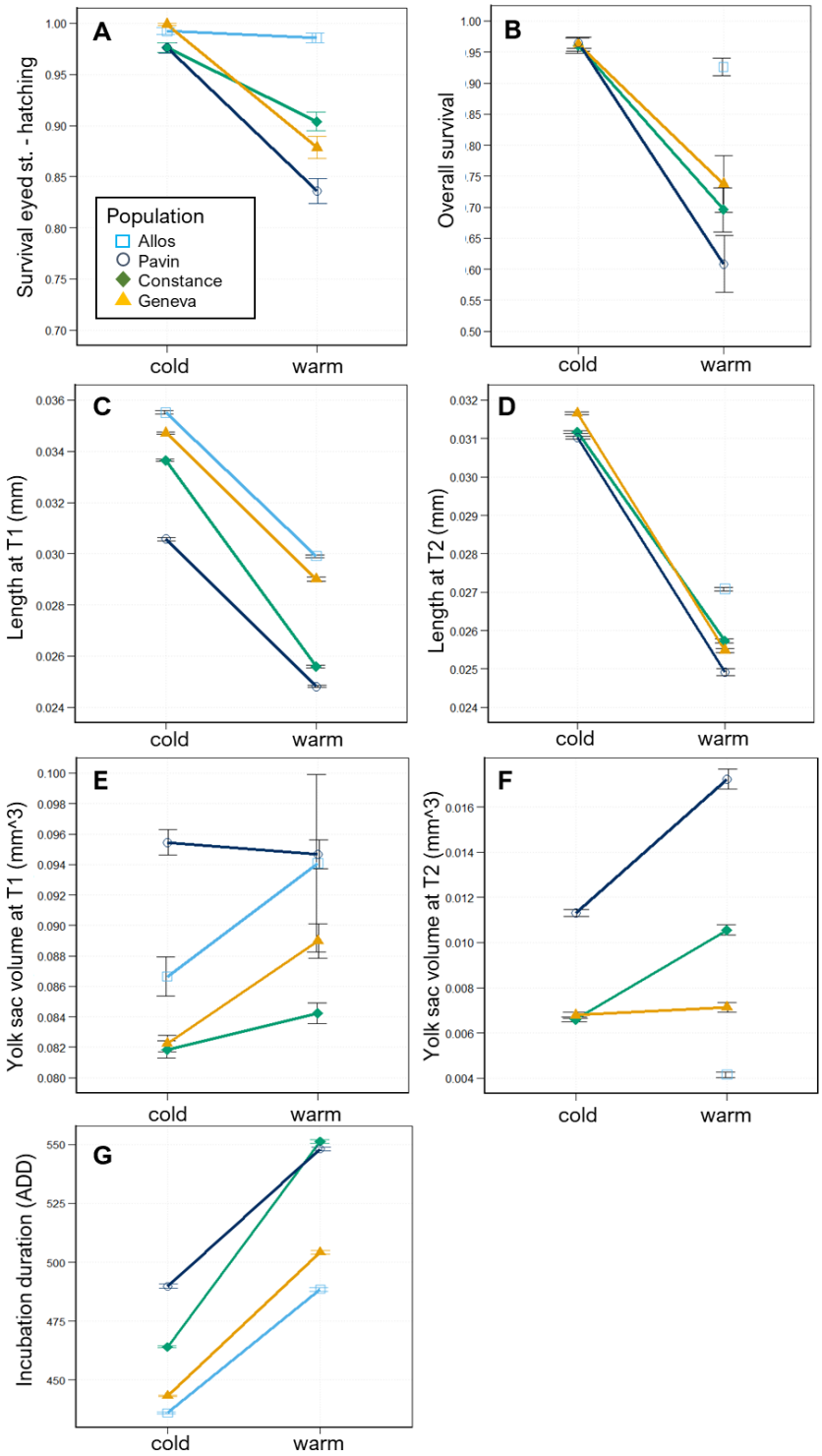

Figure 2-2 | Thermal reaction norms for (A) survival at hatching, (B), overall survival, (C) length at T1, (D) length at T2, (E) yolk sac volume at T1, (F) yolk sac volume at T2, (G) incubation duration in ADD for the four study populations (light blue: Allos, dark blue: Pavin, green: Constance, orange: Geneva). Open symbols specify cold-origin populations, and filled symbols specify warm-origin populations. Values are expressed as mean +/- s.e.m.

Thermal reaction norms

The significant population x temperature interactions revealed genetically-based thermal reactions norms. We had initially hypothesized that the study populations would be adapted to the thermal regime that was closest to their local environments. Thus, we expected the two populations from warmer locations (Geneva and Constance) to outperform the two smaller populations from colder environments (Allos and Pavin) under warm conditions, *e.g.* by producing larger individuals and showing higher survival rates. Although this was not the case, the four populations showed contrasted responses at both timepoints. Intriguingly, the cold-originating population of Allos exhibited distinct reaction norms with higher survival rates, earlier hatching and bigger individuals than the other populations, clearly corresponding to a better performance than other populations. At T2, in the warm treatment, Allos alevins were bigger with smaller yolk sacs than alevins of other origins. This morphology may indicate that Allos individuals can convert yolk reserves into larval tissue more efficiently at warm than individuals of other origin ; and may reveal a strategy to cope with a warm environment that differs from the three other populations as emerging bigger with smaller yolk allows maximizing movement and predator escape (Skoglund, Einum and Robertsen, 2011).

Neutral and adaptive population divergence

Levels of pairwise population differentiation based on neutral markers were moderate to high and revealed two pairs of weakly differentiated populations, consistently with the information available on introduction events in study populations (. Overall QST estimates revealed divergent selection on all traits at hatching except survival; in all other traits, QST did not significantly differ from FST, hence divergence for those traits can be ascribed to drift. Divergent selection as seen from QST-FST comparisons in salmonids has been well documented by previous investigators (Perry, Audet and Bernatchez, 2005; Jensen *et al.*, 2008; Rogell *et al.*, 2013; McKinney *et al.*, 2014; Côte *et al.*, 2016), but evidence of adaptive divergence among recently founded populations (*e.g.* less than 100 years

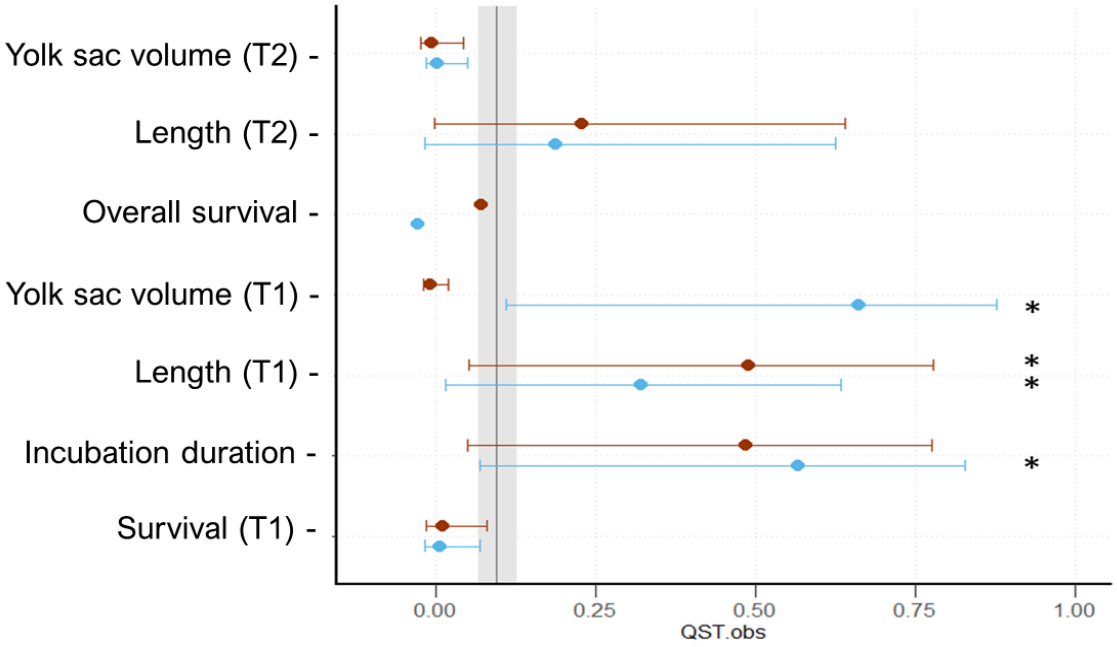

Figure 2-3 | Overall QST estimates and associated 95% CI for traits at T1 & T2 across environments (blue: cold treatment, red: warm treatment). Vertical line and shaded area indicate overall FST estimate and 95% CI calculated by bootstrapping over loci. Asterisks indicate significant differences between QST and FST values.

for Allos) has been more rarely reported (Koskinen, Haugen and Primmer, 2002; Whitney, Hinch and Patterson, 2013). In contrast, pairwise QST showed a different pattern with evidence of uniform selection in many cases. Uniform selection for survival can be expected for such a trait highly related to fitness, which should be maximized in every habitat. In contrast, such a pattern for morphological traits like yolk volume at hatching and embryo length is more surprising but could be expected between similar environments like the Geneva and Constance lakes. Other studies have documented QST < FST results (Andersen *et al.*, 2007; Chapuis *et al.*, 2007; Lamy *et al.*, 2011), but there might be a general bias toward a discreet reporting of such patterns compared to QST > FST (Lamy *et al.*, 2012). Trait conservatism can be a consequence of uniform selection that always leads to QST lower than FST. However, QST < FST patterns may emerge even under diversifying selection in small populations with reduced gene flow, where the

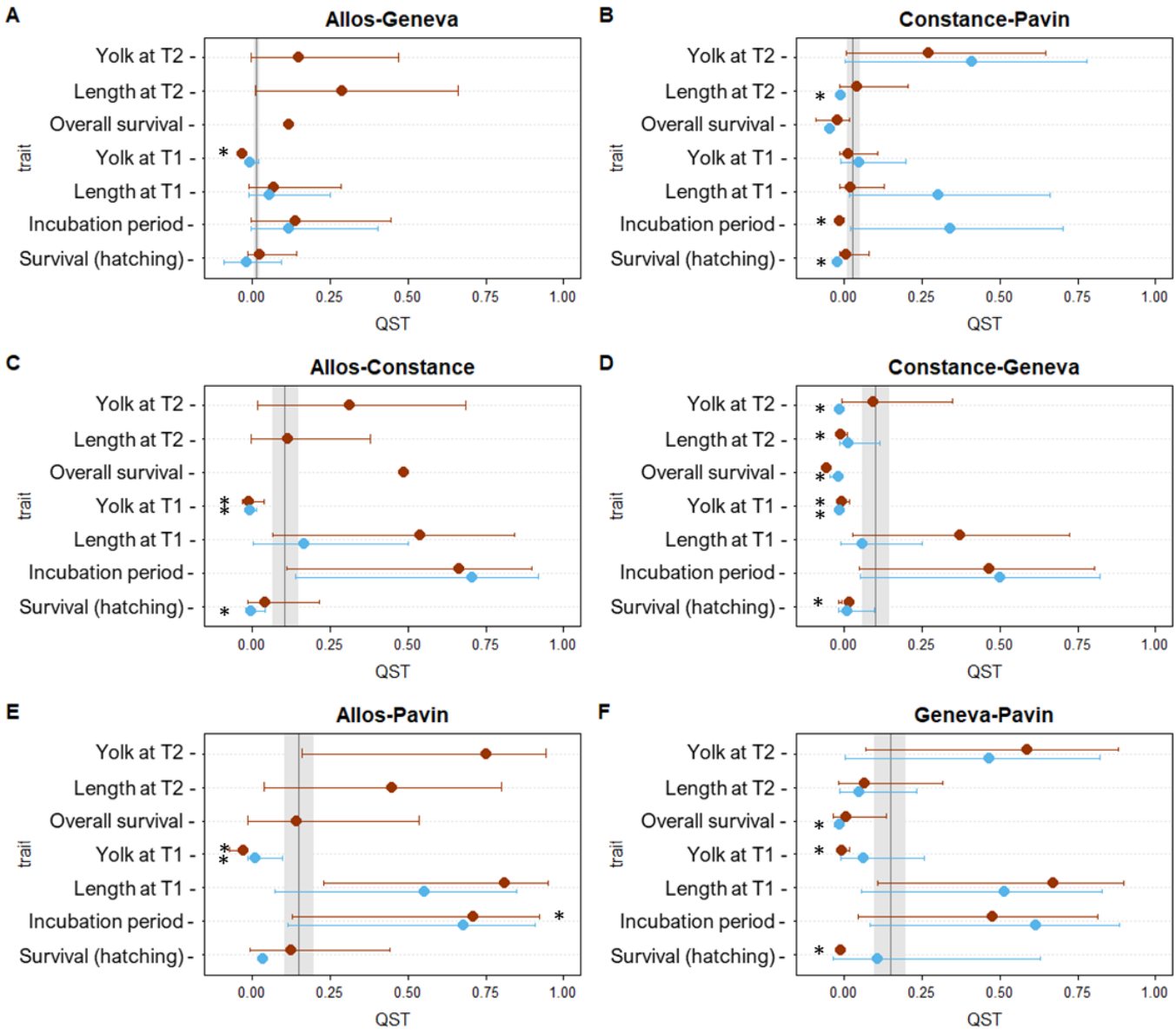

Figure 2-4 | QST estimates and associated 95% CI for traits at T1 & T2 across environments (blue: cold treatment, red: warm treatment) between pairs of populations: (A) Allos – Geneva (B) Constance – Pavin (C) Allos – Constance (D) Constance – Geneva (E) Allos – Pavin (F) Geneva – Pavin. Vertical lines and shaded areas indicate pairwise FST estimates and 95% CI calculated by bootstrapping over loci. Asterisks indicate significant differences between QST and FST values.

among-population variance due to drift can become larger than among-population variance due to differences in local optima (Lamy *et al.*, 2012; Moore *et al.*, 2017). In particular, the divergence among recently founded populations (Allos and Pavin) may be low due to a stronger role of drift relative to selection in such small populations. Accordingly, the fact that many overall and pairwise comparisons showed a pattern of QST not differing from FST further supports this hypothesis. Alternatively, canalization could be another explanation to QST < FST, as traits could share similar genetic constraints rather than identical selective optima (Flatt, 2005; Lamy *et al.*, 2012).

Finally, it is important to consider that the different patterns we observed between overall and pairwise QST-FST comparisons, may be explained by statistical reasons. First, it has been acknowledged that it is more difficult to detect significant differences between QST and FST in pairwise comparisons due to a loss of statistical power (Gilbert and Whitlock, 2015). Second, the significance of QST can be estimated with several approaches that can reveal contrasted results (*e.g.* (Leinonen *et al.*, 2013)), hence it may be interesting to compare QST estimates and their confidence intervals using several methods, *e.g.* bootstraping *versus* Bayesian approaches.

Within population diversity

Local adaptation also depends on the adaptive potential of populations, which relates to the amount of genetic variance, itself linked to trait heritability. We observed very low heritability values for survival at T1, and almost null heritability for overall survival. Such fitness related traits are expected to have a lower heritability than morphological traits because they are supposedly under stronger selective pressures that tend to reduce additive genetic variance (Merila and Sheldon, 1999). In addition, we observed lower heritability estimates in the warm treatment for most traits, which is consistent with conclusions from Charmantier and Garant (2005) who reported a trend for lower heritability values for traits measured under stressful conditions in wild populations.

Table 2-3 | Mixed models testing for temperature, population, and temperature x population effects on traits (survival at hatching, overall survival, incubation period, and the following corrected by age at corresponding timepoint: length at hatching (T1), length at T2, yolk sac volume at hatching and yolk sac volume at T2). Bolded values indicate significant (<0.05) p-values obtained from likelihood ratio tests.

		Traits at hatching							
		Survival (n=6955)		Incubation (n=6954)		Length (n=4946)		YSV (n=4946)	
fixed effects	df	x^2	p value	x^2	p value	x^2	p value	x^2	p value
T°C	1	14.817	**<0.001**	101.69	**<0.001**	100.95	**<0.001**	6.207	**0.012**
population	3	4.5568	0.207	80.771	**<0.001**	67.825	**<0.001**	6.511	0.089
T°C x population	3	3.27	0.351	25.875	**<0.001**	34.712	**<0.001**	23.457	**<0.001**
egg dry weight	1	0.224	0.636	0.805	**0.369**	12.508	**<0.001**	36.06	**<0.001**
ADD	1	-	-	-	-	-	-	-	-
		Traits at T2							
		Overall survival (n=224)		Length (n=4946)		YSV (n=4946)			
fixed effects	df	x^2	p value	x^2	p value	x^2	p value		
T°C	1	22.94	**<0.001**	143.86	**<0.001**	15.026	**<0.001**		
population	3	13.674	**0.003**	28.671	**<0.001**	56.469	**<0.001**		
T°C x population	3	2	1	5.555	0.062	16.092	**<0.001**		
egg dry weight	1	ns	ns	36.255	**<0.001**	20.168	**<0.001**		
ADD	1	-	-	-	-	-	-		

Neutral and additive diversity were stronger for the Constance-Pavin pair compared to the Geneva-Allos pair. Theoretically, high neutral diversity can be associated with a strong adaptive potential. In the case of Pavin, despite strong admixture (Figure S1) and high neutral diversity (He, Table 1), the population does not seem to benefit from this diversity as reflected by lower performances of this population under stressful conditions. We can hypothesize that the history of introductions of allochthonous fish might have increased neutral diversity, but not the adaptive potential in a context of climate change.

The lower VA estimates for Allos and Geneva populations compared to Pavin and Constance mirror the patterns of heterozygosity and allelic richness, which could be linked to their respective effective population sizes and management history (*i.e.* introduction events and stocking practices). In theory, small populations suffer from reduced genetic variation (Koskinen, Haugen and Primmer, 2002; Willi, Van Buskirk and Hoffmann, 2006; Abbott, Doak and Peterson, 2017) and hence a reduced adaptive potential relative to populations of greater size (Willi, Van Buskirk and Hoffmann, 2006; Chevin, Lande and Mace, 2010). In addition, adaptive potential

can be further reduced by the accumulation of deleterious mutations through inbreeding, a phenomenon that cannot be excluded in the four study populations where significant inbreeding coefficients were observed. In addition, the detection of significant temperature x sire interactions in a bigger set of traits for Constance and Pavin compared to Allos and Geneva, may also suggest a higher adaptive potential to temperature variations in those two populations (Table S2).

Table 2-4 | Narrow sense heritability (h^2), additive genetic variance (VA), and phenotypic variance (VP) estimates measured for all traits at both stages among the four populations. Note that heritabilities could not be calculated for the Allos population at cold because individuals were lost.

			cold			warm		
			VP tot	VA	h^2	VP tot	VA	h^2
T1								
	Survival	Allos	0,037	0,0025	0,066	0,014	7,57E-06	0,0005
		Pavin	0,023	0	0	0,147	0,024	0,161
		Constance	0,027	2,76E-15	1,02E-13	0,089	0,008	0,097
		Geneva	0,001	0	0	0,106	0	0
	Incubation period	Allos	111,28	37,502	0,337	451,93	0	0
		Pavin	1075,58	231,633	0,215	528,08	74,938	0,142
		Constance	144,799	64,678	0,446	718,68	166,775	0,232
		Geneva	73,055	14,44	0,198	8497,199	1,78E-09	2,10E-13
	Length	Allos	1,31E-06	2,78E-07	0,212	1,78E-06	6,68E-20	3,75E-14
		Pavin	2,69E-06	7,00E-07	0,286	1,38E-06	4,21E-07	0,305
		Constance	2,38E-06	8,43E-07	0,354	2,60E-06	7,09E-07	0,27
		Geneva	1,49E-06	2,32E-07	0,155	3,11E-06	2,71E-19	8,70E-14
	YSV	Allos	0,0005	0	0	0,024	0	0
		Pavin	0,0004	0	0	0,0005	2,15E-18	4,18E-15
		Constance	0,0003	1,40E-05	0,038	0,0004	3,25E-05	0,076
		Geneva	0,0002	1,46E-05	0,055	0,0005	0	0
T2								
	Overall survival	Allos				0	0	0
		Pavin	0,013	0	0	0,197	0,02	0,1
		Constance	0	0	0	0	0	0
		Geneva	0	0	0	0	0	0
	Length	Allos				0,906	0,114	0,126
		Pavin	0,867	0,074	0,086	2,771	0	0
		Constance	0,876	0,294	0,33	2,304	0,637	0,276
		Geneva	0,752	0,171	0,228	1,662	0,279	0,168
	YSV	Allos				5,778	0	0
		Pavin	10,887	0,515	0,047	67,159	4,725	0,07
		Constance	6,07	1,358	0,224	29,667	8,987	0,303
		Geneva	7,941	0,579	0,073	21,935	0	0

Impacts of stocking

Unlike the populations of Geneva, Constance, and Pavin, the Allos population has never been managed, despite having been introduced for fishing purposes (Yoichi Machino and Rivier, 2002). Besides, the three managed populations have known periodic declines over the second half of the 20th century, due to overfishing and poor water quality during eutrophication periods; following which supportive breeding practices have been set up to attempt compensating fisheries catches decrease (Caudron *et al.*, 2014; Desmolles, 2016). The Allos population has hence been considered as holder of the lake Geneva genetic diversity prior to the eutrophication-induced population decline in the 1970s (Machino and Rivier, 2002; Potvin and Bernatchez, 2003).

Thus, we hypothesize that the observed responses might have been partly caused by the genetic stress induced by supportive breeding practices in these lakes. Supportive breeding can affect the genetic quality of a population because i) it relies on randomly selecting a limited number of spawners, and ii) egg and larval rearing in hatchery conditions are often more optimal than they should be in the wild, likely leading to maladapted individuals being released into the wild (Meraner, Cornetti and Gandolfi, 2014; Hagen *et al.*, 2019). This is particularly concerning considering stocked individuals have been shown to constitute up to half of the catches in lake Geneva (see Caudron *et al.*, 2014). In the same lake, a recent study (Savary *et al.*, 2017) reported that the neutral genetic diversity of the arctic charr population fluctuated over time, but returned to pre-eutrophication levels following supportive breeding, and that within-lake population genetic structure has not changed, a result that corroborates the similarity we observed between Allos and Geneva from our STRUCTURE plot and low FST estimate. We suspect hatchery selection might alter the adaptive potential of populations under future stressful conditions.

Conclusion

We expected temperature to have drastic effects on early life history traits, and reveal genetic differences in favor of populations from warmer environments. Our results do not support the latter pattern, and highlight a higher sensitivity to temperature of warm-origin populations instead, associated with a lower genetic diversity and limited adaptive potential. We showed genetically-based population divergence during early life that relates to different environments, but we cannot exclude that drivers other than temperature could play an important role in population differentiation. These results also raise questions on local stocking practices that benefited the neutral diversity of populations, but not their adaptive potential. The recent stocking efforts in alpine lakes might have long-term detrimental effects on the populations' capacity to respond to unfavorable thermal conditions, and management protocols should be tailored accordingly.

Acknowledgements

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Supplementary files

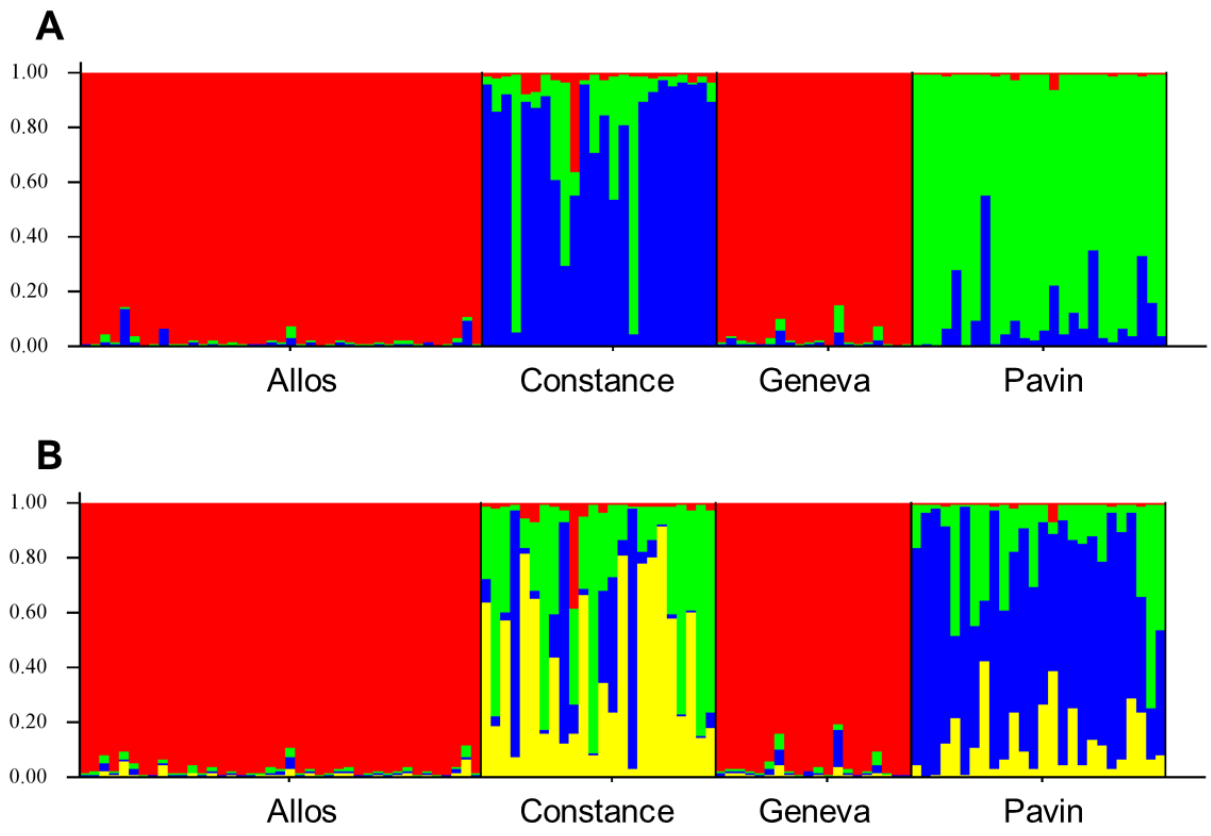

Figure 2-S1 | Genetic cluster barplots identified from STRUCTURE runs (length of burnin: 10 000, 10 000 MCMC reps), representing the probability of assignment of individual genotypes from each population with (A) 3 assumed genetic clusters (K=3), (B) 4 assumed genetic clusters (K=4).

Table 2-S1 | Summary statistics for the seven microsatellite loci in each population with: N/ number of genotyped individuals, A: number of alleles, Ar: allelic richness, Ho: observed heterozygosity, He: expected heterozygosity, HWE: deviation from H-W equilibrium, FIS, FIS_low, and FIS_high: inbreeding coefficient and associated lower and higher confidence intervals.

population		Cocl3-N	OtsG253b	Ssa456	Sfo8	Sfo23	Ssa85	Omy301	Overall
Allos									
	N	111	111	111	120	120	120	119	116
	A	13	9	6	15	10	15	10	78
	%	48.15	64.29	37.5	68.18	50	45.45	62.5	53.72
	Ar	6.4	5.39	4.96	6.97	5.86	7.8	5.44	6.12
	Ho	0.46	0.83	0.67	0.68	0.42	0.78	0.58	0.63
	He	0.8	0.74	0.69	0.75	0.74	0.8	0.65	0.74
	HWE	0	0.781	0.702	0.068	0	0.887	0.165	0
	Fis	0.4249	-0.1202	0.0345	0.1053	0.4364	0.0372	0.1072	0.1499
	Fis_Low	0.2923	-0.195	-0.0861	0.0012	0.3191	-0.0476	-0.0129	0.1094
	Fis_High	0.5471	-0.0423	0.1592	0.2116	0.5558	0.1304	0.2256	0.1909
Constance									
	N	20	20	20	24	24	24	24	22.29
	A	14	8	12	13	15	19	10	91
	%	51.85	57.14	75	59.09	75	57.58	62.5	62.59
	Ar	9.07	6.29	8.39	8.48	9.07	11.51	6.82	8.52
	Ho	0.75	0.85	0.9	0.5	0.71	0.88	0.46	0.72
	He	0.89	0.82	0.88	0.87	0.83	0.9	0.81	0.86
	HWE	0.282	0.832	0.593	0	0	0.013	0.001	0
	Fis	0.1608	-0.0398	-0.0256	0.4234	0.1438	0.0326	0.4316	0.1589
	Fis_Low	-0.0377	-0.2083	-0.152	0.2006	-0.0246	-0.0761	0.1825	0.0948
	Fis_High	0.3752	0.1377	0.123	0.638	0.3122	0.1494	0.6591	0.2228
Geneva									
	N	20	20	20	20	20	20	20	20
	A	8	6	4	8	7	12	7	52
	%	29.63	42.86	25	36.36	35	36.36	43.75	35.57
	Ar	6.2	4.73	3.71	6.29	5.19	7.49	5.53	5.59
	Ho	0.65	0.55	0.65	0.55	0.35	0.8	0.6	0.59
	He	0.75	0.66	0.67	0.71	0.67	0.76	0.76	0.71
	HWE	0.215	0.159	0.722	0	0	0	0.145	0
	Fis	0.1376	0.1729	0.0244	0.224	0.4747	-0.0475	0.2144	0.1679
	Fis_Low	-0.1702	-0.068	-0.3353	-0.0206	0.1429	-0.1884	-0.0918	0.0536
	Fis_High	0.4179	0.4435	0.352	0.4884	0.7626	0.1064	0.5072	0.2793
Pavin									
	N	25	25	25	26	26	26	26	25.57
	A	13	9	11	7	12	14	6	72
	%	48.15	64.29	68.75	31.82	60	42.42	37.5	50.42
	Ar	8.6	7.46	8.27	5.54	8.06	9.28	5.32	7.5
	Ho	0.64	0.92	0.92	0.81	0.5	0.88	0.5	0.74
	He	0.87	0.83	0.84	0.75	0.82	0.88	0.77	0.82
	HWE	0	0.129	0.124	0.136	0.003	0.001	0.018	0
	Fis	0.2681	-0.1111	-0.0921	-0.0812	0.3937	-0.0067	0.3469	0.1022
	Fis_Low	0.0482	-0.2067	-0.2229	-0.3289	0.2096	-0.1232	0.0786	0.0262
	Fis_High	0.4849	0.0048	0.05	0.1433	0.5998	0.1421	0.6021	0.177

Table 2-S2 | Model testing for temperature, dam, sire, and interactions effects within each population for all traits at hatching.

Geneva

	Survival		Incubation		Length		YSV	
	x^2	p value	x^2	p value	x^2	p value	x^2	p value
fixed effects								
temperature	74.767	**<0.001**	287.07	**<0.001**	202.69	**<0.001**	7.928	**0.004**
dam	166.26	**<0.001**	225.07	**<0.001**	5.623	**0.017**	1.803	0.179
sire	9.831	0.364	47.864	**<0.001**	0.40	0.526	18.252	**<0.001**
dam x sire	11.255	0.734	16.814	0.33	3.848	**0.049**	3.849	0.146
temperature x dam	3.355	0.85	135.01	**<0.001**	1.945	0.163	0.031	0.859
temperature x sire	4.384	0.957	87.135	**<0.001**	1.462	0.226	0.144	0.705
temperature x dam x sire	16.027	ns	47.726	**0.021**	1.99	0.158	2.132	0.344
egg dry weight	-	-	-	-	-	-	-	-
ADD	-	-	-	-	-	-	-	-

Constance

	Survival		Incubation		Length		YSV	
	x^2	p value	x^2	p value	x^2	p value	x^2	p value
fixed effects								
temperature	25.199	**<0.001**	349.52	**<0.001**	324.39	**<0.001**	0.676	0.411
dam	110.51	**<0.001**	297.06	**<0.001**	9.805	**0.002**	27.44	**<0.001**
sire	30.081	**<0.001**	65.339	**<0.001**	20.422	**<0.001**	10.468	**0.001**
dam x sire	39.266	**0.003**	29.554	**0.042**	4.948	**0.026**	0.287	0.592
temperature x dam	21.693	**0.001**	93.389	**<0.001**	3.87	**0.049**	0.796	0.372
temperature x sire	22.171	**0.023**	110.43	**<0.001**	8.115	**0.004**	0.773	0.679
temperature x dam x sire	5.135	0.999	45.377	**<0.001**	3.309	0.069	1.094	0.578
egg dry weight	-	-	-	-	-	-	-	-
ADD	-	-	-	-	-	-	-	-

Pavin

	Survival		Incubation		Length		YSV	
	x^2	p value	x^2	p value	x^2	p value	x^2	p value
fixed effects								
temperature	53.464	**<0.001**	209.93	**<0.001**	260.82	**<0.001**	0.203	0.652
dam	86.208	**<0.001**	131.12	**<0.001**	1.156	0.282	0.029	0.864
sire	34.64	**<0.001**	43.633	**<0.001**	3.494	0.061	13.602	**<0.001**
dam x sire	26.381	**0.034**	17.487	0.291	6.72	**0.01**	1.779	0.182
temperature x dam	13.652	**0.018**	147.11	**<0.001**	0.057	0.81	0.119	0.73
temperature x sire	23.286	**0.0161**	49.109	**<0.001**	0.322	0.57	0.158	0.691
temperature x dam x sire	8.472	0.904	34.345	0.267	1.71	0.679	1.876	0.391
egg dry weight	-	-	-	-	-	-	-	-
ADD	-	-	-	-	-	-	-	-

Allos

	Survival		Incubation		Length		YSV	
	x^2	p value	x^2	p value	x^2	p value	x^2	p value
fixed effects								
temperature	1.118	0.29	208.03	**<0.001**	188.06	**<0.001**	0.415	0.519
dam	1.579	0.979	53.577	**<0.001**	0.4436	0.505	5.834	**0.015**
sire	11.26	0.422	3.934	0.863	2.175	0.14	1.452	0.228
dam x sire	6.5093	0.883	11.181	0.192	27.051	**<0.001**	0.033	0.855
temperature x dam	ns	ns	19.77	**0.006**	1.117	0.29	0.034	0.752
temperature x sire	ns	ns	29.131	**0.023**	0.054	0.817	0.069	0.793
temperature x dam x sire	ns	ns	45.765	**<0.001**	0.564	0.453	0.034	0.983
egg dry weight	-	-	-	-	-	-	-	-
ADD	-	-	-		-	-	-	-

Table 2-S3 | Model testing for temperature, dam, sire, and interactions effects within each population for all traits at swimup. Note: the temperature effect could not be tested in the Allos population.

Geneva

	Survival		Length		Yolk sac volume	
	x^2	p value	x^2	p value	x^2	p value
fixed effects						
temperature	6.987	**0.008**	326.43	**<0.001**	0.344	0.557
dam	16.847	**0.032**	65.896	**<0.001**	48.4	**<0.001**
sire	8.658	0.372	38.904	**<0.001**	11.352	0.252
dam x sire	18.697	**0.044**	36.74	**0.006**	23.399	0.176
temperature x dam	1.797	0.616	18.653	**0.009**	26.356	**<0.001**
temperature x sire	6.063	0.532	16.574	0.121	19.458	0.078
temperature x dam x sire	ns	ns	23.255	0.505	121.93	**<0.001**
egg dry weight	-	-	-	-	-	-
ADD	-	-	-	-	-	-

Constance

	Survival		Length		Yolk sac volume	
	x^2	p value	x^2	p value	x^2	p value
fixed effects						
temperature	15.991	**<0.001**	296.8	**<0.001**	64.689	**<0.001**
dam	13.469	0.09	113.13	**<0.001**	95.51	**<0.001**
sire	15.172	0.174	36.267	**<0.001**	44.508	**<0.001**
dam x sire	ns	**0.024**	33.245	**0.016**	30.492	**0.03**
temperature x dam	ns	**<0.001**	46.795	**<0.001**	42.805	**<0.001**
temperature x sire	ns	**<0.001**	84.635	**<0.001**	13.007	**0.043**
temperature x dam x sire	ns	**<0.001**	38.053	**0.002**	69.53	**<0.001**
egg dry weight	-	-	-	-	-	-
ADD	-	-	-	-	-	-

Pavin

	Survival		Length		Yolk sac volume	
	x^2	p value	x^2	p value	x^2	p value
fixed effects						
temperature	22.463	**<0.001**	284.79	**<0.001**	64.26	**<0.001**
dam	26.909	**<0.001**	40.651	**<0.001**	23.379	**0.003**
sire	13.434	0.1439	12.091	0.208	10.22	0.333
dam x sire	17.114	0.104	20.156	0.166	16.982	0.32
temperature x dam	1.867	0.76	7.661	0.363	20.829	**0.04**
temperature x sire	ns	ns	10.545	0.482	45.599	**<0.001**
temperature x dam x sire	ns	ns	63.395	**0.036**	65.096	**<0.001**
egg dry weight	-	-			-	-
ADD	-	-	-	-	-	-

Allos - warm only

	Survival		Length		Yolk sac volume	
	x^2	p value	x^2	p value	x^2	p value
fixed effects						
temperature						
dam	5.191	0.637	88.877	**<0.001**	74.436	**<0.001**
sire	4.332	0.888	26.029	**0.001**	20.434	**0.009**
dam x sire	1.397	0.706	8.23	0.411	14.312	0.074
temperature x dam						
temperature x sire						
temperature x dam x sire						
egg dry weight	-	-			-	-
ADD	-	-	-	-	-	-

3. **Temperature as a constraint on tolerance to other stressors** – the example of fine sediments

Foreword

Although thermal environment can have important consequences on the growth and development of arctic charr until emergence, early life stages are in fact exposed to a variety of abiotic pressures. In freshwater systems, fine sediment deposition is a major concern as it can smother the habitat of benthic species and benthic life stages of pelagic species, limiting oxygen access and rendering the environment hypoxic. If we consider the separate impacts of temperature and sediment from a physiological perspective, temperature induces an increase in metabolic rates and oxygen demand, while sediment reduces oxygen availability. We can therefore expect, if the two pressures were to be experienced simultaneously, that they would negatively affect the performance of embryos in a synergistic way.

In salmonids, the detrimental effects of fine sediment have been long studied due to their concerning impact on vulnerable early life stages, but knowledge is essentially limited to assessing it a single stressor. An updated view of the impacts of sediment on fish in a global warming context is needed.
Aiming at assessing whether temperature could modulate the effects of sediment on embryonic traits, the experiment presented in the following article brings insights into the consequences of multiple stressor interactions and is the first study to examine the influence of temperature on the impacts of fine sediment in early life traits of fish.

Article information

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The accompanying dataset is accessible online at https://data.mendeley.com/datasets/9scmvsmjk3/1. DOI: 10.17632/9scmvsmjk3.1

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Higher temperature exacerbates the impact of sediments on embryo performances in a salmonid

Mari, Lisandrina; Garaud, Laura; Evanno, Guillaume; Lasne, Emilien

Abstract

In a warming climate, higher temperatures are likely to modulate positively or negatively the effect of other environmental factors on biota, although such interactions are poorly documented. Here, we explore under controlled conditions the combined effects of two common stressors in freshwater ecosystems, higher temperature and sediment load, on the embryonic development of arctic charr (*Salvelinus alpinus L.*). In the warm treatment, embryos had a lower survival, a longer incubation period and a smaller body size with a bigger yolk sac volume. Our data show a significant interaction between temperature and sediment load with temperature increasing dramatically the negative effects of sediment load on fitness-related traits. In the climate change context, these findings highlight the importance of taking into account different thermal scenarios when examining the effect of environmental or anthropogenic stressors.

Introduction

Freshwater ecosystems offer numerous services to human societies but are nowadays facing multiple stressors and are generally threatened (Dudgeon *et al.*, 2006). Impacts of stressors including higher temperatures, reduced dissolved oxygen concentrations or water pollution have been largely considered separately but a growing body of literature shows that they may combine in various manners, including additive, antagonistic or synergistic interactions (Cramp *et al.*, 2014; Piggott, Townsend and Matthaei, 2015a). In a multiple stressors context, understanding how distinct pressures interact is critical to predict their consequences on ecosystems and develop efficient management and risk assessment tools (Ormerod *et al.*, 2010).

Temperature is a key driver of many biological processes in aquatic ecosystems. In ectotherms (*i.e.* most aquatic organisms), temperature directly influences metabolism, development and growth rates and energy allocation to maintenance and synthesis, and subsequently determines body size and age at maturity (Zuo *et al.*, 2012). Temperature also drives major physico-chemical processes, including the solubility of gases such as oxygen. As climate warms, average water temperature increases following air temperature, and analyzing the role of temperature in modulating other stressors is a key issue that remains poorly explored (Heugens and Hendriks, 2001; Piggott *et al.*, 2015).

Fine sediments, a common pressure in freshwaters, are a major stressor for incubating eggs and yolk sac alevins of benthic spawners such as salmonids (S. M. Greig, Sear and Carling, 2007). Sediments may clog up substrate, rendering the environment hypoxic around the egg (Kemp *et al.*, 2011). Effects of temperature–hypoxia interactions have often been demonstrated in fishes (reviewed in McBryan *et al.* [2013]), yet most studies assessed such effects on metabolic rates solely, and none addressed their impact on early life-history traits or the interaction between temperature and sediment-induced hypoxia.

Here, we investigate the individual and combined effects of temperature and sediments on embryonic life-history traits in arctic charr (*Salvelinus alpinus L.*) under experimental conditions. Considering that i) high temperatures are associated with an increased aerobic metabolic demand and ii) increased sediment loads reduce oxygen availability, we predicted that the two pressures combined would alter embryo performance synergistically.

Material and methods

Study species

The arctic charr is a stenothermic cold-water salmonid that requires temperatures under 8°C for optimal spawning and egg incubation (Elliott and Elliott, 2010). Spawning grounds consist of stones and gravels, which can be clogged with sediments (Miller *et al.*, 2014). In Lake Geneva (lat. 46°25'59.99"N, long. 6°32'59.99"E, France/Switzerland), at the southern edge of the species' distribution, temperature records in historical spawning grounds range from 5.2°C to 7.1°C (J. F. Rubin and Buttiker, 1992) but recent observations show that temperature may frequently reach 8°C during spawning season (Figure S1).

Experimental design

Eggs were obtained from spawners of the Thonon-les-Bains INRA station broodstock, originating from the Lake Geneva wild population with no wild fish input since 1995. Four males and four females were crossed to produce four independent full-sib families. Thirty ova were collected per female and their dry weights were measured. Remaining ova were fertilized at 5°C and then individually distributed into 24-well cell culture microplates (Nunc MicroWell™), a common experimental incubating system for fish eggs (Wilkins *et al.*, 2015; Côte *et al.*, 2016).

Sediments

We created five sediment treatments corresponding to sedimentation rates observed in Lake Geneva (Loizeau, Girardclos and Dominik, 2012): 0, 0.007 (sediment load 1), 0.014 (load 1 x 2), 0.029 (load 1 x 4), 0.059 (load 1 x 8) and 0.12 (load 1 x 16) g/cm² per three months (a three month period roughly encompasses the duration of arctic charr egg incubation in the lake). Sediments were collected by decanting water pumped at 51 m deep in Lake Geneva, around 1 m above the lake bed. For the sake of reproducing field conditions, we kept the less than 200 mm grain-size fraction as it constituted more than 95% of collected sediments, and consisted mainly of silty and clayey particles. Sediments were oven-dried (60°C for 72 h), resuspended in mineral water (Volvic ©) and 2 ml of a solution of each sediment load was distributed in microplate wells (Figure 1).

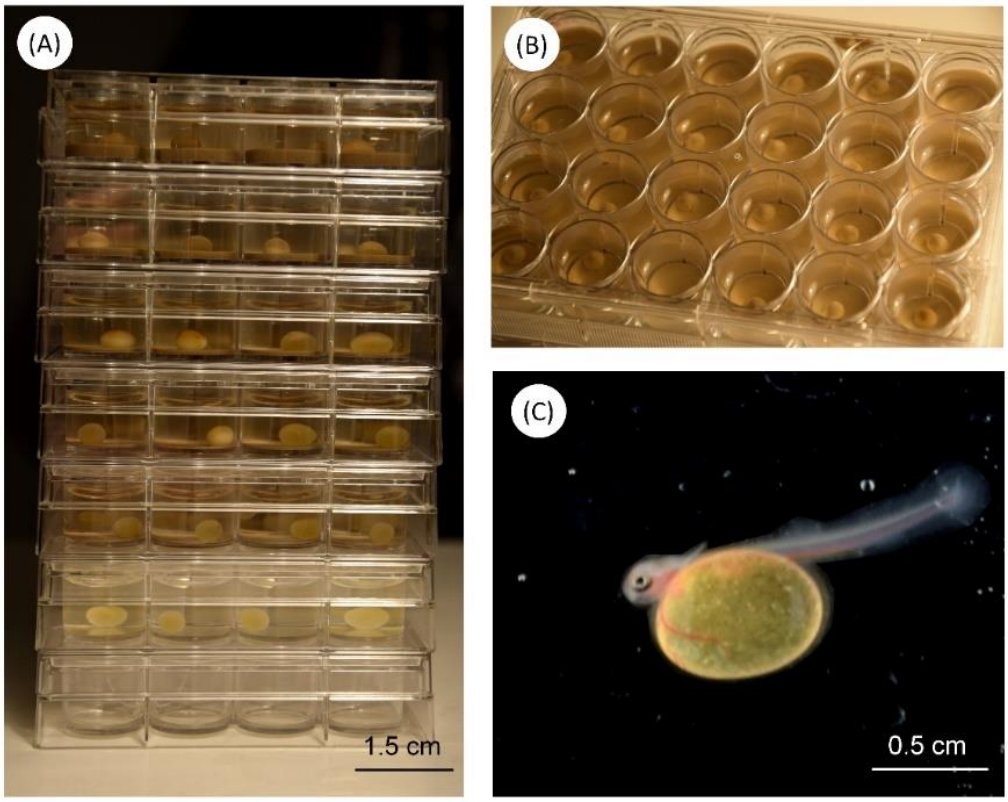

Figure 3-1 | (A) Stack of seven 24-well plates used for egg incubation, with increasing sediment load in the wells from bottom to top, (B) plate containing sediment seen from above, (C) hatched embryo. Photographs by E.Lasne and L.Garaud.

Temperature

Microplates were separated into two identical batches and incubated in climate chambers at either 5°C (5.10 ± 0.062°C) or 8.5°C (8.66 ± 0.048°C), 5°C being optimal for arctic charr egg development and survival, and 8.5°C being supposedly stressful (Elliott and Elliott, 2010). For each temperature–sediment combination, we used duplicates of 12 eggs per family randomly distributed on a rack (total = 1152 eggs).

Life-history traits

Survival was estimated as the per cent of hatched embryos from the total initial number of eggs. Incubation period was calculated as a development rate (Koops and Tallman, 2004) in accumulated degree days (ADD) from fertilization to hatching: ADD = mean daily temperature x number of days since fertilization. Each newly hatched fish was anaesthetized with 0.35 ml per litre eugenol (clove oil diluted 1 : 10 into 95% ethanol) and photographed (Nikon D5300 and Nikon DX 18–105 mm lens). Length and yolk sac volume (YSV) were measured using IMAGEJ (http://rsbweb.nih.gov/ij/download.html). Length was measured from the snout to the end of the tail (total length), and YSV was calculated as assuming the shape of an ellipsoid: YSV = pi/6 x L x H^2 (with L, major axis and H, minor axis [Kamler, 2008]).

Statistical analyses

The relationship between life history traits and treatments was analysed using linear mixed models with the lme4 package (Bates *et al.*, 2015) in R (R Core Team, 2016). Length at hatching, YSV and incubation period were log-transformed and analysed with a Gaussian error distribution whereas survival was analysed with a binomial error distribution. Temperature, sediment load and temperature sediment interaction were set as fixed effects. Average egg dry weight per female was included as a fixed covariate. Similarly, incubation period was included as a fixed

covariate in the length and YSV models. Family and plate position were set as random effects. All models were fitted under maximum-likelihood estimation. Residuals were checked graphically and did not deviate from assumptions of normality and homoscedasticity. The significance of each explanatory variable was tested with likelihood ratio tests comparing models including or not the variable of interest. Critical p-values were set at 0.05. All means are presented as ± standard error of mean. To estimate pairwise differences in sediment treatments between thermal groups, we performed Tukey's post hoc tests using the *lsmeans* library (Lenthe, 2016).

Results

Temperature significantly influenced survival at hatching with lower survival rates at 8.5°C than 5°C (χ^2 = 517.52, p<0.001, Table 1), and no individual hatched at the two highest sediment treatments (Figure 2a). At 5°C, survival declined significantly with increasing sediment loads (χ^2 = 64.66, p<0.001) and the difference in survival between the lowest and highest sediment treatments was larger at 8.5°C, as shown by the significant temperature sediment interaction (χ^2 = 47.99, p<0.001; Table 2). Incubation was longer at 8.5°C than at 5°C (521 ± 22 ADD versus 463 ± 21 ADD, respectively; χ^2 = 420.16, p<0.001) and hatching was delayed with increasing sediment load (χ^2 = 354.37, p<0.001) in both thermal groups (Figure 1b).
The temperature sediment interaction (χ^2 = 13.3, p<0.001) was significant for every sediment load (Figure 2b). Hatched embryos were longer at 5°C than at 8.5°C (13.7 ± 0.7 mm and 12.7 ± 0.6 mm, respectively, figure 1c; χ^2 = 144.87, p<0.001). Likewise, embryos reared at high sediment concentrations were smaller than those hatched at low concentrations (χ^2 = 132.73, p<0.001). Although the temperature sediment interaction was retained in the final model (χ^2 = 7.04, p<0.01), it was only significant at sediment load 2. YSVs were significantly different according to temperature (χ^2 = 61.49, p<0.001), with bigger sacs at 8.5°C (0.077 ± 0.015 cm3) than at 5°C (0.065 ± 0.014 cm3, Figure 2d). Only temperature and egg weight were retained in the final model, and both effects were significant (Table 2).

Table 3-1 | Results from linear mixed models testing the effects of temperature (T°C), sediment load, temperature x sediment interaction, egg dry weight and

		Survival (n=1152)		Incubation period (n=546)		Length (n=543)		Yolk sac volume (n=543)	
	df	x^2	p value	x^2	p value	x^2	p value	x^2	p value
fixed effects									
T°C	1	517.52	**<0.001**	420.16	**<0.001**	144.874	**<0.001**	61.49	**<0.001**
sediment load	5	64.66	**<0.001**	354.376	**<0.001**	132.738	**<0.001**	5.114	0.402
T°C x sediment load	5	47.995	**<0.001**	13.3	**<0.001**	7.0415	**0.02**	7.0023	0.5342
egg dry weight	1	2.633	0.104	2.072	0.149	0.1775	0.673	10.87	**<0.001**
ADD	1	-	-	-	-	0.000	1	0.1063	0.74

Discussion

Higher temperatures were associated with higher mortality, longer incubation period in ADD, smaller embryo size and bigger YSV compared with individuals from the cold treatment. These findings are in accordance with previous studies on the impact of temperature (Elliott and Elliott, 2010) on fish egg development, notably in salmonids. In addition, increasing sediment load led to lower survival rates, delayed hatching and smaller alevins with similar YSV, reflecting a lower conversion efficiency of yolk into tissues. The possible modes of action of sediment are manifold: it can smother the surrounding environment and render it hypoxic (S. M. Greig, Sear and Carling, 2007); form a thin layer around the egg, thus restricting gas flux through the egg membrane (Kemp *et al.*, 2011); contain microbial communities that can compete for oxygen (S. M. Greig, Sear and Carling, 2007); and induce a pathogenic stress (Kemp *et al.*, 2011). In our experiment, environmental constraints seemingly induced a partitioning of yolk reserves towards metabolic maintenance instead of growth, which ultimately delayed hatching. Both lower survival and delayed hatching following hypoxia or pathogen exposure have been documented in other salmonids (Côte *et al.*, 2012; Wilkins *et al.*, 2015). Most interestingly, our study demonstrates for the first time that temperature exacerbates the impact of sediments on fish embryonic life history traits, as reflected by the significant effects of sediment load on survival in the warm group.

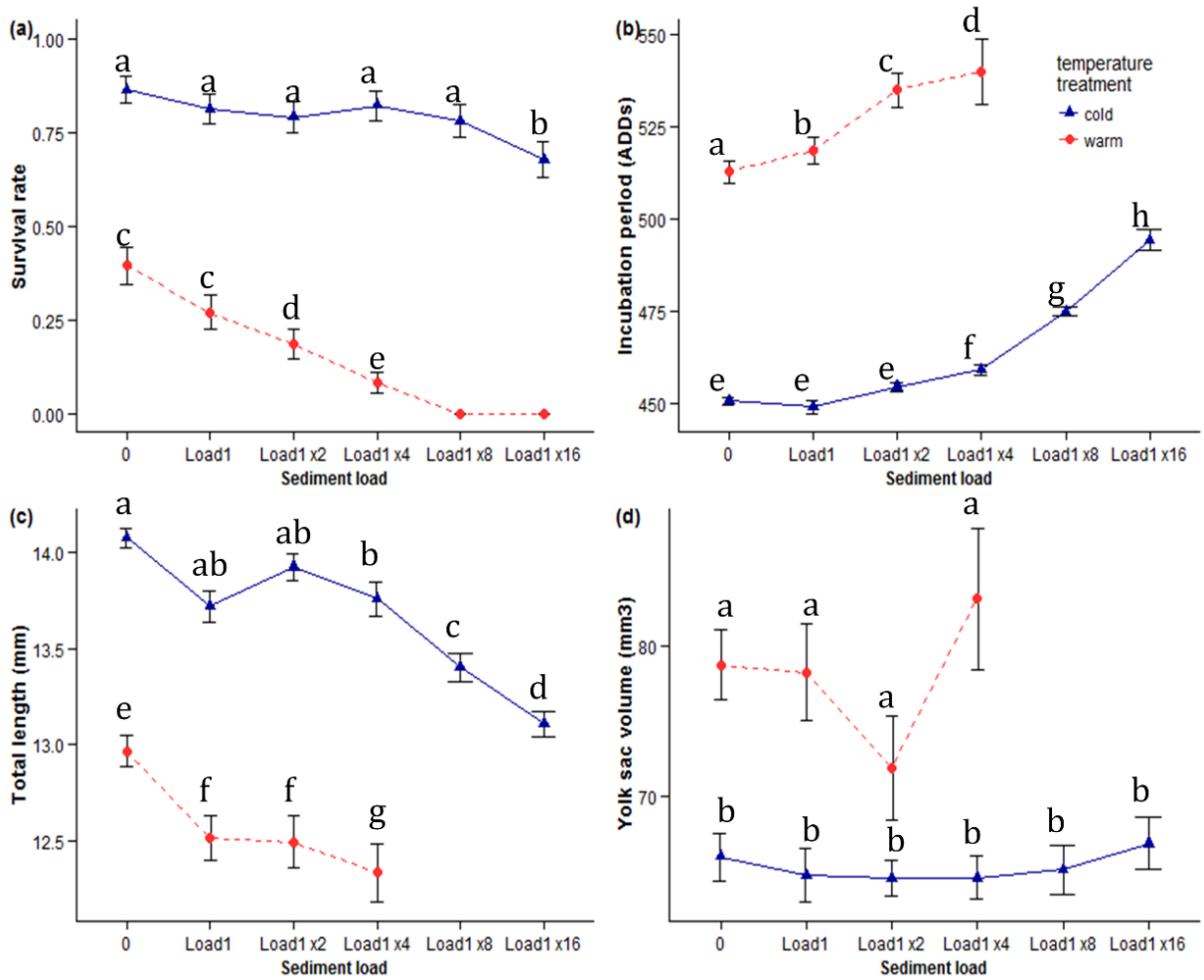

Figure 3-2 | Relationships between values of (a) survival, (b) incubation period, (c) total length, (d) yolk sac volume and sediment load in the two temperature treatments (solid line: cold [5°C] and dashed line: warm [8.5°C]). Different letters indicate significant differences at $p < 0.05$ (Tukey's post hoc tests). Means are presented ± s.e.m.

Table 3-2 | Fixed effects estimates, standard errors and associated statistics of mixed models for survival, incubation period, length and yolk sac volume (YSV). Italic values indicate p < 0.05. Estimates for interaction between temperature and sediment load 1 x 8 and sediment load 1 x 16 were not available as no fish exposed to

	Survival (n=1152)			ADD (n=546)			LT (n=543)			YSV (n=543)		
model term	estimate	z-value	p-value	estimate	t-value	p-value	estimate	t-value	p-value	estimate	t-value	p-value
intercept	1.893±0.34	5.530	**<0.001**	6.109±0.006	958.208	**<0.001**	0.339±0.01	28.925	**<0.001**	0.86±0.49	1.725	0.15
temperature	-2.329±0.36	-6.328	**<0.001**	0.131±0.006	22.299	**<0.001**	-0.076±0.008	-9.335	**<0.001**	0.139±0.017	8.067	**<0.001**
sediment load 1	-0.394±0.39	-0.986	0.324	-0.005±0.005	-1.034	0.313	-0.026±0.006	-3.987	**<0.001**	-		-
sediment load 1*2	-0.528±0.39	-1.343	0.179	0.008±0.005	1.750	0.081	-0.012±0.007	-1.899	**0.05**	-		-
sediment load 1*4	-0.323±0.4	-0.800	0.424	0.018±0.005	3.591	**<0.001**	-0.026±0.007	-3.907	**<0.001**	-		-
sediment load 1*8	-0.591±0.39	-1.515	0.13	0.05±0.005	9.924	**<0.001**	-0.051±0.007	-7.693	**<0.001**	-		-
sediment load 1*16	-1.136±0.37	-3.046	**0.002**	0.09±0.005	17.885	**<0.001**	-0.074±0.007	-10.570	**<0.001**	-		-
temperature×sediment load 1	-0.186±0.51	-0.365	0.715	0.017±0.009	1.966	**0.04**	-0.006±0.012	-0.538	0.590	-		-
temperature×sediment load 1*2	-0.535±0.51	-1.033	0.301	0.03±0.01	3.091	**0.002**	-0.03±0.013	-2.674	**<0.001**	-		-
temperature×sediment load 1*4	-1.681±0.58	-2.863	**0.004**	0.04±0.01	3.201	**<0.001**	-0.01±0.017	-0.962	0.336	-		-
temperature×sediment load 1*8	-18.503±32.18	-0.575	0.565	NA	NA	NA	NA	NA	NA	NA		NA
temperature×sediment load 1*16	-18.01±29.71	-0.606	0.544	NA	NA	NA	NA	NA	NA	NA		NA
egg dry weight	-		-	-		-	-		-	1.003±0.138	7.272	**0.002**

Regardless of the mode of action, this suggests a synergistic interaction and the existence of a critical threshold of sediment tolerance when embryos experience both stressors in combination. As both factors affect embryos via aerobic metabolism, a combined exposure will reduce the aerobic scope (*i.e.* the difference between basal and maximal oxygen consumptions, which represents the capacity to fuel critical functions) and involve complex mechanisms to respond to oxygen demand through modulation of ventilatory and cardiovascular functions. In salmonids, oxygen availability is likely to be critical in determining response to thermal stress (McBryan *et al.*, 2013).

Environmental conditions experienced during development do greatly influence later life-history trajectories, performances and reproductive success (Jonsson and Jonsson, 2014). In fish, smaller juveniles have a lower overall fitness than their larger conspecifics (Kamler, 2008). Emergence at a smaller size with a bigger YSV is associated with reduced swimming performance, high vulnerability to predators, as well as a reduced ability for intraspecific competition (Louhi *et al.*, 2011). Hence, consequences later in life are expected and should be further explored.

Overall, our results demonstrate a synergistic interaction between temperature and sediment load, such that moderate exposure to sediments that would not be considered as stressful independently of temperature can have dramatic impacts on the embryonic development and survival of arctic charr. As we used a range of sediment loads naturally experienced by the species in Lake Geneva, our findings have important implications for the conservation of this population. In the light of the climate change context, these results advocate the necessity for future studies to re-examine the effects of common stressors in freshwaters under different thermal scenarios.

Acknowledgements

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Supplementary file

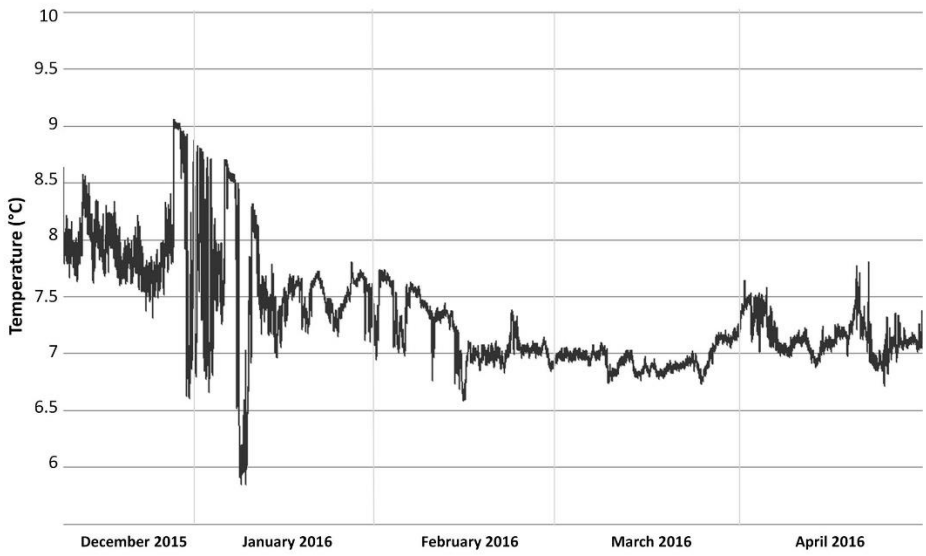


Figure 3-S1 | Temperatures recorded over winter 2015-2016 on a 40m deep spawning ground of Lake Geneva.

4. Response of wild populations in a multiple stressors context

Foreword

Investigating the impacts of combined pressures on species life history is crucial to set up appropriate mitigation measures and preserve freshwater biodiversity. In addition to warming that occurs at a global scale, lakes are threatened by an array of local pressures (*e.g.* degradation of water quality due to eutrophication, pollution, or sediment accumulation).

The previous chapter drew important conclusions on the potential of an interaction between temperature and sediment on early life history traits in a captive charr broodstock, showing for the first time that sediment loads that do not induce significant effects under optimal temperature conditions can become detrimental under warm temperature scenarios. These results, along with the contrasted thermal reaction norms observed in four wild populations in Chapter 2, led to the following questions: how do embryos originating from wild populations respond to two stressors that we know can synergistically affect their traits? Could we expect populations that are sensitive to temperature increase to exhibit poorer performance under exposure to both stressors, or could we expect all populations to respond in a similar fashion?

In the following article, I present a common garden experiment that aimed at examining the effects of a combined exposure to warm temperature and a sediment load on early life traits in hatchlings (survival, incubation duration, size and yolk sac volume) of four wild populations. Initially, I aimed at testing the effects of the two stressors on the populations studied in Chapter 2. It would have been particularly interesting to include the Allos population to this study, since individuals showed distinct phenotypic responses compared to Geneva, Constance and Pavin individuals. Unfortunately, the poor meteorological conditions met during Fall 2016 did not allow sampling over the short spawning period of arctic charr (around two weeks). Thus, the fourth study population in the present chapter originates from the low elevation reservoir lake of Sainte-Croix (France) and experiences relatively high temperatures all year long as water temperature remains over 7-8°C even in the deepest waters. The arctic charr was introduced in this lake in the 1990s from

lake Geneva individuals for fishery purpose, hence very little is known on this recently founded population.

Because sedimentation conditions are difficult to characterize in lakes (and particularly at the depths of arctic charr spawning grounds), this experiment did not attempt to investigate local adaptation to sediment exposure. The common garden approach could nonetheless reveal, to some extent, genetic effects underlying differences between populations.

Article information

Status: manuscript in preparation (target journal: Freshwater Biology).
Authors contribution: Conception and design: LM and EL. Data acquisition: LM. Data analysis: LM with input from EL and GE. Interpretation of data: LM and EL. Drafting of manuscript: LM. Writing of manuscript: LM, EL, GE, MD, JG.

Combined exposure to elevated temperature and sediment load reveals contrasted early life responses among arctic charr southernmost populations

Mari, Lisandrina; Daufresne, Martin; Guillard, Jean; Evanno, Guillaume; Lasne, Emilien

Abstract

Multiple stressors have been a topic of interest due to the unpredictable consequences of stressor interactions on ecosystems. As freshwater fish populations are declining worldwide, cold-water adapted stenotherms will be most at risk to suffer the combined impacts of global warming and local stressors. Sediment deposition is an important pressure in river and lake systems where it can affect local habitat quality of benthic species, but knowledge on the effects of sediment in a global warming context is lacking.

Alpine and peri-alpine populations of the cold water-adapted arctic charr (*Salvelinus alpinus*) constitute the Southern edge of the species range in Europe. Due to their intrinsic sensitivity and inability to migrate to more suitable environments, arctic charr early life stages (eggs and alevins) are highly vulnerable to climate change at such latitudes. Taking this into consideration, we used a common garden approach to examine the effects of combined exposure to elevated temperature and deposited sediment on early life history traits in offspring of four wild arctic charr populations, originating from geographically isolated lakes.

Temperature impacted negatively all traits and was associated with a decrease in survival, and earlier hatching of smaller individuals. Although sediment alone effects were less pervasive, affecting mostly size and yolk sac reserves, we report important interactive effects of temperature and sediment. Temperature exacerbated the negative effects of sediments on the duration of the incubation period and on the body length - yolk expenditure tradeoff during development. Furthermore, our results highlight that although the performance of individuals

seemed globally reduced, populations exhibited distinct response patterns, indicating they are likely set up specific strategies to meet energetic demands when oxygen availability is limited.

Introduction

For the past decades, climate change-induced temperature elevations have been threatening ecosystems and challenging species, leading to severe morphological and phenological changes, as well as geographic range shifts or contractions (Parmesan, 2006; Bellard *et al.*, 2012; Comte, Murienne and Grenouillet, 2014; Comte and Olden, 2016). The capacity of species to adjust in respect to one or several of these changes will determine their persistence in non-optimal thermal environments (Hendry, Gotanda and Svensson, 2017). In addition to increasing thermal stress, ecosystems ought to cope with additional local pressures. Co-occuring global and local constraints can have interactive effects on the system and its communities (Piggott *et al.*, 2015; Piggott, Townsend and Matthaei, 2015a), raising management and conservation issues. Stressors can interact additively, antagonistically, or synergistically (Piggott, Townsend and Matthaei, 2015b; Lange *et al.*, 2018) but cases of counterintuitive interactions have been reported (Ormerod *et al.*, 2010), with growing evidence that interpreting the ecological consequences of multiple stressor interactions from single-stressor effects alone can be misleading (Holmstrup *et al.*, 2010; Côté, Darling and Brown, 2016).

Multiple stressors have already been hailed as one of the causes of the current worldwide decline of freshwater fish populations (Côté, Darling and Brown, 2016; Gordon *et al.*, 2018). Assessing the effects of multiple stressors in a fast-changing climate context seems therefore crucial to set up efficient mitigation measures (Hering *et al.*, 2015). The magnitude of multiple stressor impacts can however vary substantially across fish species as well as through ontogeny (Lange *et al.*, 2018). More precisely, embryo-larval stages are known to be more sensitive to environmental changes than later life stages (Rombough, 1988, Pankhurst and Munday, 2011).

As a vast majority of aquatic organisms, fish are ectotherms meaning that their body temperature depends on the thermal environment, and metabolism, growth and development rates are firmly linked to temperature (Angilletta *et al.*, 2003), thus influencing energy allocation and ultimately individual life-history and fitness

(Zuo *et al.*, 2012; Hemmer-Brepson *et al.*, 2014). Among the array of biotic and abiotic pressures freshwater fish are exposed to, fine sediment accumulation remains a key pressure that can affect the system at all levels/biological scales (*e.g.* Townsend, Uhlmann and Matthaei, 2008). Although naturally present in freshwaters, both anthropogenic activities (agriculture, land use, and urbanization) and natural events (*e.g.* floods frequency, although impacted by climate change themselves) can boost the sediment input into lakes (Suttle *et al.*, 2004). Once deposited, fine sediment affects habitat structure, food availability and important ecological processes that can in turn alter the system functionality, *e.g.* organic matter processing (Piggott, Townsend and Matthaei, 2015a) or trophic relationships (Louhi, Richardson and Muotka, 2017). Deposited sediment can be further detrimental to benthic organisms and benthic life stages of pelagic species by 'smothering' the lake or stream bed environment, thus limiting oxygen availability (Greig, Sear, and Carling 2007). Adverse effects of deposited sediment have been notably observed on the embryo survival, development, timing of hatching, and growth of many fish species (see Kemp *et al.*, 2011; Chapman *et al.*, 2014), particularly in salmonids that are highly sensitive to breeding habitat degradation (Greig, Sear, and Carling 2007; Jensen et al. 2009; Louhi et al. 2011; Suttle et al. 2004).

Since temperature affects the oxygen demand and fine sediment reduces oxygen availability in the environment, it is reasonable to hypothesize these two stressors will interact when experienced in combination, and will likely affect primarily cold-water benthic species in their embryo-larval stages. Among at-risk species is the arctic charr *Salvelinus alpinus*, a cold-adapted stenotherm (Power, Reist and Dempson, 2008) that belongs to the diverse family of Salmonidae, of cultural and economic importance throughout Northern America and Europe (Klemetsen et al. 2003; Winfield, Berry, and Iddon 2018). In Europe, Arctic charr populations of the alpine and peri-alpine region are at the Southernmost limit of distribution of the species, and embryo-larval life stages already experience temperatures close to their known upper thermal limit (8°C, [Elliott and Elliott, 2010]). The lack of dispersal opportunities forces these landlocked populations to withstand the impacts of environmental pressures. In deep lakes, adults can potentially migrate in the water

column to track more suitable conditions, but eggs must adjust to immediate environmental conditions. Typically, spawning areas require cold, well-oxygenated water, and hard substratum constituted of stones or gravels devoid of fine sediment (Klemetsen et al. 2003). Besides, as for other salmonids, charr fry behavior is specifically demersal (Wallace and Aasjord, 1984). Hatched individuals remain within the substratum, exhibiting very limited swimming behavior until they reach the first exogenous feeding stage that follows yolk sac absorption (Teletchea and Fontaine, 2010). Compared to the pelagic larvae of other species, arctic charr embryos remain exposed to sediment for extended periods of time (until emergence from gravel), and might be particularly at risk to this pressure.

In a recent study, Mari et al. (2016) showed that temperature influences negatively fine sediment tolerance of embryos in an arctic charr captive broodstock. However, the adaptation to a captive environment might bias the effect of thermal and sediment stresses on embryo development compared to natural conditions. Much is known about early development and growth of arctic charr in hatchery settings (H. Jeuthe, Brännäs and Nilsson, 2015; Sæther, Siikavuopio and Jobling, 2016), but few studies have examined the effects of environmental stressors on early life history traits in natural populations (*e.g.* (Swift 1965; Jungwirth and Winkler 1984; Humpesch 1985). Here, we investigate the combined effects of multiple stressors - increased temperature and deposited fine sediment - on embryonic life history traits of wild arctic charr populations. We conducted a common garden experiment, rearing embryos of four different origins either at an optimum developmental temperature or at a stressful thermal regime close to their upper thermal limit, and in absence or presence of deposited sediment. We hypothesized that i) sediment would alter embryo traits more strongly under warm conditions than at optimal temperature and that, ii) because the populations originate from geographically distant lakes with distinct morphologies and thermal profiles, they would exhibit different sensitivities to the two stressors.

Material & Methods

Study system and populations

The arctic charr (*Salvelinus alpinus L*.) is the northernmost freshwater fish species on the planet, distributed across the Northern hemisphere up to 80°N (Klemetsen, 2013). The species has a more limited thermal tolerance range than other salmonids (Sæther, Siikavuopio, and Jobling, 2016; Baroudy and Elliott, 1994; Elliott and Elliott, 2010). Literature mentions that the optimal egg incubation occurs at 4-7°C (see Sæther, Siikavuopio, and Jobling 2016; Janhunen, Piironen and Peuhkuri, 2010), and egg mortality increases dramatically when temperature reaches 8°C (Jungwirth and Winkler, 1984). However, southernmost populations are likely to better resist warmer temperatures as functional spawning grounds are often found in warmer environments than their northern counterparts (7-8°C in lake Geneva, [Rubin & Buttiker 1992], over 8°C in lake Sainte-Croix, Figure 1B). Nevertheless, strong evidence of local adaptation is still lacking (Larsson *et al.*, 2005). We collected spawning individuals in December 2016 and January 2017 from four geographically isolated lakes located around the Southern limit of the species distribution range in Europe (Figure 1A): lake Geneva (France/Switzerland) and lake Constance (Switzerland/Germany/Austria), where arctic charr is native, and lake Pavin (France) and lake Sainte Croix (France), where the species has been introduced in 1859 and 1992, respectively. These four populations inhabit contrasted thermal habitats (Figure 1B).

Rearing experiment

Adults were caught with gillnets on one spawning ground per lake. Spawning males and females were crossed to produce 25 independent families in total (Pavin: 8 families, Constance: 5 families, Geneva: 6 families, Sainte-Croix: 6 families). All ova were fertilized in the field at temperatures comprised between 3 and 5° C and brought back to the INRA facilities in Thonon-les-Bains, where they were individually distributed into 24-well cell culture microplates (Nunc MicroWell™).

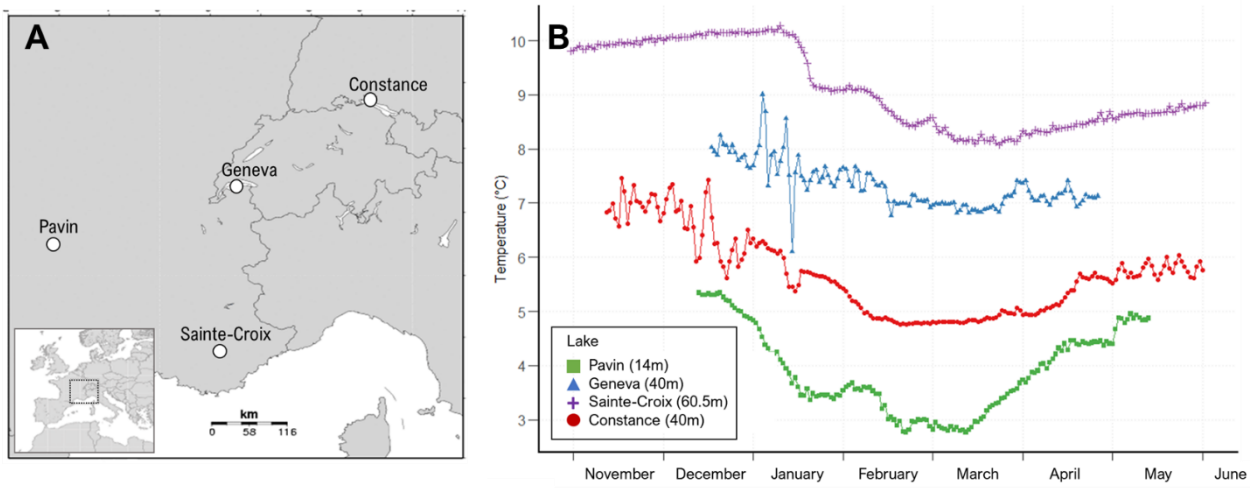


Figure 4-1 | (A) Map of the four source lakes from which spawning individuals were caught and (B) recordings of temperature from November to June 2015/2016. For Pavin, Geneva and Constance, depths correspond to depths of arctic charr spawning grounds. For Sainte-Croix, temperatures were recorded at maximum depth.

The use of microplates is now common in experimental rearing of salmonid eggs to monitor embryonic development at the individual level (Côte *et al.*, 2012; Wilkins *et al.*, 2015).

We set up two sediment treatments (absence/"no sediment" and presence/" sediment exposed"). In a previous study (Mari *et al.*, 2016), we observed significant effects on survival at a minimal sediment load of 0.015 g.cm^{-2} along a gradient of sediment exposure. We thus selected this load for the exposed treatment in the current study. It represents a low, but realistic sedimentation rate (Loizeau, Girardclos and Dominik, 2012) over a three months period, which corresponds to the length of the arctic charr egg incubation period. We collected sediments with water pumped above the lake bed near a 40m deep spawning ground in Lake Geneva. Sediment was mesh-sieved to retain the <200 mm diameter grain-size fraction, known to be the most harmful to fish early life stages (Kjelland *et al.*, 2015). Sediments were then oven-dried (60°C for 72 h) and resuspended in mineral water (Volvic ©; Mari *et al.*, [2016]) to obtain a dilution corresponding to our chosen

sediment load. 2 ml of either clear water or resuspended sediment solution was distributed in each well of the corresponding microplates. When sediment deposited at the bottom of wells, plates were split into two batches and incubated in separate climate chambers. In each chamber, plates were randomly distributed on a rack.

We set up a cold treatment of 5°C (+/- 0.90°C), an optimal temperature for development, and a warm treatment of 8.5°C (+/- 0.038°C) that is supposedly stressful (Elliott and Elliott, 2010; Sæther, Siikavuopio and Jobling, 2016), but experienced by the species on spawning grounds of lakes Geneva and Sainte-Croix (Mari *et al.*, [2016], Figure S1). Temperature was recorded from fertilization to hatching using HOBO loggers (HOBO Onset, #UX100-001).

We used duplicates of 12 eggs per family per population in plates containing no sediments and triplicates of 12 eggs in sediment plates as we were expecting higher mortality in sediment-exposed individuals (total of 3288 eggs, two families per plate). For the Sainte-Croix population, we could only test the sediment effect on 3 families out of 6 because 3 females produced clutches of less than 100 eggs.

Traits measurements

Each plate was photographed at the beginning of the experiment (Nikon D5300 and Nikon DX 18–105mm lens). Individual egg size was measured as the mean egg diameter (average of two perpendicular diameters). Plates were initially left to incubate in the dark not to disturb the first stages of development. Plates were then monitored daily (every morning) during hatching period. Each newly hatched fish was anaesthetized with 0.35ml per liter eugenol (clove oil diluted 1:10 into 95% ethanol) and photographed individually using the same set-up as described above. Length was measured from the snout to the end of tail (total length), and yolk sac volume (YSV) was calculated using the following formula: YSV = p /6x L x H^2 (with L, major axis and H, minor axis, see Kamler[2005]). Individual egg size, length and yolk sac axes at hatching were measured using IMAGEJ (http://rsbweb.nih.gov/ij/download.html). Because sediment-exposed eggs were fully covered by a

sediment layer, we could not measure fertilization success and early-life mortality at eyed-stage. Thus, survival was estimated as the number of hatched embryos divided by the number of eggs per family. While including unfertilized eggs might bias survivorship rates downwards, it allows to take into account any mortality that occurred before the eyed stage. However, fertilization success can vary among and within populations. Thus to allow populations comparisons, we standardized survival estimates relative to the trait value in the most optimal environment for egg development, *i.e.* the control (cold, no sediment) treatment as: standardized survival = $x_{treatment}$ / $\mu_{control}$ with x: mean survival of a given family; treatment: one of the four treatments (cold, cold exposed, warm, warm exposed); $\mu_{control}$: mean survival of given family in the control treatment. Incubation period was calculated as a development rate in accumulated degree days (ADD) from fertilization to hatching: ADD = mean daily temperature x number of days between fertilization and hatching date.

Statistical analyses

All analyses described below were performed in R, using critical significance threshold of 0.05.

We analyzed the relationship between the relative changes in life history trait values and treatments within each population using linear mixed models with the *lme4* package (Bates *et al.*, 2015). We used a Gaussian error distribution following log transformation to improve normality and homoscedasticity. We ran one mixed model per trait to test for global effects of treatments, as well as differences among populations. Treatments and their interaction (temperature, sediment) and population origin were specified as fixed effects, whereas family identity was set as random effect. Then, for each trait, we ran one mixed model per population to test the impacts of temperature, sediment and their interaction on traits at the population level. We set family identity, family x temperature, family x sediment and family x temperature x sediment interactions as fixed effects.

Plate identity was set as random effect in all models except survival, because survival was calculated at the family level instead of individually. Incubation duration was included as a fixed covariate in all length and YSV models. Due to the importance of maternal investment in egg size in salmonids (Polymeropoulos, Elliott, and Frappell 2016; Perry, Audet, and Bernatchez 2005), individual egg size was included as a fixed covariate in all models. All mixed models were fitted under maximum-likelihood estimation. The significance of independent variable was tested by comparing models including or not the explanatory variable(s) of interest with likelihood ratio tests, and all residuals for selected models were inspected visually (Q/Q plots) to ensure they did not deviate from assumptions of normality and homoscedasticity.

We then performed post-hoc multiple pairwise populations comparisons in each treatment and between treatments in each population. We estimated pairwise differences using the *emmeans* package in R (Lenthe et al 2018), applying a Benjamini-Hochberg correction to account for false discovery rate (Benjamini and Yekutieli 2001).

Results

Global trends

Temperature had a significant effect on all traits (Table 1), whereas sediment exposure only affected embryo length. The temperature x sediment interaction was significant in the incubation and length models. We found significant temperature x population and sediment x population interactions for all traits except survival. No two-way interactions were detected but responses varied significantly within each population. We detail below the results for each trait separately.

Survival

Overall, temperature had a significant negative effect on survival (Table 1). Within-population models and post-hoc testing revealed significantly lower survival in the

Constance and Pavin populations in the warm exposed treatment (both with or without sediment) (Figure 2, Table 2).

Table 4-1 | Linear mixed model testing for the overall effects of temperature, sediment exposure, origin, and one and two-way interactions on relative changes in survival, incubation duration, length and yolk sac volume at hatching. Bolded

		Survival (n=2808)		Incubation (n=1601)		Length (n=1596)		Yolk sac volume (n=1596)	
	df	x^2	p value	x^2	p value	x^2	p value	x^2	p value
fixed effects									
T°C	1	32.537	**<0.001**	320.03	**<0.001**	181.01	**<0.001**	19.127	**<0.001**
sediment load	1	2.219	0.136	0.2	0.655	119.19	**<0.001**	0.74	0.39
T°C x sediment load	1	3.808	0.051	6.74	**0.009**	14.107	**<0.001**	0.229	0.632
population	3	16.947	**<0.001**	29.785	**<0.001**	21.231	**<0.001**	14.02	**0.003**
T°C x population	3	3.931	0.27	20.532	**<0.001**	12.839	**0.005**	32.19	**<0.001**
sediment load x pop	3	5.297	0.506	29.2	**<0.001**	5.988	**0.1122**	10.86	**0.03**
T°C x sediment x pop	3	6.99	0.136	3.542	0.315	1.087	0.78	1.048	0.902
egg size	1	0.162	0.687	0.167	0.683	48.564	**<0.001**	64.186	**<0.001**
ADD	1	-	-	-	-	72.292	**<0.001**	0.348	0.555

There was significant variation among families in response to temperature and sediment within the Pavin and Geneva populations, as shown by significant interactions between family identity and treatment.

Incubation period

Incubation period, measured in ADDs, was significantly longer in the warm treatment for all populations (Figure 2, Table 1 & 2). There was no effect of sediment exposure on incubation period overall, but the temperature x sediment interaction was significant (Table 1).

The global model showed differences among populations in their responses to temperature and sediment exposure (significant one-way interactions, Table 1). Temperature had a strong effect in all populations. The sediment effect was found to significantly increase incubation period in the Geneva population both under cold and warm conditions (significant post-hoc testing, Figure 2). Conversely, post-hoc testing also revealed a significant decrease in incubation period due to sediment in the warm treatment for the Constance and Pavin populations. The Sainte-Croix population was unaffected by sediment. There was significant variation in the length

of incubation period among families, as well as significant family x temperature and family x sediment interactions in all populations (Table 2). Individual egg size generally did not have an effect on the duration of the incubation period, except in the Constance population.

Body size

Sediment exposed individuals were smaller than individuals in the control treatment. Length at hatching decreased even more substantially under warm conditions (Table 1 and significant post-hoc testing, Figure 2). The individuals were smallest when they experienced both stressors in combination (10% reduction of size compared to control, Figure 2), as evidenced by a significant temperature x sediment interaction. We detected significant temperature x population and sediment x population interactions (Table 1), meaning that populations differed in their response to the two stressors.

Initial egg size and incubation period had a significant effect on overall change in length (Table 1). For all populations, both temperature and sediment had significant effects when experienced separately (Table 2). An interactive effect was only found for the Pavin population (Table 2).

There was significant variation among families in all populations, with temperature x family interactions found within the Geneva and Sainte-Croix populations. Sediment x family interactions were significant for Constance and Sainte-Croix populations. We further detected a significant temperature x sediment x family in the Sainte-Croix population.

Yolk sac volume

Overall, temperature and sediment did not affect yolk sac volume (Table 1). However we found a significant population effect as well as significant interactions between population and treatments.

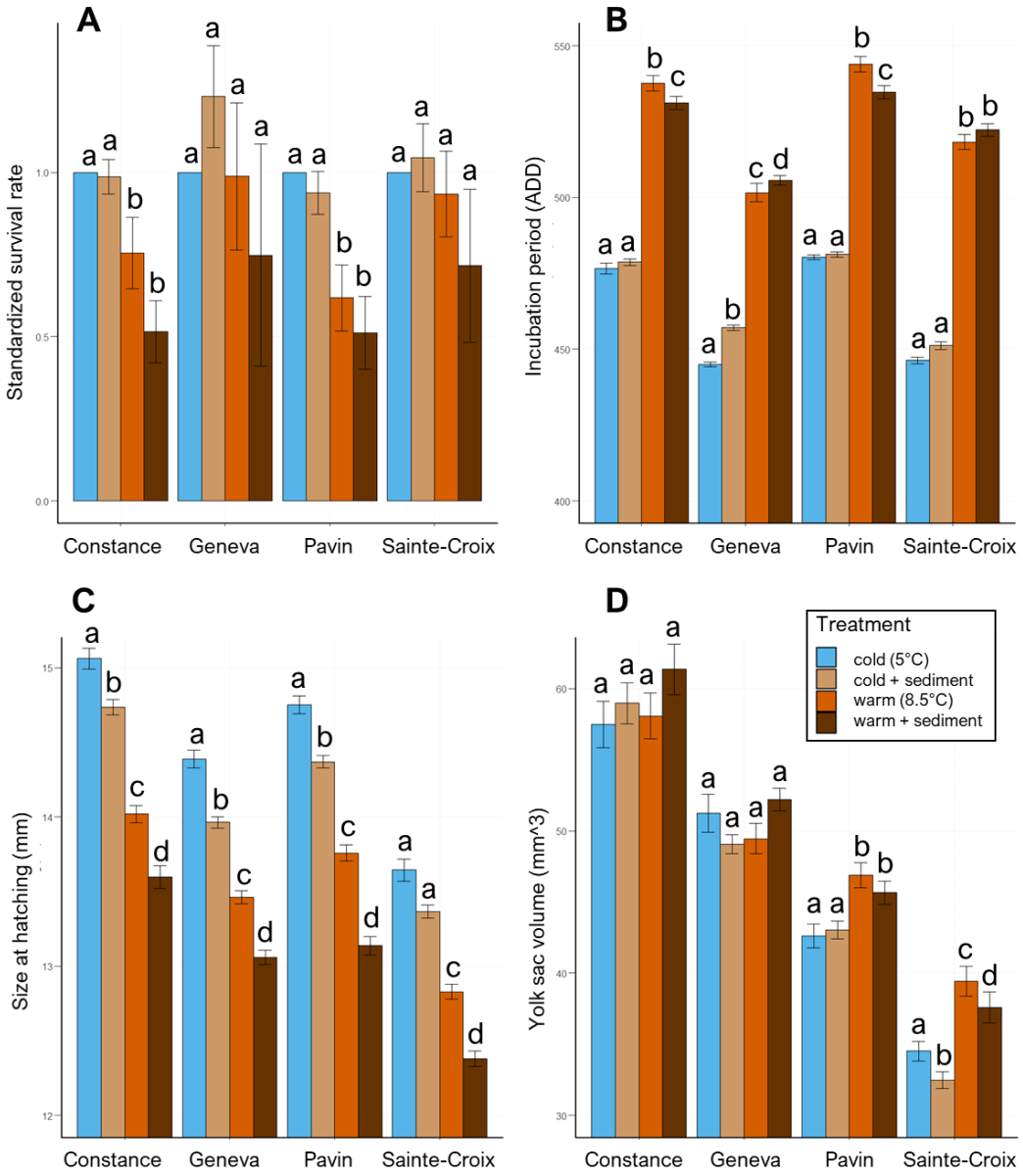


Figure 4-2 | Effects of the four treatments on (A) standardized survival rate (B) length of incubation period (C) length at hatching (D) yolk sac volume at hatching for the four study populations. In blue: control – cold temperature ; light brown: cold + sediment; orange: warm temperature; dark brown: warm + sediment. Values are expressed as mean +/-SEM. Letters indicate significant post hoc differences between treatments within each population ($p<0.05$, Benjamini-Hochberg correction).

Pavin and Sainte-Croix individuals had bigger yolk sacs in the warmer treatment (significant T°C effect, Table 2, Figure 2). Sediment exposure had a negative effect on the yolk volume of Sainte-Croix individuals, observable both at cold and at warm (Figure 2). We did not detect any temperature x sediment interaction within any population. Family identity was significant in all populations, but we only detected one significant interaction including the family term (sediment x family in Geneva individuals, Table 2). Egg size had a significant effect for all populations (Table 2).

Discussion

This study focused on the influence of two major pressures in freshwaters, deposited sediment and increased temperature, on early life stages of southernmost populations of the cold-water adapted arctic charr. It illustrates that, first, combined elevated temperatures and sediment-rich waters can affect fitness-related traits interactively, and second, that wild alpine *S. alpinus* populations have different susceptibilities to these pressures.

Separate effects of temperature and sediment

The effect of temperature was strong, affecting all traits in several but not all populations. Our results reflect what is typically associated with thermal stress in salmonids, that is a slower development (in ADDs) with hatching occurring prematurely (in days, Figure S2) under warmer conditions (Ojanguren and Braña, 2004). However, elevated temperatures were associated with smaller individuals but no change in yolk sac volume in the Constance and Geneva populations. This indicates that while hatching occurred at an earlier stage of development (as for other populations), yolk reserves were not allocated to growth, likely revealing a physiological stress response (Valdimarsson, Skulason and Snorrason, 2002).

Table 4-2 | Linear mixed model testing for the effects of temperature, sediment exposure, family, and one and two-way interactions on survival, incubation period, length and yolk sac volume within the four study populations. Egg size and duration of incubation period were added as covariates in survival, length and yolk volume models. Bolded values indicate p-value <0.05 (likelihood ratio tests).

		Population							
		Geneva		Constance		Pavin		Sainte-Croix	
		x^2	p value	x^2	p value	x^2	p value	x^2	p value
Survival	fixed effects								
	T°C	2.639	0.104	6.611	**0.01**	19.234	**<0.001**	0.982	0.322
	sediment load	0.005	0.942	4.779	**0.029**	1.630	0.202	0.169	0.681
	T°C x sediment load	5.918	**0.015**	2.252	0.133	1.684	0.431	1.665	0.435
	family	65.032	**<0.001**	70.132	**<0.001**	131.56	**<0.001**	49.915	**<0.001**
	T°C x family	17.839	**0.003**	2.663	0.616	29.254	**<0.001**	7.266	**0.026**
	sediment x family	27.569	**<0.001**	2.213	0.7	15.109	0.057	1.815	0.612
	T°C x sed x fam	33.19	**<0.001**	10.687	0.637	27.233	**0.039**	5.965	0.427
	egg size	3.06	0.08	0	0.985	2.368	0.124	2.779	0.095
	ADD	-	-	-	-	-	-		
Incubation		x^2	p value	x^2	p value	x^2	p value	x^2	p value
	fixed effects								
	T°C	61.815	**<0.001**	94.174	**<0.001**	97.021	**<0.001**	78.363	**<0.001**
	sediment load	3.359	**0.012**	0.591	0.442	4.598	**0.032**	4.998	**0.025**
	T°C x sediment load	1.253	0.263	2.8376	0.242	4.604	**0.03**	0.237	0.626
	family	127.04	**<0.001**	74.041	**<0.001**	105.89	**<0.001**	7.131	**0.028**
	T°C x family	131.63	**<0.001**	16.322	**0.003**	55.258	**<0.001**	8.847	**0.012**
	sediment x family	13.087	**0.023**	11.615	**0.04**	25.879	**<0.001**	21.727	**<0.001**
	T°C x sed x fam	4.499	0.343	11.315	**0.045**	10.882	0.1439	3.447	0.328
	egg size	0.345	0.557	20.625	**<0.001**	6.863	**0.009**	4.492	**0.033**
	ADD	-	-	-	-	-	-		
Length		x^2	p value	x^2	p value	x^2	p value	x^2	p value
	fixed effects								
	T°C	52.949	**<0.001**	53.745	**<0.001**	55.972	**<0.001**	16.128	**<0.001**
	sediment load	24.126	**<0.001**	15.062	**<0.001**	25.711	**<0.001**	5.440	**0.02**
	T°C x sediment load	0.006	0.936	0.3477	0.555	6.775	**0.009**	2.901	0.09
	family	46.835	**<0.001**	44.353	**<0.001**	299.76	**<0.001**	9.5	**0.009**
	T°C x family	24.029	**<0.001**	9.954	**0.041**	8.634	0.28	11.026	**0.004**
	sediment x family	5.556	0.352	10.637	**0.031**	8.457	0.294	8.875	**0.012**
	T°C x sed x fam	10.748	0.293	5.076	0.406	29.303	0.107	8.918	**0.03**
	egg size	1.996	0.158	69.074	**<0.001**	75.932	**<0.001**	8.136	**0.004**
	ADD	13.025	**<0.001**	0.001	0.973	5.622	**0.017**	0.358	0.55
Yolk sac volume		x^2	p value	x^2	p value	x^2	p value	x^2	p value
	fixed effects								
	T°C	1.259	0.262	0.018	0.892	8.816	**0.003**	14.665	**<0.001**
	sediment load	0.982	0.058	1.821	0.177	0.001	0.972	6.17	**0.013**
	T°C x sediment load	1.221	0.269	0.064	0.968	0.069	0.966	0.066	0.796
	family	61.891	**<0.001**	85.671	**<0.001**	221.37	**<0.001**	15.444	**<0.001**
	T°C x family	5.293	0.507	6.309	0.277	9.124	0.244	1.176	0.555
	sediment x family	27.568	**<0.001**	67.196	0.206	5.573	0.695	3.097	0.213
	T°C x sed x fam	4.875	0.899	16.92	0.324	23.877	0.4107	1.758	0.624
	egg size	129.41	**<0.001**	257.8	**<0.001**	113.6	**<0.001**	33.956	**<0.001**
	ADD	0.445	0.505	2.134	0.144	1.306	0.253	5.7	**0.017**

From a metabolic perspective, basal metabolism and respiration rates increase under warmer conditions, reflecting a greater need for energy (ATP) production to balance organism development, growth and maintenance (Angilletta *et al.*, 2003). As energy is expended differently at warm, yolk conversion efficiency is lower, thus reducing growth rates (Heming, 1982; Ojanguren and Braña, 2004; Kamler, 2008).

In the populations in which hatched individuals exhibited bigger yolks (Pavin, Sainte Croix), embryos are much smaller and the energetic savings might not be enough to compensate the strong reduction in hatchling size. Furthermore, hatching at a smaller size can have deleterious later-life consequences for fish. Although a bigger yolk sac may prevent starvation risk (Skoglund *et al.*, 2011) and allow compensatory growth, reduced size has been correlated with a reduced foraging efficiency and swimming performance, linked to lower intraspecific competitivity and predator escape capacity (Jonsson and Jonsson 2014; Louhi et al. 2011), and hence a fitness reduction in general (Kamler, 2008).

Sediment effects alone were less pervasive than temperature effects. Sediment has been linked with a decrease in embryo survival in Atlantic salmon (Levasseur *et al.*, 2006) and brown trout (Sear *et al.*, 2016) but we did not detect any changes in survival which might either i) be due to a too low sediment load, or ii) indicate that wild populations are better adapted than captive fish to such a sediment load. The amount of sediment to which embryos were exposed was rather low compared to known sedimentation rates in our study lakes (*e.g.* up to 1 $g.cm^{-2}.yr^{-1}$ in lake Geneva, [Loizeau, Girardclos and Dominik, 2012]). We deliberately used a low but realistic sediment level because we had no prior hypothesis on the local sedimentation conditions in the study populations. Sediments were found to affect length at hatching but not yolk reserves, except for Sainte-Croix embryos that had lower yolk reserves when exposed to sediments at both temperature treatments. This is consistent with our previous findings using a similarly low sediment level on charr embryos (Mari *et al.*, 2016), and with other studies that showed that sediment exposure can affect fish larval development and growth (Greig, Sear, and Carling 2005; Sear et al. 2014; Chapman et al. 2014). Incubation period did not vary with

sediment exposure, except in the Geneva population for which hatching was slightly delayed. Fine sediment is essentially considered a threat to benthic organisms due to its smothering properties (Greig, Sear and Carling, 2005; Greig, Sear and Carling, 2007; Kemp *et al.*, 2011) as it can clutter the surface of lake and stream bed, creating an anoxic layer over benthic organisms habitat and benthic spawners spawning grounds. In early life stages of salmonids, hypoxia has been shown to increase mortality, reduce the development rates and size of embryos (Roussel, 2007; Greig, Sear, and Carling 2007; Malcolm, Youngson, and Soulsby 2002) and induce either premature (Polymeropoulos, Elliott and Frappell, 2017) or delayed hatching (Côte *et al.*, 2012).

In addition, sediment can exert a direct mechanical stress to embryo-larval stages by blocking egg pores (for particles of less than 63µm of diameter) and reduce gas exchanges through the egg membrane (Greig *et al.*, 2005; Franssen *et al.*, 2012). Embryonic development is a period that requires high protein turnover for tissue remodeling. The blockage of exchanges can prevent expulsion of metabolites through the chorion and can, in turn, hinder proper embryonic development (Bennett, Connor and Eaton, 2003; Chapman *et al.*, 2014).

Interactive effects of temperature and sediment

In accordance with our initial hypothesis, *i.e.* that sediment would exert more harmful effects on life-history traits under warm conditions, we detected sublethal effects of sediment in the warm treatment on all traits. The results also show that the treatments affected the populations in a broadly similar magnitude, but the patterns were population-specific. The interactive effects were less spectacular than we had expected based on what was previously observed in a captive charr broodstock (Mari *et al.*, 2016), where the two stressors combined had induced a 25% decrease in survival, and a significantly shorter incubation period/ longer in ADD. In the present study, temperature alone affected survival strongly, but temperature and sediment did not show significantly interactive effects on survival. When higher temperature and sediment were experienced simultaneously, incubation was significantly shorter than in the control treatment. Compared to

temperature effects, stressors interaction induced either a shortened (for Constance and Pavin) or longer (for Geneva) incubation.

Interestingly, the four populations exhibited specific patterns in response to the two stressors: smaller individuals with larger yolk sac and much earlier hatching (Pavin) ; smaller individuals with no changes in yolk volume and much earlier hatching (Constance) ; smaller individuals with no changes in yolk volume and earlier hatching, with a slight delay compared to the warm-no sediment treatment (Geneva) ; and smaller individuals with larger yolk sacs than controls but smaller than in the warm-no sediment treatment and earlier hatching (Sainte-Croix). Individuals might hatch earlier to compensate the high metabolic costs of an unfavorable environment (Storm and Angilletta 2007). Compared to the energy demand of embryonic development, the hatching process itself is energetically costly (Kamler, 2008). Thus, hatching can also be delayed because meeting the energy demand necessary for hatching under warm conditions with limited oxygen availability can be more difficult. This will be likely linked to the individuals capacity to partition yolk among different physiological functions.

Advantageous plasticity in early life history traits consists in either maximizing fry mobility by emerging and starting feeding on preys at a larger size with a small yolk sac, or favoring energy saving by emerging with large yolk sacs (Skoglund *et al.*, 2011). Because our experiment focused on hatchlings, we cannot infer consequences at emergence, but these different response types likely illustrate different trade-off strategies to cope with the physiological stress induced by the combination of temperature and fine sediment. In addition, average egg size was contrasted among populations (Figure S3). Such differences might be due to local conditions as well as genetic divergence or age-related shifts (Lasne et al. 2017), and since yolk reserves constitute the only nutritional source available to individuals, egg size variation could promote response variability among populations. The crossing design was not drawn to disentangle genetic from maternal effects, but in such a common garden experiment with individual egg size as a covariate in statistical analyses, differences among populations reflect to some extent genetic effects.

In our study, a single sediment type was used, sampled from a spawning ground of lake Geneva. Recent studies (*e.g*. Sear *et al.*, 2016) highlighted the importance of sediment source and in particular the oxygen demand of communities contained in the sediment, which are site-specific. Using sediments of different origins (source lakes) could hence provide more detailed insight to the mode of action of sediment. Furthermore, sediment may contain microbial communities of given pathogenic activity (Greig, Sear and Carling, 2007), which may become more virulent under elevated temperatures (Wilkins *et al.*, 2015). However, Geneva embryos did not perform better under stressful sediment and temperature conditions than other populations hence our results do not suggest local adaptation to sediment type and associated microbial communities.

Conservation implications for arctic charr populations at the Southern edge

Three out of the four study populations (Constance, Geneva and Pavin) have suffered demographic declines in the second part of the 20th century. Still not considered as self-sustaining (Caudron *et al.*, 2014; Desmolles, 2016), they have been managed by supportive breeding (*i.e.* stocking using hatchery-reared alevins, produced from field-caught spawners from the same lake) for several decades. Investigators have previously attempted to identify the factors at cause in arctic charr decline in alpine lakes, notably pinpointing a decrease in the success of natural spawning because of poor water quality and sub-optimal oxic conditions within spawning grounds (Rubin 2005; Daniel Gerdeaux 2011; Desmolles 2016).

Over the past decades, alpine lakes water quality has improved greatly, owing to stricter enforcement of regulations on phosphorus input and improvement of waste water management (Müller, Gächter and Wüest, 2014). While water quality increased, leading to significant changes in the abundance and structure of aquatic communities (Straile and Geller, 1998; Anneville and Pelletier, 2000; Jeppesen *et al.*, 2005; Gerdeaux, Anneville and Hefti, 2006), sedimentation rates remained stable (Loizeau, Girardclos and Dominik, 2012). According to recent reports of field

observations in lakes, arctic charr spawners seem to consistently target small areas within spawning grounds that are less affected by sediment (SILA, 2015), and can remove some of the sediment present on the substratum by swimming, as part of spawning behavior (J. F. Rubin and Buttiker, 1992). Thus, spawners lay eggs on clean gravel-bed, but sediment settles over the spawning area during egg incubation as arctic charr does not display parental care following spawning. As such, the spatio-temporal dynamics of exposure are certainly more complex than we can assess in controlled laboratory settings. Sediment trapping on a Lake Geneva spawning ground during winter 2016 showed a 0.49 ± 0.05 $g.cm^{-2}.yr^{-1}$ sedimentation rate (Mari *et al*., unpublished data), a much higher load that the one used in the present study. Additionally, a major issue with sediment deposition during egg development is the risk of fry entombment (Franssen, Lapointe and Magnan, 2014). A large part of alevin and fry mortality might indeed occur upon emergence from the gravel due to fine sediment clogging the surface of the redd, preventing fish to exit the substratum.

Our results showed that temperature has a considerable impact on early life history traits. In salmonids, early life is tightly linked to maternal provisioning (Perry et al. 2004; Leblanc, Kristjánsson, and Skúlason 2014), that we took into account as individual egg size in this study, although we did not measure egg quality. Arctic charr is known to have a lower thermal tolerance and lower capacity to acclimate to higher temperatures than other salmonids (Baroudy and Elliott, 1994; Anttila *et al.*, 2015), and processes such as vitellogenesis that are particularly influenced by water temperature can greatly affect spawning success and offspring quality (Gillet 1991; Gillet and Breton 2009; Gillet et al. 2011; Jeuthe, Brännäs, and Nilsson 2015). In the alpine region, current and projected temperature increases are a concerning issue (Adrian *et al.*, 2009; Lieberherr and Wunderle, 2018; Salmaso, Anneville and Straile, 2018). In deep, low-elevation lakes, temperature might exceed arctic charr tolerance thresholds levels and induce lethal and sublethal effects on embryos, with implications for populations dynamics. Nonetheless, several studies report evidence of thermoregulatory behavior in salmonids, including in sister species *S. fontinalis* (Bertolo *et al.*, 2011; Goyer *et al.*, 2014). Similarly, arctic charr adults may be able to seek thermal refugia in deeper waters and a priority might thus be to

maintain good overwintering habitat quality and cold water refugia. In shallow high elevation lakes, temperature in spawning grounds during incubation is several degrees below the stressful condition of 8.5°C tested here. Although mountain lakes are highly vulnerable to climate change, with projected faster warming and reduction of ice and snow cover (Thompson, Kamenik and Schmidt, 2005), temperature elevation is probably not a critical short-term threat for arctic charr embryos yet. However, stressors other than temperature may be of concern, including sediment that can exert destructive effects on fish regardless of thermal conditions.

Conclusions

As discussed above, sediment can affect fish early life stages in numerous ways and through a variety of mechanisms, although all will affect aerobic metabolism and allocation of yolk resources, likely carrying significant consequences into later life stages. Future studies should aim at improving our understanding of the physiological processes underpinning the observed trade-offs in morphology, yolk resources use and incubation period in multiple stressors framework. Lastly, a proper understanding of the hierarchy of stressors, *i.e.* in addition to the mechanisms by which multiple stressors interact, which stressors constitute a dominant pressure for vulnerable species, remain essential to increase our predictive capacities and implement proper management strategies.

To sum up, our study provides evidence that temperature and fine sediment can jointly exert important sublethal effects on arctic charr early life stages, such as reduction in hatchling size and less efficient use of yolk reserves. These concerning findings underscore that a co-occurrence of elevated temperatures and poor spawning ground quality can impede optimal early-life development and growth, which may in turn have critical consequences on the maintenance of the species in lakes at the Southern edge of its range. This study also revealed that wild

populations are likely to set up different response strategies when exposed to both pressures, highlighting the need to take into account population level specificities.

Acknowledgements

The experiment was designed in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and no special permission was required. ONEMA provided funding for the experiment. LM was supported by a French Ministry for Research fellowship. We thank the APERA (Association pour la mise en valeur piscicole des Plans d'Eaux en Rhône-Alpes), the Parc National du Mercantour, LAZBW (Landwirtschaftliches Zentrum für Rinderhaltung, Grünlandwirtschaft, Milchwirtschaft, Wild und Fischerei Baden-Württemberg) and the Fédération de Pêche du Puy de Dôme staff for their contribution of arctic charr spawners and logistical assistancefor their contribution of spawning individuals; Laurent Espinat and Jean-Christophe Hustache for assistance on the field; the Environmental Physics group at the Limnological Institute of the University of Konstanz for providing temperature data for Lake Constance ; and Ayala Loisel for providing temperature data for lake Sainte-Croix.

Supplementary files

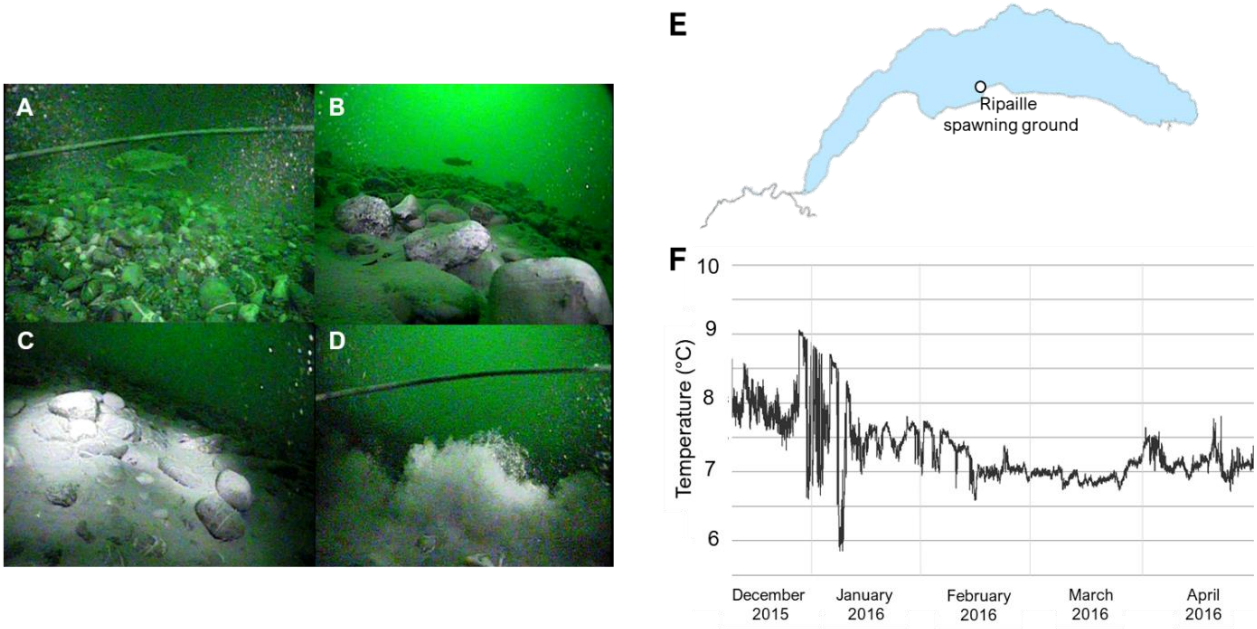


Figure 4-S1 | Information on the Lake Geneva Ripaille spawning ground (35-40m deep) from which sediment was sampled for the present study. Photographs were taken during winter 2014-2015 and show: (A) clean spawning grounds devoid of sediment (B) spawning ground with moderate sediment deposition (C) area completely clogged by deposited sediment (D) resuspended sediments following landing of the camera. (E) location of spawning ground (F) average daily temperatures recorded on spawning ground at 35m deep during winter 2015-2016.

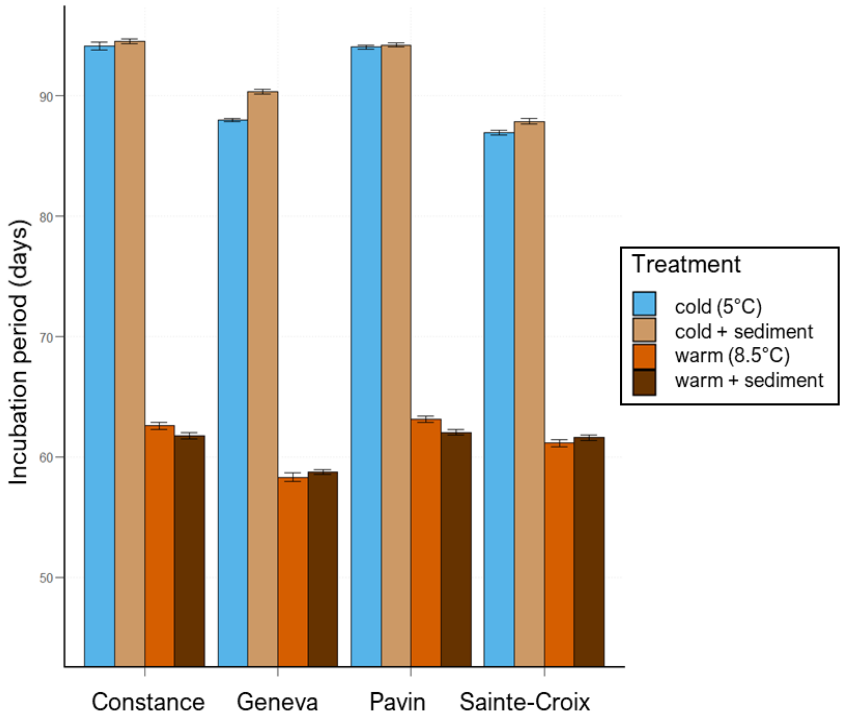
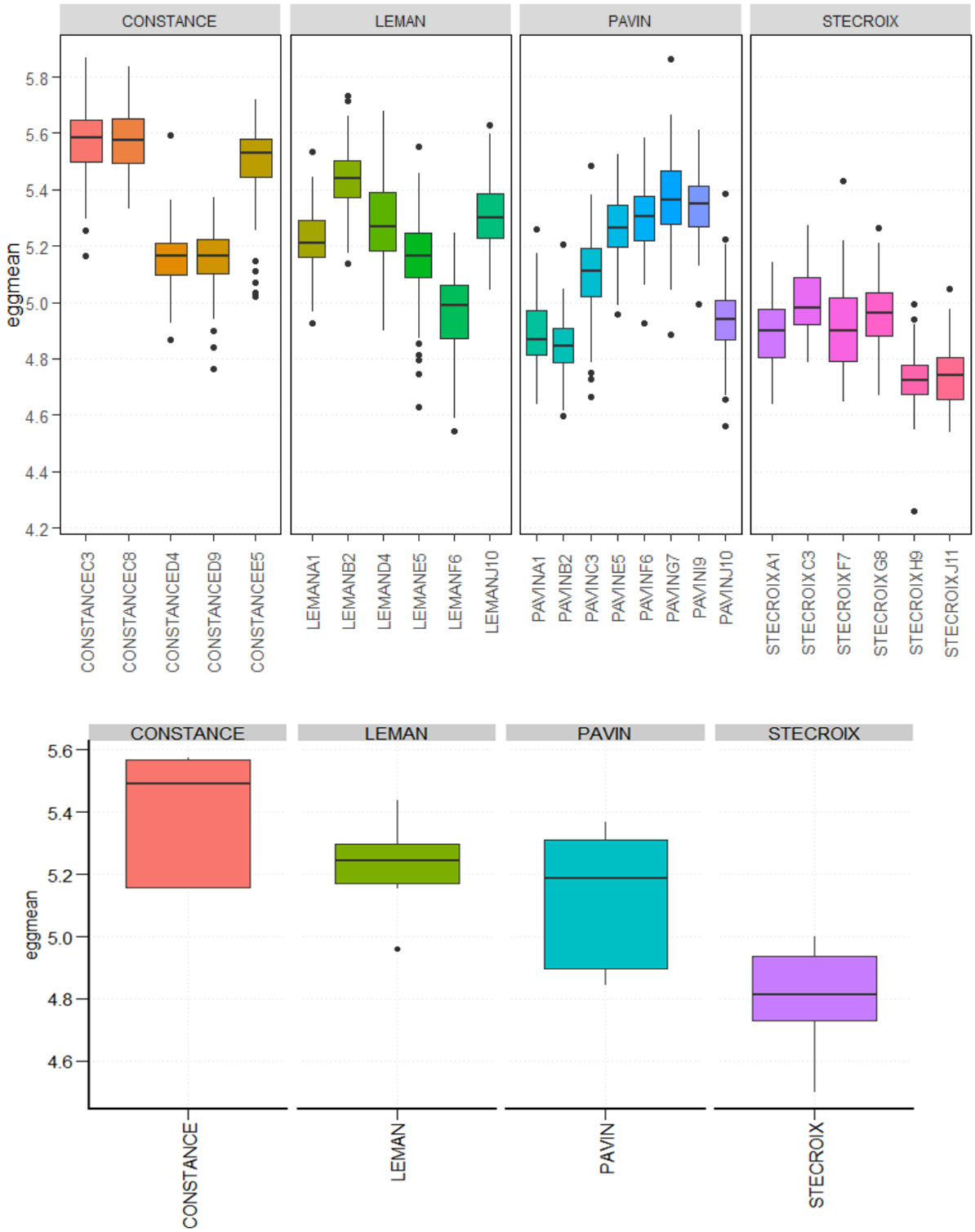


Figure 4-S2 | Effects of the four treatments on length of incubation period in days for the four study populations. In blue: control – cold temperature ; light brown: cold + sediment; orange: warm temperature; dark brown: warm + sediment. Values are expressed as mean +/- SEM.

Figure 4-S3 | Top: mean egg size per female, for all dams used in the present study. Values are represented as mean +/- 95% CI. Bottom: mean egg size per population.



5. General discussion

Effects of multiple stressors on the early life stages of a cold-water adapted fish, physiological considerations on the importance of temperature and implications for the conservation of Southernmost charr populations

Throughout this thesis, I have endeavored to study the impacts of environmental changes on Southernmost European populations of arctic charr in a global change context. I sought to tackle two important questions:

- the existence of divergence and adaptive potential among native and introduced populations in response to temperature, and
- the existence of an interaction between two critical pressures for early life stages: temperature and fine sediment.

In **Chapter 2**, I first investigated population divergence in early life fitness related traits in response to temperature. The use of a common garden experiment to rear embryos from native and introduced populations from lakes located along an altitudinal gradient, combined with quantitative genetics tools, revealed contrasted thermal reaction norms and genetic divergence. Interestingly, results did not fully support the hypothesis of local adaptation to temperature, as populations from warmer environments did not outperform populations from colder locations. Divergence in traits at hatching and emergence was driven by homogeneous selection and drift among pairs of populations, rather than directional selection. Altogether, these results showed that temperature was seemingly not the major selective pressure at play in population differentiation, other drivers (overfishing, stocking practices, environmental factors other than temperature) being critical in small, isolated populations.

While **Chapter 2** focused on the evolutionary consequences of temperature, **Chapter 3** and **Chapter 4** offered a more ecological perspective by tackling the effects of multiple stressors on early life history traits. Leaving evolutionary questions aside, I studied the influence of elevated temperature on the impacts of fine sediment, another common stressor in freshwaters, on arctic charr embryos,

both in a broodstock and in wild populations of the species. I brought strong evidence for an interaction between the two pressures on key early life traits such as the duration of egg incubation, body size and use of yolk reserves. For the first time, I showed that temperature can modulate the impacts of fine sediment on fish early life traits.

In this section, I bring together the results from these three chapters, discuss their implications and caveats, and go over future research directions.

Adaptation and plasticity in response to temperature and considerations on thermal tolerance

Much like a large body of literature, the results presented in this thesis corroborate that temperature increases will affect negatively the early life history of fish (see O'Dea *et al.*, 2018) and particularly salmonids (Pankhurst and Munday, 2011). The three results chapters showed that for life history stages for which dispersal opportunities are restricted, temperature can affect survival, duration of incubation, body size and use of yolk reserves at hatching and near-emergence. Even though the populations studied exhibited important phenotypic changes, results did not entirely confirm local adaptation patterns among populations (**Chapter 2**). Reaction norms were contrasted, and I did find occurrences of directional selection in overall QST-FST comparisons; but the responses of populations in pairwise comparisons were apparently also driven by processes other than directional selection such as drift, bottlenecks and stocking practices.

Temperature is the major environmental parameter that dictates species distributions at small and large spatial scales. In literature, our predictive capacities on the distribution of species are generally relying on information related to the thermal tolerance of species (Sunday *et al.*, 2014), a parameter measured in classic physiological studies (Speers-Roesch and Norin, 2016). It can also be used as a proxy for local adaptation to temperature to a certain extent because thermal

tolerance limits indeed constrain species response to climate change. Through this work, I showed that a continuous exposure to a temperature close to thermal tolerance limit during egg incubation can exert important sublethal effects, that will vary among populations, and provided evidence of adaptive divergence. It is likely though, that the thermal tolerance of a species can vary at several geographical scales, among populations across the species distribution, and across populations at similar latitudes. In regard to future warming, it is difficult to interpret whether alpine arctic charr will be able to cope with temperature elevations, even if adaptive potential to temperature exists (Diamond, 2017). The physiological limits of species at warm are relatively invariant and species might need to rely on behavioral tactics to cope with temperature increases (Sunday *et al.*, 2014). The fact that I have assessed the effects of only two temperatures is limiting. Even though 8.5°C is acknowledged in the literature as a limit for arctic charr early life stages, I report rather high survival rates (**Chapter 2**), and effects were essentially sublethal even in a multiple stressors context (**Chapter 4**). Phenotype and fitness can in fact vary importantly across a gradient of temperature. The evaluation of embryo response along a finer thermal gradient, including warmer and colder temperatures, could allow identifying tipping points and thermal threshold values for the different populations (Swift, 1965; Humpesch, 1985). Investigating thermal tolerance limits might be an additional approach to investigate whether or not southernmost populations are locally adapted to temperature as we lack indicators of physiological performance and knowledge on thermal tolerance would be complementary to the life history traits that were measured.

The temperature regimes used in the three experiments were homogenous. However, temperature variability during the incubation period is contrasted between populations/lakes (see Figure 1-4, Introduction section). Thermal regime variance has already been shown to affect metabolic performance, swimming capacity, and embryo development (Lim *et al.*, 2017) with strong ecological consequences for migratory salmonids (Steel, Tillotson and Larsen, 2012). Temperature variability having a role in arctic charr life history is unsure and its role in the expression of plasticity should be further explored.

Lastly, it would be interesting to investigate transgenerational effects of temperature. In arctic charr, vitellogenesis is a highly temperature sensitive process that occurs during summer and temperatures experienced throughout the year might influence female physiology and thus, maternal investment into eggs (Gillet and Breton, 2009; Gillet *et al.*, 2011; H. Jeuthe, Brännäs and Nilsson, 2015; Henrik Jeuthe, Brännäs and Nilsson, 2015). Transgenerational effects of temperature are thus likely, but a major hurdle to this type of experiment is the long generation time of arctic charr (two to three years), that complicates the feasibility of such experiments.

Response to sediment exposure and assessment of the sediment risk

In **Chapters 3** and **4**, my research clearly illustrates that fine sediment is a stressor that can affect fitness related traits in early life stages, and that these detrimental effects i) are weak but increase with very high sediment loading under optimum thermal conditions and ii) are exacerbated under warmer conditions according to a synergistic interaction. Even though literature makes mentions of sediment rates remaining stable over time in several large lakes (Asselman, Middelkoop and Van Dujk, 2003; Loizeau, Girardclos and Dominik, 2012), sediment deposition will vary according to the lake geomorphology and hydrology and case by case studies are needed to assess the dynamics of sediment exposure properly. Conducting complete studies that combine field observation and experimental research rather than relying solely on experimental results would allow a more robust assessment of the sediment risk. So far, research efforts on the effects of fine sediment on salmonid early life have been carried out in shallow habitats, *e.g.* rivers and streams. For logistic reasons, the applicability of such studies in lakes is restricted to accessible habitats, and is unfortunately impracticable in large peri-alpine lakes (*e.g.* Geneva, Constance) where spawning grounds are typically located at depths over 30-40m.

My work focused solely on fine sediment, that is, the fraction of sediment of less than 200µm grain size. Studies focusing on fish generally either use this fraction or even finer sediment (less than 63µm, see Argent and Flebbe [1999]), that has been deemed the most harmful for fish, but there has been debate around the importance of a harmful size fraction (Franssen *et al.*, 2012; Chapman *et al.*, 2014; Sear *et al.*, 2016). As a matter of fact, not only sediment granulometry, but a large number of factors can come at play in influencing the impacts of sediment: factors directly linked to the geomorphological nature of sediment such as particle size and percentage of fines; and factors linked to location and water quality, such as microbial contamination and sediment-bound pollutants. Depending on the topography of the area, the mode of action of sediment may vary (Greig, Sear and Carling, 2007; Chapman *et al.*, 2014). I only focused on direct contact with eggs, but the impacts of sediment are manifold and another critical risk might be the one of entombment of surviving individuals, preventing them to exit from the redd (Franssen *et al.*, 2012). However, entombment may also favor only larger surviving individuals that are capable of swimming more efficiently than their conspecifics.

Size at emergence from the gravel might further influence later life phenotype. In migratory salmonids, differences in early life development rate and yolk conversion efficiency have been linked to divergent later life phenotypes and behavioral syndromes (Angilletta *et al.*, 2008; Régnier *et al.*, 2012; Metcalfe, Van Leeuwen and Killen, 2016; Van Leeuwen *et al.*, 2017). **Chapter 4** showed that when temperature and sediment were experienced simultaneously, populations exhibited phenotypic responses that resembled different strategies of response. Considering these observations were made at hatching, it is difficult to infer of the consequences these phenotypes will have in later stages. Because the experimental set up consisted of cell culture plates, individuals could not be reared beyond hatching, we can only envisage that the strong differences in size and timing of hatching observed in this work can have consequences past the first feeding stage, notably in behavior and swimming capacity, and this will impact escape capacity and foraging.

Life history trade-offs in early life: growth, development, and maintenance

The previous three chapters have all successfully demonstrated that environmental stressors can affect embryo development with important reduction in size and differential use of yolk sac reserves among populations and treatments. Arctic charr embryos – as most salmonid embryos in general – rely on endogenous feeding until they emerge from gravel (Rubin and Buttiker, 1992; Teletchea and Fontaine, 2010). Thus, the limited yolk resources must be allocated towards tissue production for growth, organogenesis for development, or organism maintenance. Life history theory predicts that the reallocation of reserves towards one specific biological function over another indubitably leads to tradeoffs (Stearns, 1992), and changes in environmental conditions will be reflected on competitive energy partitioning.

Physiological responses to temperature and to hypoxia can be profound. They include, but are not limited to, changes in cardiac frequency, respiratory ventilation (in arctic charr notably, see Anttila *et al.* [2015]), and metabolic rates. Elevated temperatures induce an increase in respiration rates and metabolism, leading to a higher turnover rate at the cellular and mitochondrial level. As physiological processes can underlie tradeoffs between traits, one hypothesis would be that yolk resources were expended for organism maintenance instead of growth and that the responses to the environment were mediated by changes in the oxidative balance. A part of my work was devoted to explore this assumption (see Box 1). Results were inconclusive and it was not possible to disentangle whether they reflected biological variation or methodological limitations. As a matter of fact, oxidative stress biomarkers can exhibit extremely high intrinsic variability, requiring the use of a high number of biomarkers to encompass the system variability (Sepp *et al.* [2012] recommend using over eight). However, we can expect that tradeoffs might occur at other levels tightly linked to the expression of oxidative stress, by affecting, as example, mitochondrial acclimation potential (Chung and Schulte, 2015).

Box 1 | Complementary investigation on oxidative balance

What is oxidative stress?

At the cellular level, ATP production is accompanied by the ubiquitous production of reactive oxygen species (ROS), that are released from the mitochondrial electron transport chain as ATP synthesis byproducts. ROS are paradoxically essential and hazardous to cell function. At moderate cellular concentrations, they play a role in the mediation of signaling processes, thus forming a core component of cellular homeostasis (Metcalfe and Alonso-Alvarez, 2010). When in excess, ROS can induce significant damage to cell membranes, proteins and DNA, and threaten cell integrity and survival, a situation referred to as oxidative stress. Under non stressful conditions, the production of ROS is balanced with the production of enzymatic anti-oxidant defenses to prevent. Over the past two decades (Alonso-Alvarez *et al.*, 2004; Monaghan, Metcalfe and Torres, 2009; Costantini *et al.*, 2010) research has shown that oxidative balance might be playing a key role in mediating life history trade-offs, as oxidative stress can be seen as a universal physiological constraint (Dowling and Simmons, 2009; Metcalfe and Alonso-Alvarez, 2010). However, oxidative stress is largely understudied in fish compared to other taxa (Birnie-Gauvin *et al.*, 2017) and one of the initial goals of the experiment presented in **Chapter 2** was to investigate the relationship between temperature, traits and the oxidative balance among the study populations.

Hypotheses

We hypothesized that i) temperature will induce at least an increase in antioxidant defenses at warm, if not an increase of both defenses and oxidative damages, ii) synthesis of antioxidant defenses is costly, and will be traded-off against growth, likely resulting in smaller individual and/or individual of same size as their conspecifics in the cold treatment, but with reduced yolk sizes, and iii) such tradeoff will be less observed less markedly in populations originating from warmer lakes.

Methods

20-25 individuals per population were sampled at T2 (end of the experiment, near emergence), euthanized by eugenol overdose, and immediately frozen in liquid nitrogen. The high lipid content of the yolk sac made it nearly impossible to obtain robust and repeatable measurements. Thus, the yolk was systematically removed from individuals prior to thawing, and analyses were performed on larval tissue only. Larval tissue was homogenized on ice using a mortar and pestle in PBS 1X buffer. We measured the concentration in SOD (enzymatic defense) and TBARS (damages to cell membrane lipids) of samples using assay kits (Cayman Chemical, USA). We performed the three assays according to manufacturer's instructions.

Box 1 – Continued | Complementary investigation on oxidative balance

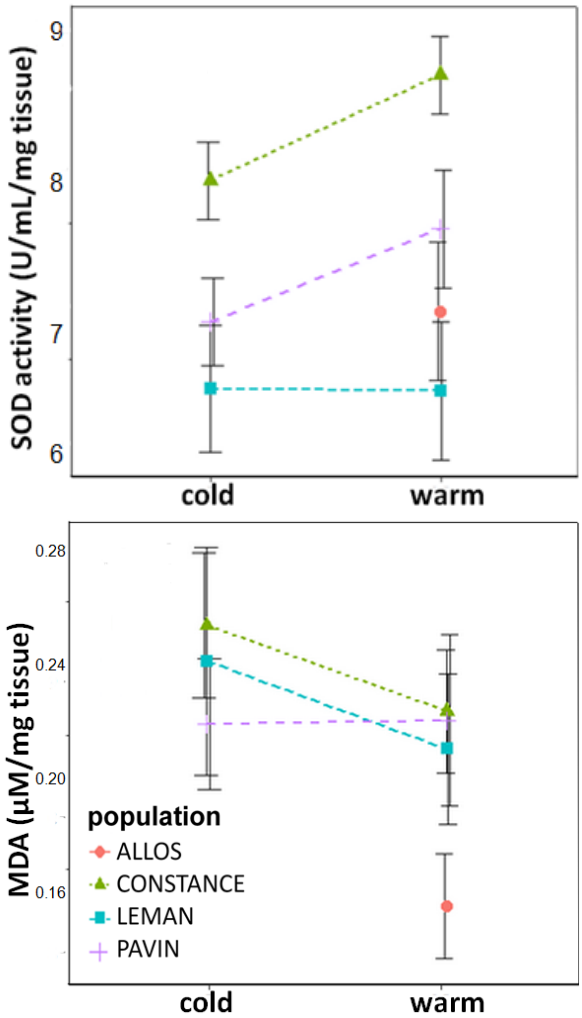


Figure 5-1 | Top: enzymatic anti-oxydant activity of SOD. Bottom: quantified damages to membranes with the TBARS method.

Summary of results

The results show a trend in enzymatic defense (SOD activity) increase for the populations of Constance and Pavin under warm conditions, although significance was marginal. No differences between thermal treatments were detected for Geneva. There were no differences in measured damages (TBARS) among thermal treatments.

While our sample size is average for physiological studies, there was great variability among individuals and patterns were not significant. It is nonetheless interesting to note that Allos individuals, that outperformed other populations, show less damages at warm.

It is certain that the environment can affect phenotypic and evolutionary responses, going beyond life history traits to affect several levels of biological complexity *e.g.* cell, physiological performance, life history, or behavior. These responses themselves stem from changes in gene expression (Heath et al 1995) and tradeoffs might occur at the gene expression level as well (St-Cyr, Derome and Bernatchez, 2008; Mäkinen *et al.*, 2016). The past decade has seen a rapid growth of a body of literature on the use of high-throughput sequencing tools – particularly transcriptomics – to investigate the impacts of abiotic variables on non-model

species (Qian *et al.*, 2014; Logan and Buckley, 2015; Todd, Black and Gemmell, 2016; Oomen and Hutchings, 2017). Thus, a major perspective of this thesis is to assess the physiological underpinnings of response to temperature at the molecular level with a transcriptomics approach. In Fall 2017, I have initiated a collaboration with the University of Iceland, where I have spent two months to perform the laboratory work necessary for sample and library preparation for RNA-sequencing. A total of 96 samples (9-12 individuals per population and temperature treatment) from the common garden experiment described in **Chapter 4** were sequenced. As the data is still being analyzed to this day, no related results will be discussed here. The analysis of RNA-Seq data should nonetheless provide comprehensive insights to aid us interpret the results observed at the life history level in response to temperature by i) identifying co-regulated sets of genes involved in the response, ii) assessing individual variation within each population, and iii) whether conserved gene expression patterns exist among populations originating from similar thermal habitats or from similar genetic background.

Implications of this work for Southernmost arctic charr populations

Multiple stressors affecting arctic charr and management of multiple stressors

A fundamental aspect to the management of multiple stressors is the characterization of the nature and quantification of stressors interactions. Establishing a hierarchy of stressors is instrumental to set up appropriate management measures as identifying the dominant stressors affecting a system is necessary (MARS 2018, Jeppesen et al 2017). The environment is complex and it is known that temperature can modulate the effects of many other pressures (Heino, Virkkala and Toivonen, 2009; Lange *et al.*, 2018). By showing that temperature can modulate the sensitivity of charr embryos to fine sediment, **Chapter 3** underscores the importance to reconsider the impacts of 'classic' stressors under the light of several thermal scenarios that reflect future thermal

conditions. This might lead to a more realistic, albeit pessimistic, assessment of the impacts of the environment on freshwater biota.

The rate of warming is a major concern, but many other environmental stressors are likely to affect arctic charr population dynamics in alpine lakes and should not be neglected (Champigneulle and Gerdeaux, 1995; Rubin, 2005; Gerdeaux, 2011; Caudron *et al.*, 2014; Tiberti and Splendiani, 2019). While habitat degradation, habitat loss and lack of thermal refugia are critical abiotic pressures, biotic interactions are equally important but receive less attention in a multiple stressors framework. With climate change, the species will be subjected to increased predation and competition for habitat or food resources with native and invasive generalists and/or eurythermal species that will thrive under warmer conditions (*e.g.* competition between charr juvenile and invasive stickleback in Greenland [Svenning, Aas and Borgstrøm, 2015]). However, the number of environmental stressors we take into account increases the number of possible two-way interactions (Côté, Darling and Brown, 2016) and a proper assessment of long term and large-scale consequences will require a combination of experiments with a modelling approach.

Genetic structure of populations and influence of stocking practices

As observed among the native vs. introduced populations studied in **Chapter 2,** and if we were to compare the response to multiple stressors among the captive stock studied in **Chapter 3** and the wild populations of **Chapter 4**, the sensitivity to environmental differs between wild and captive fish, and among managed and non-managed populations. This research thus raises two important issues on fish management practices (Figure 2):

i) the consequence of domestication (maladaptation of captive individuals released in the wild), and

ii) the consequences of stocking practices (as a source of genetic stress).

Figure 5-2 | Mechanisms by which current supportive breeding practices can affect wild populations' genetic integrity and adaptive potential.

Chapter 2 did not directly focus on the genetic structure of the four study populations (Allos, Pavin, Constance, and Geneva) per se, but the microsatellite genotyping and clustering approach (STRUCTURE) demonstrated interesting dynamics in population structure that are worth reporting here (Figure 3). Assignment probability to genetic clusters revealed that the structure of lake Geneva was strikingly similar to the structure of Allos. The Allos population was founded from Geneva individuals in the 1920s. If we consider Allos as representative of the Geneva population's genetic diversity, this would confirm the findings of Savary *et al.*, 2017, who report that despite eutrophication-induced population declines and allochthonous charr introductions in the 70s, the structure of the Geneva population recovered and is now essentially similar to the structure prior to these events. While intriguing, this result is interesting because it suggests a form of resilience that occurred particularly rapidly within the population, since allochthonous genotypes were not assimilated on the long term. However, the thermal reaction norms observed between Allos and Geneva were contrasted, with Allos outperforming Geneva.

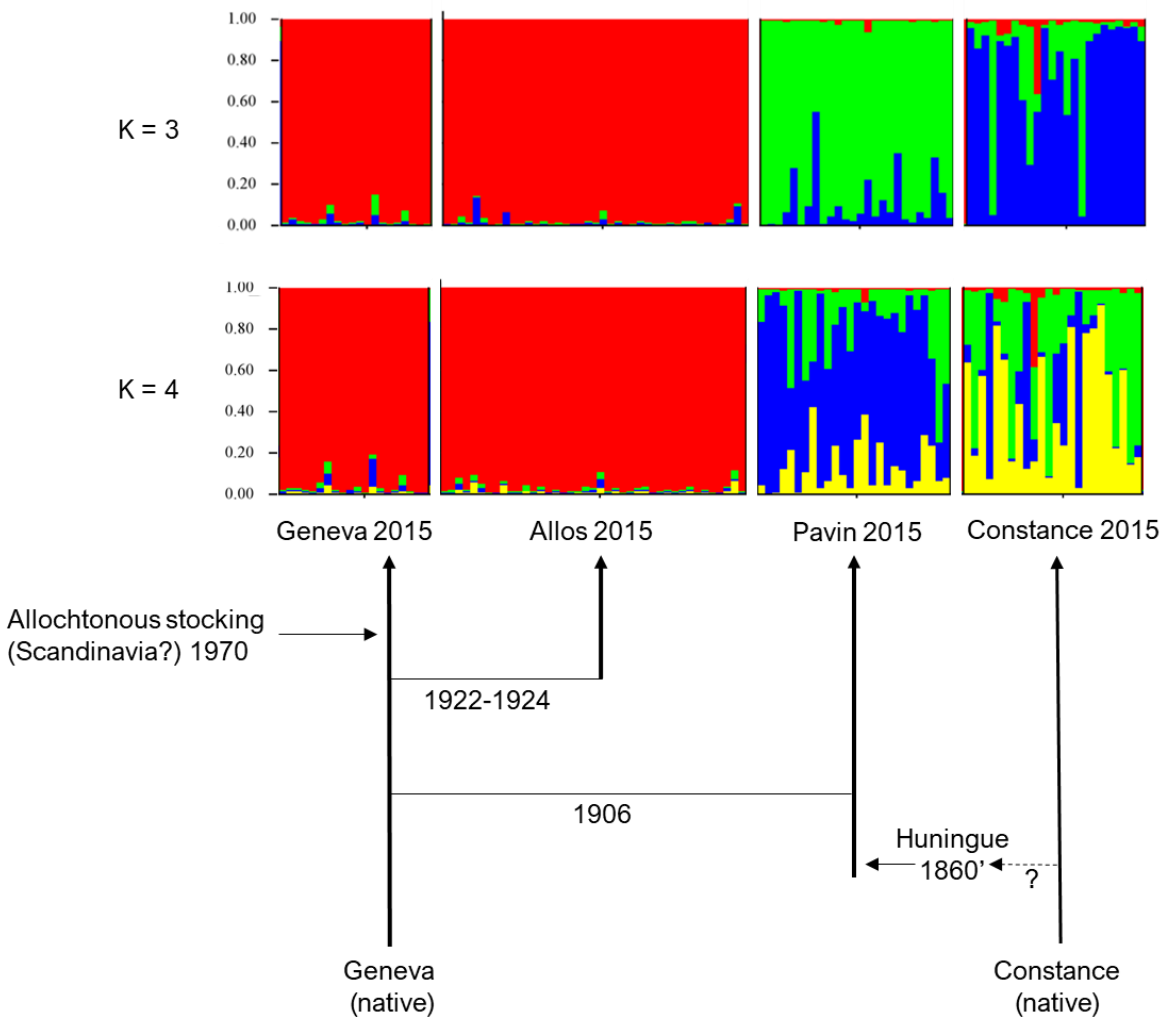


Figure 5-3 | Barplots of individual assignment probability within each population to 3 genetic clusters (top) or 4 genetic clusters (bottom) in Chapter 2; and links with dates of arctic charr introductions within each population. Refer to the Material & Methods section of Chapter 2 for references.

Stocking practices, including supportive breeding, can lead to genetic stress (Heath *et al.*, 2003; Christie *et al.*, 2011, 2016). Although it is supported by a growing number of investigations, this reasoning remains speculative and future research should attempt to address the impacts of stocking on the adaptive potential of charr populations and the consequences of different approaches to population management. Both well-suited hatchery rearing conditions and parsimonious stocking are paramount to successful management programs.

Spawners used in the **Chapter 3** experiment were captive but originated initially from Lake Geneva, although no introduction of wild individuals occurred in this stock for the past 20 years. Microsatellite genotyping of individuals from this captive stock (using the same methods as in Chapter 2) revealed a rather high FST between captive fish and wild Geneva individuals (FST = 0.083). It is extremely likely that hatchery selection took place over the several past generations, which might explain why this population is so differentiated from the wild one. Many studies report that captive fish are not suitable to being released into the wild ; in captivity, chances of survival are much higher because environmental conditions are optimum (Einum and Fleming, 2000). But as hatchery reared organisms experience different conditions (e.g. high densities, restricted swimming, different temperatures), the phenotypes of hatchery reared individuals ultimately deviate from wild conspecifics in terms of growth, physiology, behavior and neurophysiology (Rosengren *et al.*, 2016). Captive exposure has been shown to induce significant plastic and genetic changes over short time scales, notably over the course of a single generation in brook trout (Fraser *et al.*, 2018).

Hatchery settings will never be fully representative of natural conditions, but even though preventing artificial selection is nearly impossible, it would be interesting to evaluate how introducing more complexity (e.g. fluctuations in temperature, exposure to sediment) to create more realistic environments could help limiting maladaptation and conserving the adaptive potential of populations.

Much like many other salmonid species, arctic charr populations in large alpine lakes have suffered the consequences of overfishing during the 20th century but are still exploited to this day. Except for the lake Geneva population, information is lacking regarding the historical genetic structure of charr populations. It is known that intense fishing pressure can induce significant phenotypic changes in fish reproductive traits, and investigators have highlighted occurrences of fishery induced evolution (Dunlop, Feiner and Höök, 2018; Hollins *et al.*, 2018) including in perialpine lakes salmonids (Nusslé, Bornand and Wedekind, 2009; Thomas *et al.*, 2009). If stocking practices can induce plastic and genetic changes, fisheries

pressure can indubitably contribute to shaping the genetic structure of populations as well.

Concluding remarks

In summary, this work brought important insights to the study of the impacts of multiple stressors on the early life stages of an iconic freshwater stenotherm, with a particular attention to the species' capacity to respond to temperature, whether adaptively or not.

The way forward might however be in aiming at depicting a more exact picture of the potential of response of populations to environmental changes, not by investigating the responses but the architecture of response (Bay *et al.*, 2017). Pursuing such endeavors would greatly improve our predictive capacities, even though it would require a sustained effort to gather robust, long-term data on the characterization of adaptive alleles, genetic architecture, and dynamics of populations.

In regard to the conservation of arctic charr and our role in preserving vulnerable populations or species in the context of fast environmental changes, there is a long history of human-induced disturbances in the arctic charr populations of large alpine lakes (overfishing, eutrophication, intense stocking practices) and finding solutions for sustainable management can be challenging.

While some authors are in favor of a more proactive approach to conservation, does the "quest for a good Anthropocene" (Madliger *et al.*, 2018) truly imply to try setting up management measures at all costs? The past decade has seen investigators start questioning management practices (Brannon *et al.*, 2004), even though when carried out carefully, stocking can help populations recover to a certain extent. A recently published review on the current status of arctic charr in Italian Alps (Tiberti and Splendiani, 2019) suggests that we reevaluate the conservation value of the species at this latitude – especially in lakes where the charr was introduced – and re-think our approach to conservation. Prior to decision-making, a holistic approach integrating life-history, physiological performance and information on the genetic background of populations, remains essential.

6. References

Abbott, R. E., Doak, D. F. and Peterson, M. L. (2017) 'Portfolio effects, climate change, and the persistence of small populations: analyses on the rare plant Saussurea weberi', *Ecology*, 98(4), pp. 1071–1081. doi: 10.1002/ecy.1738.

Adams, C. E., Wilson, A. J. and Ferguson, M. M. (2008) 'Parallel divergence of sympatric genetic and body size forms of Arctic charr, Salvelinus alpinus, from two Scottish lakes', *Biological Journal of the Linnean Society*, 95(4), pp. 748–757. doi: 10.1111/j.1095-8312.2008.01066.x.

Adrian, R. *et al.* (2009) 'Lakes as sentinels of climate change', *Limnology and Oceanography*, 54(6 part 2), pp. 2283–2297.

Alonso-Alvarez, C. *et al.* (2004) 'Increased susceptibility to oxidative stress as a proximate cost of reproduction', *Ecology Letters*, 7(5), pp. 363–368. doi: 10.1111/j.1461-0248.2004.00594.x.

Amundsen, P. A. and Knudsen, R. (2009) 'Winter ecology of Arctic charr (Salvelinus alpinus) and brown trout (Salmo trutta) in a subarctic lake, Norway', *Aquatic Ecology*, 43(3), pp. 765–775. doi: 10.1007/s10452-009-9261-8.

Andersen, D. H. *et al.* (2007) 'Divergence at neutral and non-neutral loci in Drosophila buzzatii populations and their hybrids', *Evolutionary Ecology*, 22(5), pp. 593–605. doi: 10.1007/s10682-007-9184-6.

Angilletta, M. J. *et al.* (2003) 'Tradeoffs and the evolution of thermal reaction norms', *Trends in Ecology and Evolution*, 18(5), pp. 234–240. doi: 10.1016/S0169-5347(03)00087-9.

Angilletta, M. J. *et al.* (2008) 'Big dams and salmon evolution: changes in thermal regimes and their potential evolutionary consequences', *Evolutionary Applications*, 1(2), pp. 286–299. doi: 10.1111/j.1752-4571.2008.00032.x.

Anneville, O. *et al.* (2013) 'L ' empreinte du changement climatique sur le Léman', *Archives des Sciences*, 41(0), pp. 157–172.

Anneville, O. and Pelletier, J. P. (2000) 'Recovery of Lake Geneva from eutrophication : quantitative response of phytoplankton', *Archiv für Hydrobiologie*, (148:4), pp. 607–624.

Anttila, K. *et al.* (2015) 'Warm acclimation and oxygen depletion induce species-specific responses in salmonids.', *The Journal of experimental biology*, 218(Pt 10), pp. 1471–7. doi: 10.1242/jeb.119115.

Argent, D. G. and Flebbe, P. a. (1999) 'Fine sediment effects on brook trout eggs in laboratory streams', *Fisheries Research*, 39, pp. 253–262. doi: 10.1016/S0165-7836(98)00188-X.

Asselman, N. E. M., Middelkoop, H. and Van Dujk, P. M. (2003) 'The impact of changes in climate and land use on soil erosion, transport and deposition of suspended sediment in the River Rhine', *Hydrological Processes*, 17(16), pp. 3225–3244. doi: 10.1002/hyp.1384.

Balian, E. V. *et al.* (2007) 'The Freshwater Animal Diversity Assessment: an overview of the results', *Hydrobiologia*, (595), pp. 627–637. doi: 10.1007/s10750-007-9246-3.

Baroudy, E. and Elliott, J. . (1994) 'Tolerance of parr of Arctic charr, Salvelinus alpinus, to reduced dissolved oxygen concentrations', *Journal of Fish Biology*, 44, pp. 736–738.

Baroudy, E. and Elliott, J. M. (1994) 'The critical thermal limits for juvenile Arctic charr Salvelinus alpinus', *Journal of Fish Biology*, pp. 1041–1053. doi: 10.1111/j.1095-8649.1994.tb01071.x.

Bates, D. *et al.* (2015) 'Fitting Linear Mixed-Effects Models Using lme4', *Journal of Statistical Software*, 67(1), pp. 1–48. doi: 10.18637/jss.v067.i01.

Bay, R. A. *et al.* (2017) 'Predicting Responses to Contemporary Environmental Change Using Evolutionary Response Architectures', *The American Naturalist*. The University of Chicago Press, 189(5), pp. 463–473. doi: 10.1086/691233.

Bebak, J., Hankins, J. A. and Summerfelt, S. T. (2000) 'Effect of Water Temperature on Survival of Eyed Eggs and Alevins of Arctic Char', *North American Journal Of Aquaculture*, (62:2), pp. 139–143. doi: 10.1577/1548-8454(2000)062.

Beer, W. N. and Anderson, J. J. (2011) 'Sensitivity of juvenile salmonid growth to future climate trends', *River Research and Applications*. John Wiley & Sons, Ltd, 27(5), pp. 663–669. doi: 10.1002/rra.1390.

Beer, W. N. and Anderson, J. J. (2013) 'Sensitivity of salmonid freshwater life history in western US streams to future climate conditions', *Global Change Biology*. John Wiley & Sons, Ltd (10.1111), 19(8), pp. 2547–2556. doi: 10.1111/gcb.12242.

Bellard, C. *et al.* (2012) 'Impacts of climate change on the future of biodiversity', *Ecology Letters*, 15(4), pp. 365–377. doi: 10.1111/j.1461-0248.2011.01736.x.Impacts.

Bennett, D. H., Connor, W. P. and Eaton, C. A. (2003) 'Substrate composition and emergence success of fall Chinook salmon in the Snake River', *Northwest Sci.*, 77(2), pp. 93–99.

Bertolo, A. *et al.* (2011) 'Behavioural thermoregulatory tactics in lacustrine brook charr, Salvelinus fontinalis', *PLoS ONE*, 6(4). doi: 10.1371/journal.pone.0018603.

Birnie-Gauvin, K. *et al.* (2017) 'A comparative and evolutionary approach to oxidative stress in fish: A review', *Fish and Fisheries*. John Wiley & Sons, Ltd (10.1111), 18(5), pp. 928–942. doi: 10.1111/faf.12215.

Blanquart, F. *et al.* (2013) 'A practical guide to measuring local adaptation', *Ecology Letters*, 16(9), pp. 1195–1205. doi: 10.1111/ele.12150.

Bogataj, L. (2007) 'How will the Alps Respond to Climate Change', *Alpine space–man & environment*, 3, pp. 43–51. Available at: http://www.uibk.ac.at/alpinerraum/publications/vol3/kajfezbogataj.pdf (Accessed: 29 June 2015).

Brannon, E. L. *et al.* (2004) 'The controversy about salmon hatcheries', *Fisheries*, 29(9), pp. 12–31. doi: 10.1577/1548-8446(2004)29[12:tcash]2.0.co;2.

Brook, B. W., Sodhi, N. S. and Bradshaw, C. J. A. (2008) 'Synergies among extinction drivers under global change', *Trends in Ecology & Evolution*. Elsevier, 23(8), pp. 453–460. doi: 10.1016/j.tree.2008.03.011.

Brunner, P. C. *et al.* (2001) 'Holarctic phylogeography of Arctic charr (Salvelinus alpinus L.) inferred from mitochondrial DNA sequences', *Evolution*, 55(3), pp. 573–586. doi: 10.1111/j.0014-3820.2001.tb00790.x.

Brunner, P. C., Douglas, M. R. and Bernatchez, L. (1998) 'Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr Salvelinus alpinus (Teleostei: Salmonidae) from central Alpine lakes', *Molecular Ecology*, 7(2), pp. 209–223. doi: 10.1046/j.1365-294x.1998.00341.x.

Caudron, A. *et al.* (2014) 'Thirty years of reoligotrophication do not contribute to restore self-sustaining fisheries of Arctic charr, Salvelinus alpinus, in Lake Geneva', *Fisheries Research*, 154, pp. 165–171. doi: 10.1016/j.fishres.2014.01.023.

Champigneulle, A. and Gerdeaux, D. (1995) 'Survey, management and recent rehabilitation of the Arctic charr (Salvelinus alpinus) fishery in the French-Swiss Lake Leman', in.

Soetvattenslaboratoriet. Available at: http://agris.fao.org/agris-search/search.do?recordID=SE9611184 (Accessed: 29 June 2015).

Chapman, J. M. *et al.* (2014) 'Clear as mud: a meta-analysis on the effects of sedimentation on freshwater fish and the effectiveness of sediment-control measures.', *Water research*, 56, pp. 190–202. doi: 10.1016/j.watres.2014.02.047.

Chapuis, E. *et al.* (2007) 'High quantitative and no molecular differentiation of a freshwater snail (Galba truncatula) between temporary and permanent water habitats', *Molecular Ecology*, 16(16), pp. 3484–3496. doi: 10.1111/j.1365-294X.2007.03386.x.

Charmantier, A. and Garant, D. (2005) 'Environmental quality and evolutionary potential: Lessons from wild populations', *Proceedings of the Royal Society B: Biological Sciences*, 272(1571), pp. 1415–1425. doi: 10.1098/rspb.2005.3117.

Chevin, L. M., Lande, R. and Mace, G. M. (2010) 'Adaptation, plasticity, and extinction in a changing environment: Towards a predictive theory', *PLoS Biology*, 8(4). doi: 10.1371/journal.pbio.1000357.

Christie, M. R. *et al.* (2011) 'Genetic adaptation to captivity can occur in a single generation', *Proceedings of the National Academy of Sciences*, 109(1), pp. 238–242. doi: 10.1073/pnas.1111073109.

Christie, M. R. *et al.* (2016) 'A single generation of domestication heritably alters the expression of hundreds of genes', *Nature Communications*. Nature Publishing Group, 7(1), pp. 1–6. doi: 10.1038/ncomms10676.

Chung, D. J. and Schulte, P. M. (2015) 'Mechanisms and costs of mitochondrial thermal acclimation in a eurythermal killifish (Fundulus heteroclitus)', *The Journal of Experimental Biology*, 218, pp. 1621–1631. doi: 10.1242/jeb.120444.

Cocchiglia, L. (2012) 'Evaluation of the effects of fine sediment inputs from stream culverts on brown trout egg survival through field and laboratory assessments', *Inland Waters*, 2(1), pp. 47–58. doi: 10.5268/IW-2.1.495.

Comte, L., Murienne, J. and Grenouillet, G. (2014) 'Species traits and phylogenetic conservatism of climate-induced range shifts in stream fishes', *Nature Communications*, 5, pp. 1–9. doi: 10.1038/ncomms6053.

Comte, L. and Olden, J. D. (2016) 'Evolutionary and environmental determinants of freshwater fish thermal tolerance and plasticity', *Global Change Biology*. doi: 10.1111/gcb.13427.

Costantini, D. *et al.* (2010) 'From molecules to living systems: Historical and contemporary issues in oxidative stress and antioxidant ecology', *Functional Ecology*, 24(5), pp. 950–959. doi: 10.1111/j.1365-2435.2010.01746.x.

Côté, I. M., Darling, E. S. and Brown, C. J. (2016) 'Interactions among ecosystem stressors and their importance in conservation'.

Côte, J. *et al.* (2012) 'Population differences in response to hypoxic stress in Atlantic salmon', *Journal of Evolutionary Biology*, 25(12), pp. 2596–2606. doi: 10.1111/jeb.12007.

Côte, J. *et al.* (2016) 'Adaptive divergence in embryonic thermal plasticity among Atlantic salmon populations', *Journal of Evolutionary Biology*, pp. 1–9. doi: 10.1111/jeb.12896.

Cramp, R. L. *et al.* (2014) 'Synergistic interaction between UVB radiation and temperature increases susceptibility to parasitic infection in a fish Synergistic interaction between UVB radiation and temperature increases susceptibility to parasitic infection in a fish', pp. 10–13.

Crozier, L. G. and Hutchings, J. a. (2014) 'Plastic and evolutionary responses to climate change

in fish', *Evolutionary Applications*, 7(1), pp. 68–87. doi: 10.1111/eva.12135.

Dea, R. E. O. *et al.* (2018) 'Title : Developmental temperature affects phenotypic means and variability : a meta-analysis of fish data Authors and affiliations', (December), pp. 1–113.

Desmolles, F. (2016) 'History of the Fish Fauna of Lake Pavin : A Population Heavily Influenced by man?' doi: 10.1007/978-3-319-39961-4.

Diamond, S. E. (2017) 'Evolutionary potential of upper thermal tolerance: biogeographic patterns and expectations under climate change', *Annals of the New York Academy of Sciences*. doi: 10.1111/nyas.13223.

Dowling, D. K. and Simmons, L. W. (2009) 'Reactive oxygen species as universal constraints in life-history evolution', *Proceedings of the Royal Society B: Biological Sciences*, 276(1663), pp. 1737–1745. doi: 10.1098/rspb.2008.1791.

Dudgeon, D. *et al.* (2006) 'Freshwater biodiversity: importance, threats, status and conservation challenges', *Biological Reviews of the Cambridge Philosophical Society*, 81(2), pp. 163–82. doi: 10.1017/S1464793105006950.

Dunlop, E. S., Feiner, Z. S. and Höök, T. O. (2018) 'Potential for fisheries-induced evolution in the Laurentian Great Lakes', *Journal of Great Lakes Research*. The Authors, 44(4), pp. 735–747. doi: 10.1016/j.jglr.2018.05.009.

Einum, S. and Fleming, I. A. (2000) 'Selection against late emergence and small offspring in Atlantic salmon (Salmo salar)', *Evolution*, 54(2), pp. 628–639. doi: 10.1111/j.0014-3820.2000.tb00064.x.

Elliott, J. M. and Elliott, J. a. (2010) 'Temperature requirements of Atlantic salmon Salmo salar, brown trout Salmo trutta and Arctic charr Salvelinus alpinus: Predicting the effects of climate change', *Journal of Fish Biology*, 77(8), pp. 1793–1817. doi: 10.1111/j.1095-8649.2010.02762.x.

Fitzpatrick, B. M. (2012) 'Underappreciated Consequences of Phenotypic Plasticity for Ecological Speciation', *International Journal of Ecology*, pp. 32–37. doi: 10.1155/2012/256017.

Flatt, T. (2005) 'The evolutionary genetics of canalization', *The Quarterly Review of Biology*, 80(3), pp. 288–316. doi: 10.1086/516403.

Foden, W. B. *et al.* (2013) 'Identifying the World's Most Climate Change Vulnerable Species: A Systematic Trait-Based Assessment of all Birds, Amphibians and Corals', *PLOS ONE*. Public Library of Science, 8(6), p. e65427. Available at: https://doi.org/10.1371/journal.pone.0065427.

Franks, S. J. and Hoffmann, A. A. (2012) 'Genetics of Climate Change Adaptation', *Annual Review of Genetics*, 46(1), pp. 185–208. doi: 10.1146/annurev-genet-110711-155511.

Franssen, J. *et al.* (2012) 'Asphyxiation and entombment mechanisms in fines rich spawning substrates : experimental evidence with brook trout (Salvelinus fontinalis) embryos', *Canadian Journal of Fisheries and Aquatic Sciences*, 599, pp. 587–599. doi: 10.1139/F2011-168.

Franssen, J., Lapointe, M. and Magnan, P. (2014) 'Geomorphic controls on fine sediment reinfiltration into salmonid spawning gravels and the implications for spawning habitat rehabilitation', *Geomorphology*. Elsevier B.V., 211, pp. 11–21. doi: 10.1016/j.geomorph.2013.12.019.

Fraser, D. J. *et al.* (2018) 'Population correlates of rapid captive- - induced maladaptation in a

wild fish', (May), pp. 1–13. doi: 10.1111/eva.12649.

Garcia-gonzalez, F. *et al.* (2012) 'COMPARING EVOLVABILITIES : COMMON ERRORS SURROUNDING THE CALCULATION AND USE OF COEFFICIENTS OF ADDITIVE GENETIC VARIATION', *Evolution*, (1), pp. 2341–2349. doi: 10.1111/j.1558-5646.2011.01565.x.

Gerdeaux, D. (2011) 'Does global warming threaten the dynamics of Arctic charr in Lake Geneva?', *Hydrobiologia*, 660(1), pp. 69–78. doi: 10.1007/s10750-010-0412-7.

Gerdeaux, D., Anneville, O. and Hefti, D. (2006) 'Fishery changes during re-oligotrophication in 11 peri-alpine Swiss and French lakes over the past 30 years', *Acta Oecologica*, 30, pp. 161–167. doi: 16/j.actao.2006.02.007.

Ghalambor, C. K. *et al.* (2007) 'Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments', *Functional Ecology*, 21(3), pp. 394–407. doi: 10.1111/j.1365-2435.2007.01283.x.

Gienapp, P. *et al.* (2008) 'Climate change and evolution : disentangling environmental and genetic responses', *Molecular Ecology*, (17), pp. 167–178. doi: 10.1111/j.1365-294X.2007.03413.x.

Gilbert, K. J. and Whitlock, M. C. (2015) 'Q ST – F ST comparisons with unbalanced half-sib designs', *Molecular Ecology Resources*, (15), pp. 262–267. doi: 10.1111/1755-0998.12303.

Gillet, C. (1991) 'Egg production in an Arctic charr (Salvelinus alpinus L.) brood stock: effects of temperature on the timing of spawning and the quality of eggs', *Aquatic Living Resources*, 4, pp. 109–116. doi: 10.1051/alr:1991010.

Gillet, C. *et al.* (2011) 'General and Comparative Endocrinology Disruption of the secretion and action of 17 , 20 b -dihydroxy-4-pregnen-3-one in response to a rise in temperature in the Arctic charr , Salvelinus alpinus . Consequences on oocyte maturation and ovulation', *General and Comparative Endocrinology*. Elsevier Inc., 172(3), pp. 392–399. doi: 10.1016/j.ygcen.2011.04.002.

Gillet, C. and Breton, B. (2009) 'LH secretion and ovulation following exposure of Arctic charr to different temperature and photoperiod regimes: Responsiveness of females to a gonadotropin-releasing hormone analogue and a dopamine antagonist', *General and Comparative Endocrinology*. Elsevier Inc., 162(2), pp. 210–218. doi: 10.1016/j.ygcen.2009.03.005.

Gordon, T. A. C. *et al.* (2018) 'Fishes in a changing world : learning from the past to promote sustainability of fish populations', *Journal of Fish Biology*, (92), pp. 804–827. doi: 10.1111/jfb.13546.

Goyer, K. *et al.* (2014) 'Effects of lake warming on behavioural thermoregulatory tactics in a cold-water stenothermic fish', *PLoS ONE*, 9(3). doi: 10.1371/journal.pone.0092514.

Greig, S. M. *et al.* (2005) 'Impact of clay particles on the cutaneous exchange of oxygen across the chorion of Atlantic salmon eggs', *Journal of Fish Biology*, 66(6), pp. 1681–1691. doi: 10.1111/j.0022-1112.2005.00715.x.

Greig, S. M., Sear, D. a. and Carling, P. a. (2005) 'The impact of fine sediment accumulation on the survival of incubating salmon progeny: Implications for sediment management', *Science of The Total Environment*, 344(1–3), pp. 241–258. doi: 10.1016/j.scitotenv.2005.02.010.

Greig, S. M., Sear, D. a. and Carling, P. a. (2007) 'A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos', *Hydrological Processes*, 21(3), pp. 323–334. doi: 10.1002/hyp.6188.

Greig, S., Sear, D. and Carling, P. (2007) 'A field-based assessment of oxygen supply to incubating Atlantic salmon (Salmo salar) embryos'. doi: 10.1002/hyp.

Gunderson, A. R., Armstrong, E. J. and Stillman, J. H. (2016) 'Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment', *Annual Review of Marine Science*. Annual Reviews, 8(1), pp. 357–378. doi: 10.1146/annurev-marine-122414-033953.

Hagen, I. J. *et al.* (2019) 'Supplementary stocking selects for domesticated genotypes', *Nature Communications*. Springer US, 10(99), pp. 1–8. doi: 10.1038/s41467-018-08021-z.

Harrison, I. *et al.* (2018) 'The freshwater biodiversity crisis', *Science*. Edited by J. Sills, 362(6421), pp. 1369 LP – 1369. doi: 10.1126/science.aav9242.

Heath, D. D. *et al.* (2003) 'Rapid evolution of egg size in captive salmon', *Science*, 299(5613), p. 1738. doi: 10.1126/science.1088250.

Heino, J., Virkkala, R. and Toivonen, H. (2009) 'Climate change and freshwater biodiversity : detected patterns , future trends and adaptations in northern regions', 84, pp. 39–54. doi: 10.1111/j.1469-185X.2008.00060.x.

Heming, T. A. (1982) 'Effects of temperature on utilization of yolk by chinook salmon (Oncorhynchus tshawytscha) eggs and alevins', *Canadian Journal of Fisheries and Aquatic Sciences*. NRC Research Press, 39(1), pp. 184–190.

Hemmer-Brepson, C. *et al.* (2014) 'Non-stressful temperature effect on oxidative balance and life history traits in adult fish (Oryzias latipes)', *Journal of Experimental Biology*, 217(2), pp. 274–282. doi: 10.1242/jeb.096172.

Hendry, A. P., Gotanda, K. M. and Svensson, E. I. (2017) 'Human influences on evolution , and the ecological and societal consequences'.

Hering, D. *et al.* (2015) 'Managing aquatic ecosystems and water resources under multiple stress - An introduction to the MARS project', *Science of the Total Environment*, 503–504, pp. 10–21. doi: 10.1016/j.scitotenv.2014.06.106.

Heugens, E. and Hendriks, A. (2001) 'A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment', *... Critical Reviews in ...*, 31(december), pp. 247–284. Available at: http://informahealthcare.com/doi/abs/10.1080/20014091111695.

Heywood, M. J. T. and Walling, D. E. (2007) 'The sedimentation of salmonid spawning gravels in the Hampshire Avon catchment, UK: implications for the dissolved oxygen content of intragravel water and embryo survival', *Hydrological Processes*, 21, pp. 770–788. doi: 10.1002/hyp.6266.

Hoffmann, A. A. and Sgrò, C. M. (2011) 'Climate change and evolutionary adaptation', *Nature*, 470(7335), pp. 479–485. doi: 10.1038/nature09670.

Hollins, J. *et al.* (2018) 'A Physiological Perspective on Fisheries-Induced Evolution', *Evolutionary Applications*, (September 2017), pp. 1–16. doi: 10.1111/eva.12597.

Holmstrup, M. *et al.* (2010) 'Interactions between effects of environmental chemicals and natural stressors: A review', *Science of the Total Environment*. Elsevier B.V., 408(18), pp. 3746–3762. doi: 10.1016/j.scitotenv.2009.10.067.

HumpeschU.H. (1985) 'Inter specific and intra specific variation in hatching success and embryonic development of 5 species of salmonids and thymallus thymallus', *Archiv fuer Hydrobiologie*, 104(1), pp. 129–144. Available at:

https://eurekamag.com/research/005/719/005719652.php.

Hutchings, J. A. *et al.* (2007) 'Genetic variation in life-history reaction norms in a marine fish', *Proc. R. Soc. B*, (274), pp. 1693–1699. doi: 10.1098/rspb.2007.0263.

Hutchings, J. A. (2011) 'Old wine in new bottles : reaction norms in salmonid fishes', *Heredity*. Nature Publishing Group, 106(3), pp. 421–437. doi: 10.1038/hdy.2010.166.

Jackson, M. C. *et al.* (2016) 'Net effects of multiple stressors in freshwater ecosystems: A meta-analysis', *Global Change Biology*, 22(1), pp. 180–189. doi: 10.1111/gcb.13028.

Janhunen, M., Piironen, J. and Peuhkuri, N. (2010) 'Parental effects on embryonic viability and growth in Arctic charr Salvelinus alpinus at two incubation temperatures', *Journal of Fish Biology*, 76(10), pp. 2558–2570. doi: 10.1111/j.1095-8649.2010.02648.x.

Jensen, D. W. *et al.* (2009) 'Impact of Fine Sediment on Egg-To-Fry Survival of Pacific Salmon: A Meta-Analysis of Published Studies', *Reviews in Fisheries Science*, 17(3), pp. 348–359. doi: 10.1080/10641260902716954.

Jensen, L. F. *et al.* (2008) 'Local adaptation in brown trout early life-history traits: implications for climate change adaptability.', *Proceedings. Biological sciences / The Royal Society*, 275(1653), pp. 2859–68. doi: 10.1098/rspb.2008.0870.

Jeppesen, E. *et al.* (2005) 'Lake responses to reduced nutrient loading – an analysis of contemporary long-term data from 35 case studies', *Freshwater Biology*, (50), pp. 1747–1771. doi: 10.1111/j.1365-2427.2005.01415.x.

Jeuthe, Henrik, Brännäs, E. and Nilsson, J. (2015) 'Effects of variable egg incubation temperatures on the embryonic development in Arctic charr Salvelinus alpinus', *Aquaculture Research*, pp. 1–12. doi: 10.1111/are.12825.

Jeuthe, H., Brännäs, E. and Nilsson, J. (2015) 'Thermal stress in Arctic charr *Salvelinus alpinus* broodstock: a 28 year case study', *Journal of Fish Biology*, 86(3), pp. 1139–1152. doi: 10.1111/jfb.12634.

Jonsson, B. and Jonsson, N. (2014) 'Early environment influences later performance in fishes', *Journal of Fish Biology*, 85(2), pp. 151–188. doi: 10.1111/jfb.12432.

Jungwirth, M. and Winkler, H. (1984) 'The temperature dependence of embryonic development of grayling', *Aquaculture*, 38(4), pp. 315–327. doi: 10.1016/0044-8486(84)90336-3.

Kamler, E. (2005) 'Parent–egg–progeny Relationships in Teleost Fishes: An Energetics Perspective', *Reviews in Fish Biology and Fisheries*, 15(4), pp. 399–421. doi: 10.1007/s11160-006-0002-y.

Kamler, E. (2008) 'Resource allocation in yolk-feeding fish', *Reviews in Fish Biology and Fisheries*, 18(2), pp. 143–200. doi: 10.1007/s11160-007-9070-x.

Kawecki, T. J. and Ebert, D. (2004) 'Conceptual issues in local adaptation', *Ecology Letters*, 7(12), pp. 1225–1241. doi: 10.1111/j.1461-0248.2004.00684.x.

Keenan, K. *et al.* (2013) 'diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors', *Methods in Ecology and Evolution*. John Wiley & Sons, Ltd (10.1111), 4(8), pp. 782–788. doi: 10.1111/2041-210X.12067.

Kemp, P. *et al.* (2011) 'The impacts of fine sediment on riverine fish', *Hydrological Processes*, 25(11), pp. 1800–1821. doi: 10.1002/hyp.7940.

King, H. R., Pankhurst, N. W. and Watts, M. (2007) 'Reproductive sensitivity to elevated water temperatures in female Atlantic salmon is heightened at certain stages of vitellogenesis', *Journal of Fish Biology*, 70(1), pp. 190–205. doi: 10.1111/j.1095-8649.2006.01295.x.

Kjelland, M. E. *et al.* (2015) 'A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioral, and transgenerational implications', *Environment Systems and Decisions*. Springer US, 35(3), pp. 334–350. doi: 10.1007/s10669-015-9557-2.

Klemetsen, a *et al.* (2003) 'Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): a review of aspects of their life histories', *Ecology of Freshwater Fish*, 12(1), pp. 1–59. doi: 10.1034/j.1600-0633.2003.00010.x.

Klemetsen, A. (2010a) 'The Charr Problem Revisited: Exceptional Phenotypic Plasticity Promotes Ecological Speciation in Postglacial Lakes', *Freshwater Reviews*, 3(1), pp. 49–74. doi: 10.1608/FRJ-3.1.3.

Klemetsen, A. (2010b) 'The Charr Problem Revisited: Exceptional Phenotypic Plasticity Promotes Ecological Speciation in Postglacial Lakes', *Freshwater Reviews*, 3(1), pp. 49–74. doi: 10.4290/FRJ-3.1.3.

Klemetsen, A. (2013) 'The Most Variable Vertebrate on Earth', *Journal of Ichthyology*, 53(10), pp. 781–791. doi: 10.1134/S0032945213100044.

Knopp, T. *et al.* (2007) 'Contrasting levels of variation in neutral and quantitative genetic loci on island populations of moor frogs (Rana arvalis)', *Conservation Genetics*, 8(1), pp. 45–56. doi: 10.1007/s10592-006-9147-4.

Knudsen, R. *et al.* (2006) 'Incipient speciation through niche expansion: an example from the Arctic charr in a subarctic lake', *Proceedings of the Royal Society B: Biological Sciences*, 273(1599), pp. 2291–2298. doi: 10.1098/rspb.2006.3582.

Knudsen, R. *et al.* (2016) 'The role of Salvelinus in contemporary studies of evolution, trophic ecology and anthropogenic change', *Hydrobiologia*. Springer International Publishing, 783(1), pp. 1–9. doi: 10.1007/s10750-016-2999-9.

Kondolf, G. M. *et al.* (2008) 'Assessing Physical Quality of Spawning Habitat', *American Fisheries Society*, 65, pp. 1–26.

Koops, M. A. and Tallman, R. F. (2004) 'Adaptive Variation in the Development and Mortality of Arctic Char Eggs', *Physiology Section*, (AUGUST 2004), pp. 47–68.

Koskinen, M., Haugen, T. and Primmer, C. (2002) 'Contemporary fisherian life-history evolution in small salmonid populations', *Letters to Nature*, 419(October), pp. 3–7. doi: 10.1038/nature01130.1.

Lamy, J. B. *et al.* (2011) 'Uniform selection as a primary force reducing population genetic differentiation of cavitation resistance across a species range', *PLoS ONE*, 6(8). doi: 10.1371/journal.pone.0023476.

Lamy, J. B. *et al.* (2012) 'QST< FST As a signature of canalization', *Molecular Ecology*, 21(23), pp. 5646–5655. doi: 10.1111/mec.12017.

Lange, K. *et al.* (2018) 'stressor effects on freshwater fish : Importance of taxonomy and life stage', (August). doi: 10.1111/faf.12305.

Larsson, S. *et al.* (2005) 'Thermal adaptation of Arctic charr: Experimental studies of growth in eleven charr populations from Sweden, Norway and Britain', *Freshwater Biology*. doi: 10.1111/j.1365-2427.2004.01326.x.

Larsson, S. and Berglund, I. (2005) 'The effect of temperature on the energetic growth efficiency of Arctic charr (Salvelinus alpinus L.) from four Swedish populations', *Journal of Thermal Biology*, 30(1), pp. 29–36. doi: 10.1016/j.jtherbio.2004.06.001.

Leblanc, C. A.-L., Kristjánsson, B. K. and Skúlason, S. (2014) 'The importance of egg size and egg energy density for early size patterns and performance of Arctic charr Salvelinus alpinus', *Aquaculture Research*, pp. 1–12. doi: 10.1111/are.12566.

Van Leeuwen, T. E. *et al.* (2017) 'Differences in early developmental rate and yolk conversion efficiency in offspring of trout with alternative life histories', *Ecology of Freshwater Fish*. John Wiley & Sons, Ltd (10.1111), 26(3), pp. 371–382. doi: 10.1111/eff.12281.

Lehnherr, I. *et al.* (2018) 'The world's largest High Arctic lake responds rapidly to climate warming', *Nature Communications*, 9(1), p. 1290. doi: 10.1038/s41467-018-03685-z.

Leinonen, T. *et al.* (2013) 'QST -FST comparisons: Evolutionary and ecological insights from genomic heterogeneity', *Nature Reviews Genetics*. Nature Publishing Group, 14(3), pp. 179–190. doi: 10.1038/nrg3395.

Levasseur, M. *et al.* (2006) 'Effects of silt and very fine sand dynamics in Atlantic salmon (Salmo salar) redds on embryo hatching success', *Canadian Journal of Fisheries and Aquatic Sciences*, 63(7), pp. 1450–1459. doi: 10.1139/f06-050.

Lieberherr, G. and Wunderle, S. (2018) 'Lake Surface Water Temperature Derived from 35 Years of AVHRR Sensor Data for European Lakes', *Remote Sensing* . doi: 10.3390/rs10070990.

Lim, M. Y.-T. *et al.* (2017) 'The effects of fluctuating temperature regimes on the embryonic development of lake whitefish (Coregonus clupeaformis)', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 214, pp. 19–29. doi: https://doi.org/10.1016/j.cbpa.2017.08.010.

Logan, C. a. and Buckley, B. a. (2015) 'Transcriptomic responses to environmental temperature in eurythermal and stenothermal fishes', *Journal of Experimental Biology*, 218(12), pp. 1915–1924. doi: 10.1242/jeb.114397.

Loizeau, J.-L., Girardclos, S. and Dominik, J. (2012) 'Taux d'accumulation de sédiments récents et bilan de la matière particulaire dans le Léman (Suisse-France)', *Archives des Sciences*, 65(January), pp. 81–92.

Louhi, P. *et al.* (2011) 'Does fine sediment constrain salmonid alevin development and survival?', *Canadian Journal of Fisheries and Aquatic Sciences*, 68(10), pp. 1819–1826. doi: 10.1139/f2011-106.

Louhi, P., Richardson, J. S. and Muotka, T. (2017) 'Sediment addition reduces the importance of predation on ecosystem functions in experimental stream channels', *Canadian Journal of Fisheries and Aquatic Sciences*, 74(1), pp. 32–40. doi: 10.1139/cjfas-2015-0530.

Lynch, M. and Walsh, B. (1998) *Genetics and analysis of quantitative traits*. Sinauer Sunderland, MA.

Machino, Y. (1995) 'The Status of Salvelinus in France Results and discussion', (February 1878), pp. 352–358.

Machino, Y and Rivier, B. (2002) 'Lake Allos (Alpes-de-Haute-Provence). Sanctuary and laboratory of evolution for the Arctic charr of Lake Leman (Salvelinus alpinus; salmonidae).', *Cybium*, 26(3), pp. 173–177.

Machino, Yoichi and Rivier, B. (2002) 'LE LAC D'ALLOS (ALPES-DE-HAUTE-PROVENCE). SANCTUAIRE ET LABORATOIRE DE L'ÉVOLUTION POUR L'OMBLE CHEVALIER DU LÉMAN

(SALVELINUS ALPINUS; SALMONIDAE)', *Cybium*, 26(3), pp. 173-177 (in French with English abstract). Available at: http://sfi.mnhn.fr/cybium/numeros/2002/263/03-Machino.pdf (Accessed: 29 June 2015).

Madliger, C. L. *et al.* (2018) 'Conservation physiology and the quest for a " good " Anthropocene', 5(August), pp. 1–10. doi: 10.1093/conphys/cox003.....An.

Magbanua, F. S. *et al.* (2016) 'Individual and combined effects of fine sediment and glyphosate herbicide on invertebrate drift and insect emergence: a stream mesocosm experiment', *Freshwater Science*, 35(1), pp. 139–151. doi: 10.1086/684363.

Mäkinen, H. *et al.* (2016) 'Plastic and evolutionary gene expression responses are correlated in European grayling (Thymallus thymallus) subpopulations adapted to different thermal environments', *Journal of Heredity*, 107(1), pp. 82–89. doi: 10.1093/jhered/esv069.

Malcolm, Youngson and Soulsby, C. (2002) 'Survival of salmonid eggs in a degraded gravel bed stream : effects of groundwater-surface water interactions', *River Research and Applications*. Available at: papers2://publication/uuid/C191BF0E-843C-4DC6-B6D2-2E5940F05CDF.

De March, B. G. E. (1995) 'Effects of Incubation Temperature on the Hatching Success of Arctic Char Eggs', *The Progressive Fish Culturist*, 57(April 2015), pp. 1–25. doi: 10.1577/1548-8640(1995)057.

Mari, L. *et al.* (2016) 'Higher temperature exacerbates the impact of sediments on embryo performances in a salmonid', *Biology Letters*, 12(12). doi: http://dx.doi.org/10.1098/rsbl.2016.0745.

McBryan, T. L. *et al.* (2013) 'Responses to Temperature and Hypoxia as Interacting Stressors in Fish: Implications for Adaptation to Environmental Change', *Integrative and Comparative Biology*, 53(4), pp. 648–659. doi: 10.1093/icb/ict066.

McKinney, G. J. *et al.* (2014) 'Genetic and morphological divergence in three strains of brook trout salvelinus fontinalis commonly stocked in lake superior', *PLoS ONE*, 9(12). doi: 10.1371/journal.pone.0113809.

Meraner, A., Cornetti, L. and Gandolfi, A. (2014) 'Defining conservation units in a stocking-induced genetic melting pot: unraveling native and multiple exotic genetic imprints of recent and historical secondary contact in Adriatic grayling.', *Ecology and evolution*, 4(8), pp. 1313–27. doi: 10.1002/ece3.931.

Merilä, J. and Crnokrak, P. (2001) 'Comparison of genetic differentiation at marker loci and quantitative traits', *Journal of Evolutionary Biology*. Wiley Online Library, 14(6), pp. 892–903.

Merila, J. and Sheldon, B. C. (1999) 'Genetic architecture of fitness and nonfitness traits: Empirical patterns and development of ideas', *Heredity*, 83(2), pp. 103–109. doi: 10.1046/j.1365-2540.1999.00585.x.

Metcalfe, N. B. and Alonso-Alvarez, C. (2010) 'Oxidative stress as a life-history constraint: the role of reactive oxygen species in shaping phenotypes from conception to death: Oxidative stress as a life-history constraint', *Functional Ecology*, 24(5), pp. 984–996. doi: 10.1111/j.1365-2435.2010.01750.x.

Metcalfe, N. B., Van Leeuwen, T. E. and Killen, S. S. (2016) 'Does individual variation in metabolic phenotype predict fish behaviour and performance?', *Journal of Fish Biology*, 88(1), pp. 298–321. doi: 10.1111/jfb.12699.

Miaud, C. and Ple, S. (2015) 'Phenotypic divergence of the common toad (Bufo bufo) along an altitudinal gradient : evidence for local adaptation', *Heredity*, (June 2014), pp. 69–79. doi: 10.1038/hdy.2014.71.

Milano, E. R., Kenney, A. M. and Juenger, T. E. (2016) 'Adaptive differentiation in floral traits in the presence of high gene flow in scarlet gilia (Ipomopsis aggregata)', *Molecular Ecology*, 25(23), pp. 5862–5875. doi: 10.1111/mec.13752.

Miller, H. *et al.* (2014) 'Distribution, characteristics and condition of Arctic charr (Salvelinus alpinus) spawning grounds in a differentially eutrophicated twin-basin lake', *Ecology of Freshwater Fish*, pp. 32–43. doi: 10.1111/eff.12122.

Monaghan, P., Metcalfe, N. B. and Torres, R. (2009) 'Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation', *Ecology Letters*, 12(1), pp. 75–92. doi: 10.1111/j.1461-0248.2008.01258.x.

Moore, J. *et al.* (2017) 'Migration Harshness Drives Habitat Choice And Local Adaptation In Anadromous Arctic Char: Evidence From Integrating Population Genomics And Acoustic Telemetry', *bioRxiv*. doi: 10.1101/138545.

Moore, J. *et al.* (2018) 'Premature alarm on the impacts of climate change', *Nature Communications*. Springer US, pp. 9–10. doi: 10.1038/s41467-018-06479-5.

Müller, B., Gächter, R. and Wüest, A. (2014) 'Accelerated Water Quality Improvement during Oligotrophication in Peri-Alpine Lakes', *Environmental Science & Technology*. American Chemical Society, 48(12), pp. 6671–6677. doi: 10.1021/es4040304.

Müller, R. (1992) 'Trophic state and its implications for natural reproduction of salmonid fish', *Hydrobiologia*, 243(1), pp. 261–268. doi: 10.1007/BF00007041.

Nilsson, J. *et al.* (2016) 'Effects of age and rearing environment on genetic parameters of growth and body weight and heritability of skin pigmentation in Arctic charr (Salvelinus alpinus L.)', *Aquaculture*. Elsevier B.V., 453, pp. 67–72. doi: 10.1016/j.aquaculture.2015.11.035.

Nusslé, S., Bornand, C. N. and Wedekind, C. (2009) 'Fishery-induced selection on an Alpine whitefish: Quantifying genetic and environmental effects on individual growth rate', *Evolutionary Applications*, 2(2), pp. 200–208. doi: 10.1111/j.1752-4571.2008.00054.x.

Ojanguren, A. F. and Braña, F. (2004) 'Thermal dependence of embryonic growth and development in brown trout', *Journal of Fish Biology*, 28(2), pp. 297–301. doi: 10.1046/j.0022-1112.2003.00049.x.

Oomen, R. A. and Hutchings, J. A. (2017) 'Transcriptomic responses to environmental change in fishes: Insights from RNA sequencing', *FACETS*. Canadian Science Publishing, 2(2), pp. 610–641. doi: 10.1139/facets-2017-0015.

Oomen, R. a and Hutchings, J. a (2015) 'Genetic variability in reaction norms in fishes', *Environmental Reviews*, 23(November 2014), pp. 1–14.

Van Oosterhout, C. *et al.* (2003) 'Inbreeding depression and genetic load of sexually selected traits: How the guppy lost its spots', *Journal of Evolutionary Biology*, 16(2), pp. 273–281. doi: 10.1046/j.1420-9101.2003.00511.x.

Ormerod, S. J. *et al.* (2010) 'Multiple stressors in freshwater ecosystems', *Freshwater Biology*, 55(SUPPL. 1), pp. 1–4. doi: 10.1111/j.1365-2427.2009.02395.x.

Pankhurst, N. W. and King, H. R. (2010) 'Temperature and salmonid reproduction: Implications for aquaculture', *Journal of Fish Biology*, 76(1), pp. 69–85. doi: 10.1111/j.1095-

8649.2009.02484.x.

Pankhurst, N. W. and Munday, P. L. (2011) 'Effects of climate change on fish reproduction and early life history stages', *Marine and Freshwater Research*, 62(9), pp. 1015–1026. doi: 10.1071/MF10269.

Parmesan, C. (2006) 'Ecological and Evolutionary Responses to Recent Climate Change', *Annual Review of Ecology, Evolution, and Systematics*, 37(1), pp. 637–669. doi: 10.1146/annurev.ecolsys.37.091305.110100.

Parmesan, C. and Yohe, G. (2003) 'A globally coherent fingerprint of climate change impacts across natural systems', *Nature*. Macmillan Magazines Ltd., 421, p. 37. Available at: https://doi.org/10.1038/nature01286.

Parsons, K. J., Skúlason, S. and Ferguson, M. (2010) 'Morphological variation over ontogeny and environments in resource polymorphic arctic charr (Salvelinus alpinus)', *Evolution and Development*, 12(3), pp. 246–257. doi: 10.1111/j.1525-142X.2010.00410.x.

Perry, G. M. L. *et al.* (2004) 'Shifting patterns in genetic control at the embryo-alevin boundary in brook charr.', *Evolution; international journal of organic evolution*, 58(9), pp. 2002–2012. doi: 10.1111/j.0014-3820.2004.tb00485.x.

Perry, G. M. L., Audet, C. and Bernatchez, L. (2005) 'Maternal genetic effects on adaptive divergence between anadromous and resident brook charr during early life history', *Journal of Evolutionary Biology*, 18(5), pp. 1348–1361. doi: 10.1111/j.1420-9101.2005.00954.x.

Piggott, J. J. *et al.* (2015) 'Multiple stressors and stream ecosystem functioning: Climate warming and agricultural stressors interact to affect processing of organic matter', *Journal of Applied Ecology*, 52(5), pp. 1126–1134. doi: 10.1111/1365-2664.12480.

Piggott, J. J., Townsend, C. R. and Matthaei, C. D. (2015a) 'Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics', *Global Change Biology*, 21(5), pp. 1887–1906. doi: 10.1111/gcb.12861.

Piggott, J. J., Townsend, C. R. and Matthaei, C. D. (2015b) 'Reconceptualizing synergism and antagonism among multiple stressors', *Ecology and Evolution*, 5(7), pp. 1538–1547. doi: 10.1002/ece3.1465.

Pigliucci, M. (2005) 'Evolution of phenotypic plasticity: where are we going now?', *Trends in Ecology & Evolution*, 20(9), pp. 481–486. doi: 10.1016/j.tree.2005.06.001.

Polymeropoulos, E. T., Elliott, N. G. and Frappell, P. B. (2016) 'The maternal effect of differences in egg size influence metabolic rate and hypoxia induced hatching in Atlantic salmon eggs: implications for respiratory gas exchange across the egg capsule', *Canadian Journal of Fisheries and Aquatic Sciences*, 73(8), pp. 1173–1181. doi: 10.1139/cjfas-2015-0358.

Polymeropoulos, E. T., Elliott, N. G. and Frappell, P. B. (2017) 'Hypoxic acclimation leads to metabolic compensation after reoxygenation in Atlantic salmon yolk-sac alevins', *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*. Elsevier, 213(April), pp. 28–35. doi: 10.1016/j.cbpa.2017.08.011.

Potvin, C. and Bernatchez, L. (2003) 'Étude génétique de la population d'omble chevalier (Salvelinus alpinus) du lac d'Allos. Rapport présenté au Directeur du Parc national du Mercantour Alpes-de -Haute-Provence, France'.

Power, G. (2002) 'Charrs, glaciations and seasonal ice', *Environmental Biology of Fishes*, 64(1–3), pp. 17–35. doi: 10.1023/A:1016066519418.

Power, M., Reist, J. D. and Dempson, J. B. (2008) 'C H A P T E R 14 Fish in high-latitude Arctic lakes', (December 2008).

Przeslawski, R., Byrne, M. and Mellin, C. (2015) 'A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae', *Global Change Biology*, 21(6), pp. 2122–2140. doi: 10.1111/gcb.12833.

Qian, X. *et al.* (2014) 'RNA-Seq technology and its application in fish transcriptomics.', *Omics : a journal of integrative biology*, 18(2), pp. 98–110. doi: 10.1089/omi.2013.0110.

Régnier, T. *et al.* (2012) 'Influence of energetic status on ontogenetic niche shifts: emergence from the redd is linked to metabolic rate in brown trout.', *Oecologia*, 168(2), pp. 371–80. doi: 10.1007/s00442-011-2109-7.

Reid, A. J. *et al.* (2018) 'Emerging threats and persistent conservation challenges for freshwater biodiversity', *Biological Reviews*. doi: 10.1111/brv.12480.

Rogell, B. *et al.* (2013) 'Adaptive divergence in body size overrides the effects of plasticity across natural habitats in the brown trout', *Ecology and Evolution*, 3(7), pp. 1931–1941. doi: 10.1002/ece3.579.

Rombough, P. J. (1988) 'Growth, aerobic metabolism, and dissolved oxygen requirements of embryos and alevins of steelhead, Salmo gairdneri', *Canadian Journal of Zoology*, pp. 651–660. doi: 10.1139/z88-097.

Rombough, P. J. (2007) 'Oxygen as a constraining factor in egg size evolution in salmonids', *Canadian Journal of Fisheries and Aquatic Sciences*, 64(4), pp. 692–699. doi: 10.1139/f07-047.

Rosengren, M. *et al.* (2016) 'Born to be wild: effects of rearing density and environmental enrichment on stress, welfare, and smolt migration in hatchery-reared Atlantic salmon', *Canadian Journal of Fisheries and Aquatic Sciences*. NRC Research Press, 74(3), pp. 396–405.

Roussel, J.-M. (2007) ' Carry-over effects in brown trout (Salmo trutta): hypoxia on embryos impairs predator avoidance by alevins in experimental channels ', *Canadian Journal of Fisheries and Aquatic Sciences*, 64(5), pp. 786–792. doi: 10.1139/f07-055.

Rubin and Buttiker (1992) 'Les Sites De Fraye De L ' Omble Chevalier ,' *Bulletin Français de la Pêche et de la Pisciculture*, (325), pp. 69–82.

Rubin, J.-F. (2005) 'Les sites de reproduction de l ' omble chevalier du Léman ont-ils évolué de 1981 à 2005 ?', *ARCHIVES DES SCIENCES-GENEVE*, 58(3), p. 201.

Rubin, J. (2005) 'Pourquoi la population d'ombles chevaliers diminue-t-elle dans le Léman?', *ARCHIVES DES SCIENCES-GENEVE*, 58(3), p. 247.

Rubin, J. F. and Buttiker, B. (1992) 'Les sites de fraye de l'omble chevalier (Salvelinus alpinus L.) dans le Léman', *Bulletin Français de la Pêche et de la Pisciculture*, (325), pp. 69–82. doi: 10.1051/kmae:1992015.

Sæther, B. S., Siikavuopio, S. I. and Jobling, M. (2016) 'Environmental conditions required for intensive farming of Arctic charr (Salvelinus alpinus (L.))', *Hydrobiologia*. Springer International Publishing, 783(1), pp. 347–359. doi: 10.1007/s10750-015-2572-y.

Salmaso, N., Anneville, O. and Straile, D. (2018) *European large perialpine lakes under anthropogenic pressures and climate change : present status , research gaps and future challenges*, *Hydrobiologia*. Springer International Publishing. doi: 10.1007/s10750-018-3758-x.

Saltz, J. B. *et al.* (2018) 'Why does the magnitude of genotype-by-environment interaction vary?', *Ecology and Evolution*, 8(12), pp. 6342–6353. doi: 10.1002/ece3.4128.

Savary, R. *et al.* (2017) 'Stocking activities for the Arctic charr in Lake Geneva: Genetic effects in space and time', *Ecology and Evolution*, (April), pp. 1–11. doi: 10.1002/ece3.3073.

Scheffers, B. R. *et al.* (2016) 'The broad footprint of climate change from genes to biomes to people', *Science*, 354(6313). doi: 10.1126/science.aaf7671.

Schinegger, R. *et al.* (2016) 'Untangling the effects of multiple human stressors and their impacts on fish assemblages in European running waters', *Science of the Total Environment*. The Authors, 573, pp. 1079–1088. doi: 10.1016/j.scitotenv.2016.08.143.

Sear, D. A. *et al.* (2014) 'Factors controlling the temporal variability in dissolved oxygen regime of salmon spawning gravels', *Hydrological Processes*, 28(1), pp. 86–103. doi: 10.1002/hyp.9565.

Sear, D. A. *et al.* (2016) 'Does fine sediment source as well as quantity affect salmonid embryo mortality and development?', *Science of The Total Environment*. Elsevier B.V., 541, pp. 957–968. doi: 10.1016/j.scitotenv.2015.09.155.

Sepp, T. *et al.* (2012) 'Individual Consistency and Covariation of Measures of Oxidative Status in Greenfinches', *Physiological and Biochemical Zoology*, 85(3), pp. 299–307. doi: 10.1086/664827.

SILA and Coulée douce (2015) *Campagne d'observation 2014 des omblières du lac d'Annecy*.

Skoglund, H. *et al.* (2011) 'Phenotypic plasticity in physiological status at emergence from nests as a response to temperature in Atlantic salmon (Salmo salar)', 1479, pp. 1470–1479. doi: 10.1139/F2011-056.

Skoglund, H., Einum, S. and Robertsen, G. (2011) 'Competitive interactions shape offspring performance in relation to seasonal timing of emergence in Atlantic salmon', *Journal of Animal Ecology*, 80, pp. 365–374. doi: 10.1111/j.1365-2656.2010.01783.x.

Sly, P. G. and Evans, D. O. (1996) 'Suitability of habitat for spawning lake trout', *Journal of Aquatic Ecosystem Health*, 5, pp. 153–175. doi: 10.1007/BF00124104.

Soulsby, C. *et al.* (2001) 'Fine sediment influence on salmonid spawning habitat in a lowland agricultural stream: a preliminary assessment', *Science of The Total Environment*, 265(1–3), pp. 295–307. doi: 10.1016/S0048-9697(00)00672-0.

Speers-Roesch, B. and Norin, T. (2016) 'Ecological significance of thermal tolerance and performance in fishes: new insights from integrating field and laboratory approaches', *Functional Ecology*, 30(6), pp. 842–844. doi: 10.1111/1365-2435.12652.

Spitze, K. (1993) 'Population structure in Daphnia obtusa: quantitative genetic and allozymic variation.', *Genetics*, 135(2), pp. 367 LP – 374. Available at: http://www.genetics.org/content/135/2/367.abstract.

St-Cyr, J., Derome, N. and Bernatchez, L. (2008) 'The transcriptomics of life-history trade-offs in whitefish species pairs (Coregonus sp.)', *Molecular Ecology*, 17(7), pp. 1850–1870. doi: 10.1111/j.1365-294X.2008.03696.x.

Stearns, S. C. (1992) *Stearns, Stephen C., 1992. The Evolution of Life Histories. Oxford University Press, London xii + 249 pp.* John Wiley & Sons, Ltd (10.1111). doi: 10.1046/j.1420-9101.1993.6020304.x.

Steel, E., Tillotson, a and Larsen, D. (2012) 'Beyond the mean: The role of variability in predicting ecological effects of stream temperature on salmon', *Ecosphere*, 3(November), pp. 1–11. doi: 10.1890/ES12-00255.1.

Straile, D. and Geller, W. (1998) 'Crustacean zooplankton in Lake Constance from 1920 to

1995: Response to eutrophication and re-oligotrophication', *Advances in Limnology*, 53, pp. 255–274.

Sunday, J. M. *et al.* (2014) 'Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation', *Proceedings of the National Academy of Sciences*, 111(15), pp. 5610–5615. doi: 10.1073/pnas.1316145111.

Suttle, K. B. *et al.* (2004) 'How fine sediment in riverbeds impairs growth', *Ecological Applications*, 14(4), pp. 969–974. Available at: //000223156600001.

Svenning, M.-A., Aas, M. and Borgstrøm, R. (2015) 'First records of three-spined stickleback Gasterosteus aculeatus in Svalbard freshwaters: An effect of climate change?', *Polar Biology*. Springer Berlin Heidelberg, 38(11), pp. 1937–1940. doi: 10.1007/s00300-015-1752-6.

Swift, D. . (1965) 'Effect of Temperature on Mortality and Rate of Development of the Eggs of the Windermere Charr (Salvelinus alpinus)', *Journal Fisheries Research Board of Canada*, 22(4), pp. 913–917.

Teletchea, F. and Fontaine, P. (2010) 'Comparison of early life-stage strategies in temperate freshwater fish species: trade-offs are directed towards first feeding of larvae in spring and early summer', *Journal of Fish Biology*, 77(1), pp. 257–278. doi: 10.1111/j.1095-8649.2010.02689.x.

Thomas, G. *et al.* (2009) 'Human-induced changes in the reproductive traits of Lake Constance common whitefish (Coregonus lavaretus)', *Journal of Evolutionary Biology*, 22(1), pp. 88–96. doi: 10.1111/j.1420-9101.2008.01622.x.

Thompson, R., Kamenik, C. and Schmidt, R. (2005) 'Ultra-sensitive Alpine lakes and climate change', 64(2), pp. 139–152.

Tibblin, P. *et al.* (2015) 'Evolutionary Divergence of Adult Body Size and Juvenile Growth in Sympatric Subpopulations of a Top Predator in Aquatic Ecosystems', *The American Naturalist*, 186(1), pp. 98–110. doi: 10.1086/681597.

Tiberti, R. and Splendiani, A. (2019) 'Management of a highly unlikely native fish : The case of arctic charr Salvelinus alpinus from the Southern Alps', (January 2018), pp. 1–9. doi: 10.1002/aqc.3027.

Todd, E. V., Black, M. A. and Gemmell, N. J. (2016) 'The power and promise of RNA-seq in ecology and evolution', *Molecular Ecology*, pp. 1224–1241. doi: 10.1111/mec.13526.

Townsend, C. R., Uhlmann, S. S. and Matthaei, C. D. (2008) 'Individual and combined responses of stream ecosystems to multiple stressors', *Journal of Applied Ecology*, (45), pp. 1810–1819.

Valdimarsson, S. K., Skulason, S. and Snorrason, S. S. (2002) 'The relationship between egg size and the rate of early development in Arctic charr, Salvelinus alpinus', *Environmental Biology of Fishes*, 65(4), pp. 463–468. doi: 10.1023/a:1021107712866.

de Villemereuil, P. *et al.* (2016) 'General methods for evolutionary quantitative genetic inference from generalized mixed models', *Genetics*, 204(3), pp. 1281–1294. doi: 10.1534/genetics.115.186536.

Wallace, J. C. and Aasjord, D. (1984) 'An investigation of the consequences of egg size for the culture of Arctic charr, Salvetinus alpinus (L.)', *Journal of Fish Biology*, 24, pp. 427–435. doi: 10.1111/j.1095-8649.1984.tb04813.x.

Walther, G. R. *et al.* (2002) 'Ecological responses to recent climate change', *Nature*, 416(6879), pp. 389–395. doi: 10.1038/416389a.

Weir, B. S. and Cockerham, C. C. (1984) 'ESTIMATING F-STATISTICS FOR THE ANALYSIS OF

POPULATION STRUCTURE', *Evolution*. John Wiley & Sons, Ltd (10.1111), 38(6), pp. 1358–1370. doi: 10.1111/j.1558-5646.1984.tb05657.x.

Whitney, C. K., Hinch, S. G. and Patterson, D. a. (2013) 'Provenance matters: thermal reaction norms for embryo survival among sockeye salmon Oncorhynchus nerka populations', *Journal of Fish Biology*, 82(4), pp. 1159–1176. doi: 10.1111/jfb.12055.

Whoriskey, F. (2005) 'Evolution Illuminated: Salmon and Their Relatives. Edited by Andrew P Hendry and Stephen C Stearns.', *The Quarterly Review of Biology*. The University of Chicago Press, 80(1), pp. 115–116. doi: 10.1086/431065.

Wilkins, L. G. E. *et al.* (2015) 'Embryonic gene expression of Coregonus palaea (whitefish) under pathogen stress as analyzed by high-throughput RNA-sequencing', *Fish and Shellfish Immunology*. Elsevier Ltd, 47(1), pp. 130–140. doi: 10.1016/j.fsi.2015.08.035.

Willi, Y., Van Buskirk, J. and Hoffmann, A. A. (2006) 'Limits to the Adaptive Potential of Small Populations', *Annual Review of Ecology, Evolution, and Systematics*, 37(1), pp. 433–458. doi: 10.1146/annurev.ecolsys.37.091305.110145.

Wilson, A. J. *et al.* (2009) 'Trading offspring size for number in a variable environment: Selection on reproductive investment in female Soay sheep', *Journal of Animal Ecology*, 78(2), pp. 354–364. doi: 10.1111/j.1365-2656.2008.01489.x.

Winfield, I. J., Berry, R. and Iddon, H. (2018) 'The cultural importance and international recognition of the Arctic charr Salvelinus alpinus populations of Windermere, UK', *Hydrobiologia*. Springer International Publishing, 6. doi: 10.1007/s10750-018-3814-6.

Yekutieli, D. and Benjamini, Y. (2001) 'The control of the false discovery rate in multiple testing under dependency', *The Annals of Statistics*. Institute of Mathematical Statistics, 29(4), pp. 1165–1188. doi: 10.1214/aos/1013699998.

Zuo, W. *et al.* (2012) 'A general model for effects of temperature on ectotherm ontogenetic growth and development.', *Proceedings of the Royal Society B*, 279(1734), pp. 1840–6. doi: 10.1098/rspb.2011.2000.

Appendix

Appendix 1 : Scientific contributions

Publications arising from this work (* indicates mentored undergraduate student)

Mari, L., Evanno, G., Daufresne, M., Fumagalli, L. & Lasne, E. Evidence for adaptive thermal reactions norms but also uniform selection among Arctic charr southern populations. **In preparation**.

Mari, L., Garaud, L.*, Evanno, G. & Lasne, E. (2016) Higher temperature exacerbates the impact of sediments on embryo performances in a salmonid. **Biology Letters***.* 12. (DOI: http://dx.doi.org/10.1098/rsbl.2016.0745)

Mari, L., Daufresne, M., Guillard, J., Evanno, G. & Lasne, E. Combined exposure to elevated temperature and sediment load reveals contrasted early life responses among arctic charr southernmost populations. **In preparation for Freshwater Biology**.

Oral communications

International conferences (presenter name underlined)

Phenotypic plasticity of arctic charr *Salvelinus alpinus* at the individual and population scales in response to co-occurring environmental stressors during development. **Mari, L.**, Daufresne, M., Riti, A., Evanno, G., Lasne, E. **SFEcologie 2018**, Rennes, France. **(Oral presentation)**

Synergy between temperature and fine sediments on fish early life history: a case study of southernmost arctic charr populations. **Mari, L.**, Daufresne, M., Riti, A., Evanno, G., Lasne, E. I**nternational Association for Great Lakes Research x European Large Lakes Symposium 2018**, Evian-les-Bains, France. **(Oral presentation)**

Investigating the combined effects of temperature and sediments in freshwaters: life history trade-offs in a cold-water salmonid. **Mari, L.**, Daufresne, M., Evanno, G., Lasne, E. **The Society for Experimental Biology Annual Meeting**, Florence, Italy. **(Oral presentation)**

Response of a cold-water-adapted species, the arctic charr *Salvelinus alpinus*, to thermal changes : an ecophysiological perspective. **Mari, L.**, Daufresne, M., Voituron, Y., Guillard, J., Evanno, G., Lasne, E. **SFEcologie 2016**, Marseille, France. **(Oral presentation)**

Seminars

The arctic charr and climate change in the Alps: how does a cold-water species respond to a warming environment? **Invited Seminar**, University of Iceland, Reykjavik.

Réponse d'une espèce d'eau froide – l'omble chevalier – aux facteurs environnementaux dans un contexte de changement climatique. **Séminaire Doctorants Zone Atelier Bassin du Rhône**, Irstea Villeurbanne, France.

Réponses des stades embryonnaires d'une espèce d'eau froide, l'omble chevalier, au changement climatique. **Séminaire INRA réseau Salmonidés et Poissons amphihalins migrateurs**, Saint Pée-sur-Nivelle, France.

L'omble chevalier face au réchauffement climatique. **Doctoriales de l'Université Grenoble-Alpes**, Autrans, France. **(Poster)**

Potentiel d'adaptation de l'omble chevalier *Salvelinus alpinus* dans un contexte de réchauffement climatique. **Séminaire INRA réseau Salmonidés et Poissons amphihalins migrateurs**, Thonon-les-Bains, France.

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