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RESEARCH ARTICLE

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Response of European whitefish embryos to thermal conditions diverges between peri-alpine populations

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Abstract – Peri-alpine lakes are vulnerable to climate warming. Water temperature in these lakes has increased and will likely continue in the coming decades, questioning the fate of inhabiting aquatic populations. Understanding how coregonine fishes respond to these changes is of great importance as these are economically and ecologically valuable populations. Considering the diversity of European whitefish from the *Coregonus* genus in peri-alpine lakes, we can assume that their response to rising temperatures could be diverse. We conducted an experiment to explore how embryos from four European whitefish populations from three lakes – Bourget, Geneva, and Constance – responded to contrasting incubation temperatures (7.0 °C vs. 9.0 °C) in terms of incubation duration and survival. In Constance, both pelagic and littoral populations were considered separately. Survival from the eyed-egg stage to hatching at 7.0 °C in all populations was high, ranging from 89% to 98%. At 9.0 °C, increased mortality—compared to the 7.0 °C treatment—was the lowest in Bourget (33%) and the highest in the Constance littoral (62%) population. Mortality was intermediate for the Constance pelagic (44%) and Geneva (45%) populations. The impact of warmer incubation temperature on hatching dynamics also contrasted among populations, as both Constance populations hatched more precociously in the warm treatment relative to the cold treatment (–4.6 and –2.7% Accumulated Degree Days for littoral and pelagic populations, respectively), suggesting they are more stressed than populations from Geneva and Bourget (–1.2 and +1.1%, respectively). These data indicate populations may possess the ability to respond differently to warming temperatures.

Keywords: Adaptation / climate change / temperature / common garden / reaction norm

1 Introduction

All over the world, lake water temperatures are rising due to climate change (George, 2010; Maberly *et al.*, 2020). Alpine and peri-alpine lakes are particularly vulnerable to climate warming. Surface water temperature has already increased by 0.4 °C per decade in peri-alpine lakes in Switzerland (Michel *et al.*, 2021) and is expected to increase an additional 3.8 °C by 2100 (Desgué-Itier *et al.*, 2023). Deep waters of the epilimnion

are also vulnerable to warming, although at a slower rate. This brings into question the fate of aquatic communities and their ability to respond to thermal shifts (Maberly *et al.*, 2020). The ability of many lacustrine populations to persist depends on putative phenological and physiological adjustments through plastic and (or) adaptive processes to match new conditions (Wilczek *et al.*, 2010). Understanding how fish populations respond to climatic warming is important (Anneville *et al.*, 2015) and may help inform which management options are most appropriate under certain circumstances.

In fish, embryos have a narrower thermal window than other life stages. Cardiorespiratory systems are not fully developed and thus access to oxygen is regulated by the egg membrane (Pörtner and Farrell, 2008; Pankhurst and Munday,

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2011; Dahlke *et al.*, 2020). Given the concentration of dissolved oxygen is inversely related to water temperature, embryonic development is therefore considered a thermal bottleneck. Along with spawning adults, the embryo life stage has been proposed to define the degree to which fish are vulnerable to climate change (Dahlke *et al.*, 2020; Sunday, 2020). Different approaches may be used to evaluate the fate of populations exposed to a warming environment. One common approach is to calculate water temperature projections and determine if these predictions are within the range of thermal tolerance for embryos (Hansen *et al.*, 2017; Kelly *et al.*, 2020). Thermal requirements are however population specific as physiological adaptations are known to occur at a local scale (Eliason *et al.*, 2011; McKenzie *et al.*, 2021). A better appreciation for how specific populations respond to changes in temperature could facilitate development of models for species with a broad geographic range (Hoffmann *et al.*, 2011). For instance, reaction norms (*i.e.*, the phenotypic expression pattern of specific genotypes across a range of environmental conditions) for temperature could provide scientists and managers with valuable information.

European whitefish are a stenothermal, cold-water adapted group of both phylogenetically and ecologically related coregonines. European whitefish are sometimes considered as a species complex named *Coregonus lavaretus* (L.) (*sensu* Østbye *et al.*, 2005) or even a single species (Crotti *et al.*, 2020; Öhlund *et al.*, 2020) characterized by strong phenotypic diversity. Within lakes, reproductive isolation has been shown to be poor as habitat contraction led to hybridization and speciation reversal (Vonlanthen *et al.*, 2012). Between lakes, translocation of fish has led to strong hybridization, which also supports the assumption of poor reproductive isolation (Anneville *et al.*, 2015). Considering speciation as a continuum ranging from panmixia to strict reproductive isolation (Hendry, 2009), most populations from the *Coregonus lavaretus* (L.) species complex are probably located at various positions between the two extremes.

European whitefish are widely distributed in Eurasia from 45 to 70°N (Østbye *et al.*, 2005). European whitefish found across the continent showcase substantial genetic and phenotypic diversity (Douglas and Brunner, 2002; Selz *et al.*, 2020; De-Kayne *et al.*, 2022; Selz and Seehausen, 2023). In northern areas, European whitefish live mainly in oligotrophic lakes and rivers with both anadromous and resident populations. However, southern populations are found in deep lakes that provide cold refugia. European whitefish are highly polymorphic and may be present in the same lake under various morphotypes forming populations adapted to living, feeding, and spawning in the various lacustrine habitats (*i.e.*, littoral, profundal, and pelagic; Alexander *et al.*, 2017). Ovulation and spawning often occurs in early winter when water temperatures fall below 8.0–7.0°C (Hartmann, 1984; Anneville *et al.*, 2013), but this threshold can differ within and among lakes depending on spawning habitat selection (*i.e.*, pelagic or littoral). Embryos require cold temperatures less than 7.0°C for optimal development. Temperatures greater than 7.0°C can compromise development, leading to increased mortality (Cingi *et al.*, 2010; Stewart *et al.*, 2021). Although there is now growing evidence that some European whitefish populations are threatened by warming climate (Trippel *et al.*, 1991;

Graham and Harrod, 2009; Cingi *et al.*, 2010; Karjalainen *et al.*, 2015), there is little information comparing how different populations respond to thermal constraints. These putative variations should be investigated and integrated into predictive models for the purpose of informing conservation practices (Meek *et al.*, 2023).

Using the same protocol as Stewart *et al.* (2021), we investigated the response of European whitefish embryos from three peri-alpine lakes at the southern edge of their distribution (*i.e.*, lakes Bourget, Geneva, and Constance) to two temperature treatments. Our aim was to analyze the level of variation between these populations for both survival and incubation duration. We discussed the observed patterns considering local thermal conditions among lakes and about the possible causes of variation. We then discussed our analysis in the context of similar investigations, with a particular focus on the study by Stewart *et al.* (2021).

2 Methods

2.1 Studied European whitefish populations

Our study focuses on three peri-alpine lakes: Lake Geneva, Lake Bourget, and Lake Constance (Fig. 1). These deep peri-alpine lakes exhibit notable stratification throughout most of the year and have been undergoing reoligotrophication since the 1980s (Straile *et al.*, 2007; Rimet *et al.*, 2020). Lake Bourget is 231 m above sea level, while Lake Constance and Lake Geneva have an altitude of 395 m and 372 m, respectively.

In lakes Bourget and Geneva, a littoral spawning population of European whitefish—belonging to *Coregonus lavaretus* (Kottelat and Freyhof, 2007)—is present with similar life history traits, ecological behavior, and diet (Bourinet *et al.*, 2023). Lake Constance currently supports three different populations of European whitefish: the benthivorous gangfish *Coregonus macrophthalmus*, the zooplanktivorous blaufelchen *Coregonus wartmanni* and the rare, large growing benthivorous sandfelchen *Coregonus arenicolus* (Kottelat and Freyhof, 2007). Given the rarity of sandfelchen, only gangfish and blaufelchen were used in the present study. Gangfish spawn in the shallow waters (5–30 m) of the littoral zone between November and January while blaufelchen spawn in the deeper waters (100–250 m) of the pelagic zone in December (Eckmann, 1987; Eckmann and Rösch, 1998). Vonlanthen *et al.* (2012) showed that F_{st} values between the littoral gangfish and the pelagic blaufelchen was 0.06, which suggests there is significant gene flow between populations. This flow might result from natural mixing processes in the wild or from artificial breeding practices (Gum *et al.*, 2014). To simplify terminology and avoid confusion, we refer to blaufelchen as Constance pelagic and gangfish as Constance littoral hereafter.

Temperatures in the 0–5 m depth layer (where embryos incubate near-shore) during winter in lakes Bourget and Geneva have been near 7.0°C from 1990 to 2020, but water temperatures can be marginally warmer on average in Lake Geneva (+0.24°C, SD=0.32, Bourinet *et al.*, 2023). Average winter water temperatures in Lake Constance have historically been 5.7°C at 20 m depth and 4.5–4.8°C at depths > 100 m (Roberts *et al.*, 2024).

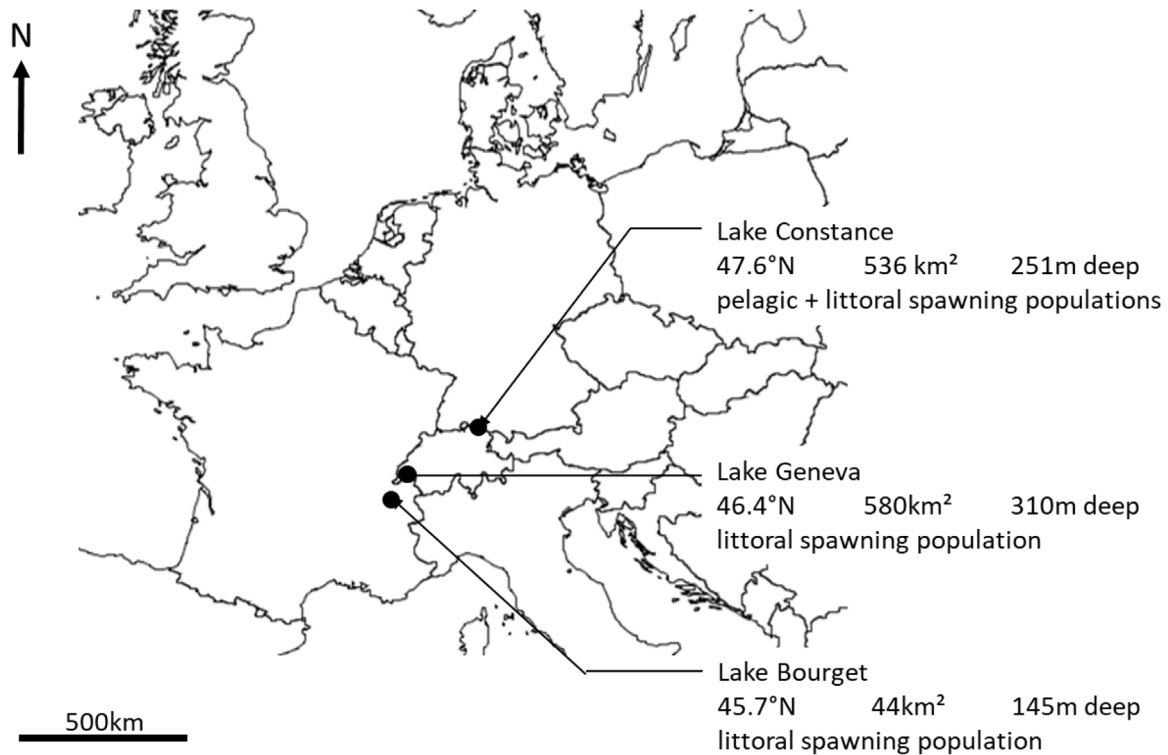


Fig. 1. Location and characteristics (latitude, surface area, and maximum depth) of the lakes where European whitefish were collected for use in the experiment.

2.2 Gamete collection

The experiment was performed during winter 2018–2019. Parental fish were caught by gillnetting on spawning shoals on the 11th, 19th and 27th of December for lakes Constance, Geneva, and Bourget, respectively. Nets were set at dusk and recovered at dawn. Fish were checked for ripeness and kept alive in tanks. Fish were anesthetized using a solution of clove oil at 35 mg l⁻¹ (first dissolved in ethanol at a ratio of 1:10) before recording total length and mass (fresh mass to the nearest g; [Tab. 1](#)). Sex of individuals was determined and gametes were stripped from ripe individuals. Gametes from Constance pelagic, Constance littoral, and Bourget fish were stored on ice at temperatures close to the lake surface temperature and immediately transported to the INRAE facilities in Thonon-les-Bains (France), whereas gametes from Geneva were processed immediately after stripping. Egg size was measured as dry mass because it is a better indicator of energy and nutrient content than wet mass or diameter ([Vrtilék *et al.*, 2020](#)). For dry mass measurements, 10 individual eggs were collected from each parental female fish, oven-dried at 60.0 °C for 4 days, and weighed (± 0.1 mg).

2.3 Crossing design and artificial fertilizations

Reconstituted freshwater ([ISO 6341, 2012](#)) was used for the entire experiment both for fertilization and incubation. Fish were fertilized by creating 3 blocks of 3 females x 4 males according to a full factorial design for Geneva, Bourget, and Constance pelagic populations, and 1 block of 3 females x 4 males for the Constance littoral population because of low

spawner catches ([Fig. 2](#)). After fertilization, each family of embryos were stored in the dark for 24 h in petri dishes at 7.0 °C. If families had low fertilization rates (no membrane detachment) they were excluded from the remainder of the experiment. Ultimately, 36 total families were obtained for the Geneva, Bourget, and Constance pelagic populations while only 12 families were obtained for the Constance littoral population.

2.4 Treatment

The embryos were placed in two walk-in, thermo-regulated chambers. The first chamber was set at 7.0 °C to mimic current temperature conditions in the three lakes. The second chamber was set at 9.0 °C to mimic temperatures at the upper end of the tolerance range ([Gillet, 1991](#)). Climate model projections suggest that 9.0 °C could become a reality, as even Lake Constance (*i.e.*, which is colder than Lake Geneva and Lake Bourget) winter temperatures at 20 m are expected to reach 8.4–8.6 °C by 2100, under ssp370 or ssp585 scenarios ([Roberts *et al.*, 2024](#)).

In each temperature treatment, 36 embryos per family were evenly split among three different 24-well cell culture microplates (Greiner Bio-One brand), to separate the treatments from a potential plate effect, resulting in each microplate having 12 embryos from two families from the same population. Embryos were transferred with a pipette to individual microplate wells filled with 2 ml of reconstituted water. For the whole experiment, a total of 360 plates were constituted representing a total of 8640 embryos.

Temperatures were monitored using three probes per chamber and recorded every hour (TG-4100, Tinytag). In the coldest chamber, the average temperature was 7.4 °C (SD= 0.2) and in the other chamber, the temperature was 9.3 °C (SD=0.02). The microplates were placed on shelves covered with opaque, plastic sheeting to ensure stable conditions with respect to temperature and darkness.

2.5 Studied traits

Two traits were studied: survival and incubation duration from fertilization to hatch. Mean embryo survival was estimated as the mean percent of embryos surviving between the eye-up (*i.e.*, eye pigmentation visible) and hatch stage to rule out unfertilized eggs, which can bias survival estimates. Incubation duration was individually assessed by daily inspection of microplates and expressed in terms of both the number of days from fertilization to hatching (days post-fertilization; DPF) and the sum of the degree-days (accumulated degree-days; ADD).

2.6 Statistical analyses

The statistical methods used follow the protocol described in Stewart *et al.* (2021). Briefly, embryo survival was analyzed as a binomial response variable, while incubation duration was analyzed as a continuous response variable. A cubic root transformation was applied to DPF and ADD to normalize the distributions. Embryo survival was analyzed with binomial generalized linear mixed-effects models, and the transformed variables were analyzed with restricted maximum likelihood linear mixed-effects models with the *lme4* package v.1.1-30 (Bates *et al.*, 2015). Population and incubation temperature treatment were included as fixed effects and female, male, female x male, fertilization block, and microplate as random effects. The maximal model for each trait (*i.e.*, survival, incubation duration expressed in terms of DPF or ADD) was selected using a backward, stepwise effect-selection process from the *buildmer* package v.2.6 (Voeten, 2023). The significance for population, incubation temperature, interaction effects, and any random effects selected were determined using a likelihood ratio test between the maximal model and reduced models with the model effect of interest removed.

To enable population comparisons, the response to incubation temperature for each trait was standardized to what we assumed was the optimal incubation temperature treatment—the 7.0 °C temperature treatment. For each trait, the within-family percent change from the optimal incubation temperature was calculated as:

$$\frac{X_2 - X_1}{X_1} \times 100$$

where x_1 = mean trait value from the 7.0 °C incubation treatment and x_2 = the mean trait value from the 9.0 °C incubation treatment. The mean among-family percent change was calculated, along with the standard error, as the among-family variation in percent change. This provides an evaluation of the mean reaction norm slope at a population scale (*i.e.*, percentage of trait value change for a 2.0 °C increase).

A-

		males												
		1	2	3	4	5	6	7	8	9	10	11	12	
females	A	X	X	X	X									
	B	X	X	X	X									
	C	X	X	X	X									
	D					X	X	X	X					
	E					X	X	X	X					
	F					X	X	X	X					
	G										X	X	X	X
	H										X	X	X	X
	I										X	X	X	X

B-

		males			
		1	2	3	4
females	A	X	X	X	X
	B	X	X	X	X
	C	X	X	X	X

Fig. 2. Crossing design. Three blocks of 4 males x 3 females (*i.e.*, a total of 36 families for each population) were produced for Geneva, Bourget, and Constance pelagic populations (A). For the Constance littoral population, only one block of 4 males x 3 females (*i.e.*, a total of 12 families) was produced due to low spawner catches (B). For each family produced, a sample of 36 eggs were exposed to a 7.0 °C treatment, and 36 other eggs were exposed to a 9.0 °C treatment.

Egg dry mass was not measured at the individual level so variance in ova mass was analyzed separately using a generalized mixed-effects model with individual ova mass as predicted variable and source population as a fixed effect and dam contribution as a random effect. Post-hoc Tukey tests were performed using the *emmeans* package to assess contrasts between pairs of populations using (α at $p < 0.05$; Lenth, 2024). All analyses were performed in R version 4.2.1 (R Core Team, 2022).

3 Results

3.1 Spawning adult and egg size

Total lengths and fresh mass of spawning adults used for gamete collection varied among study groups (Tab. 1). Both Constance littoral and Constance pelagic European whitefish were smaller than those of Geneva and Bourget. Geneva European whitefish spawners were the largest among all study groups (Tab. 1). Egg dry mass variance analysis yielded significant contrasts between populations ($F_{3,26} = 3.4998$,

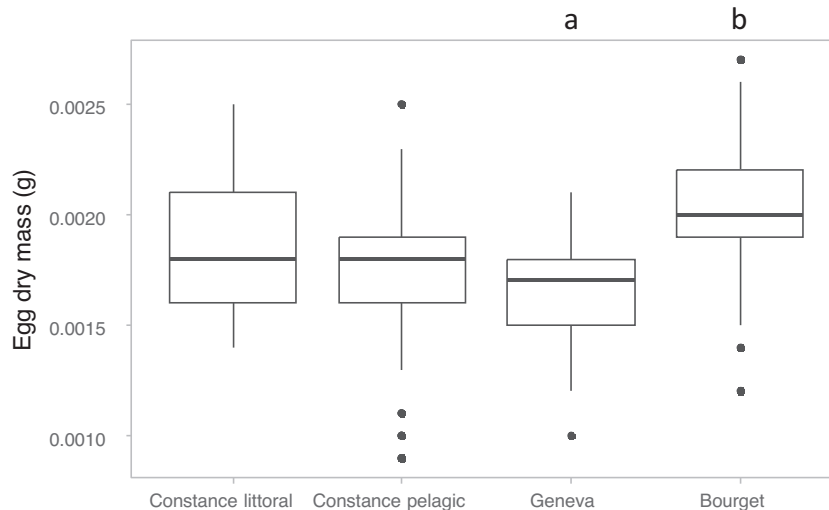


Fig. 3. Boxplots of egg dry mass (g) distribution for the four populations. A total of ten eggs were collected from each female, with 3 females sampled from the Constance littoral population and 9 females sampled from all other populations. Different letters above boxplots indicate pairwise Tukey contrasts (α at $p < 0.05$).

$p < 0.03$; Fig. 3). Egg dry mass was the highest for European whitefish from Bourget (mean = 2.0 mg, SD = 0.3) and lowest for European whitefish from Geneva (mean = 1.7 mg, SD = 0.2), which represented a significant difference between populations (post-hoc Tukey test $p < 0.02$). The egg dry mass for Constance littoral (mean = 1.8 mg, SD = 0.3) and Constance pelagic (mean = 1.7 mg, SD = 0.4) European whitefish were similar and not statistically different when compared to Bourget and Geneva eggs (post-hoc Tukey tests $p > 0.10$).

3.2 Developmental and morphological traits

All European whitefish traits examined had a significant interaction effect between population and incubation temperature (all $P < 0.001$; Tab. 2). The interaction effects precluded any interpretation of main effects but did reveal different norms of reaction for the study groups. Below we describe the interaction effects. All female, male, female \times male, block, and plate random effects were significant (maximum $P = 0.048$; Tab. 2).

3.3 Survival at hatching

Embryo survival was highest among all study groups at the 7.0 °C incubation temperature compared to the 9.0 °C treatment (Fig. 4). The effect of the highest temperature for European whitefish embryo survival depended on population. Among all populations, embryo survival was highest for Constance littoral and Constance pelagic European whitefish from Constance at 7.0 °C (98.1% and 94.6%, respectively) but lowest at 9.0 °C (40.1% and 51.5%, respectively). Embryo survival was a bit lower at 7.0 °C in Geneva and Bourget populations (93.9% and 89.2%, respectively) compared to Constance populations, but higher at 9.0 °C (63.2% and 53.0%, respectively), compared to Constance populations. The reaction norm for survival (Fig. 4) was highest for Constance littoral population (−61.6%) and lowest for the Bourget

population (−33.3%). It was intermediate for Geneva (−45%) and Constance pelagic (−44.0%) populations.

3.4 Incubation duration

The mean number of days between fertilization and hatching was highest among all study groups at the 7.0 °C incubation temperature (Fig. 4). Constance littoral European whitefish had the highest DPF at 7.0 °C (59.0 days) but the lowest DPF at 9.0 °C (43.7 days) among all study groups. The differences in DPF among study groups were more pronounced at 7.0 °C (mean = 1.8 days, SD = 1.0) than at 9.0 °C (mean = 0.6 days, SD = 0.3).

Accumulated degree-days were highest for all study groups at 7.0 °C, except for Bourget European whitefish (Fig. 4). At 7.0 °C, Bourget European whitefish ADD was the lowest (415.8 ADD) among all study groups but at 9.0 °C the highest (419.3 ADD) among all study groups. Bourget and Geneva European whitefish ADD had the lowest differential responses, but the responses were different to increasing incubation temperature: Bourget embryos had a positive response from 7.0 to 9.0 °C incubation temperatures (difference = 3.5 ADD) while Geneva embryos responded negatively (difference = −6.8 ADD). Both Constance littoral and Constance pelagic populations had a greater negative response in ADD to increasing incubation temperature (difference = 33.8 and 19.5, respectively) than Geneva and Bourget European whitefish. The Constance littoral population had the strongest response to increasing incubation temperatures compared to all other study groups.

4 Discussion

Our common garden experiment allowed us to produce thermal reaction norms for survival and incubation duration for European whitefish embryos from four populations across

Table 1. Mean total length (TL), fresh mass (FM), and sample sizes (N) of the female and male European whitefish (*Coregonus spp.*) collected from lakes Constance (littoral and pelagic populations), Geneva, and Bourget. Standard deviation in parentheses.

Sex	Constance Littoral			Constance Pelagic			Geneva			Bourget		
	TL (mm)	FM (g)	N	TL (mm)	FM (g)	N	TL (mm)	FM (g)	N	TL (mm)	FM (g)	N
Female	324.3 (1.2)	223.33 (14.2)	3	332.6 (30.7)	277.4 (78.5)	10	479.2 (42.9)	1102.3 (306.0)	12	400.9 (36.9)	571.8 (129.1)	12
Male	317.8 (28.6)	216.25 (54.9)	4	340.4 (17.7)	260.1 (48.0)	14	441.1 (56.7)	802.7 (443.3)	16	422.7 (26.2)	551.1 (66.2)	15

Table 2. Likelihood ratio test output for each model selected for embryo survival and incubation duration (number of days post-fertilization (DPF) and accumulated degree days (°C; ADD)) from European whitefish (*Coregonus spp.*) in lakes Constance (littoral and pelagic populations), Geneva, and Bourget. Effects abbreviations: t – treatment, pop – population, block – a group of four males each paired to three females (see Fig. 2). The full model was selected using a backward, stepwise effect-selection and it is bolded for each trait.

Trait	Model	Effect Tested	df	χ^2	p-value
Embryo Survival	t + pop + t:pop + female:male + female + block + plate	t	1	377.56	<0.001
	pop + female:male + female + block + plate	pop	3	4.14	0.246
	t + female:male + female + block + plate	t:pop	3	39.79	<0.001
	t + pop + female:male + female + block + plate	female:male	1	14.35	<0.001
	t + pop + t:pop + female:male + block + plate	female	1	10.39	0.001
	t + pop + t:pop + female:male + female + plate	block	1	27.27	<0.001
	t + pop + t:pop + female:male + female + block	plate	1	51.31	<0.001
	t + pop + t:pop + female:male + female + male + block + plate	t	1	780.85	<0.001
	pop + female:male + female + male + block + plate	pop	3	2.19	0.534
	t + female:male + female + male + block + plate	t:pop	3	45.53	<0.001
Incubation Period (DPF)	t + pop + t:pop + female:male + female + block + plate	female:male	1	62.23	<0.001
	pop + female:male + female + male + block + plate	female	1	3.90	0.048
	t + female:male + female + male + block + plate	male	1	7.55	0.006
	t + pop + female:male + female + male + plate	block	1	11.95	<0.001
	t + pop + t:pop + female:male + female + male + block	plate	1	197.62	<0.001
	t + pop + t:pop + female:male + female + male + block + plate	t	1	33.21	<0.001
	pop + female:male + female + male + block + plate	pop	3	1.49	0.686
	t + female:male + female + male + block + plate	t:pop	3	51.28	<0.001
	t + pop + female:male + female + male + block + plate	female:male	1	59.13	<0.001
	t + pop + t:pop + female:male + male + block + plate	female	1	4.30	0.038
Incubation Period (ADD)	t + pop + t:pop + female:male + female + block + plate	male	1	8.15	0.004
	pop + female:male + female + male + plate	block	1	17.53	<0.001
	t + female:male + female + male + plate	plate	1	199.59	<0.001
	t + pop + female:male + female + male + block + plate	t	1	33.21	<0.001
	t + pop + t:pop + female:male + male + block + plate	pop	3	1.49	0.686
	t + pop + female:male + female + male + block + plate	t:pop	3	51.28	<0.001
	t + pop + t:pop + female:male + male + block + plate	female:male	1	59.13	<0.001
	t + pop + t:pop + female:male + male + block + plate	female	1	4.30	0.038
	t + pop + t:pop + female:male + female + block + plate	male	1	8.15	0.004
	t + pop + t:pop + female:male + female + male + plate	block	1	17.53	<0.001

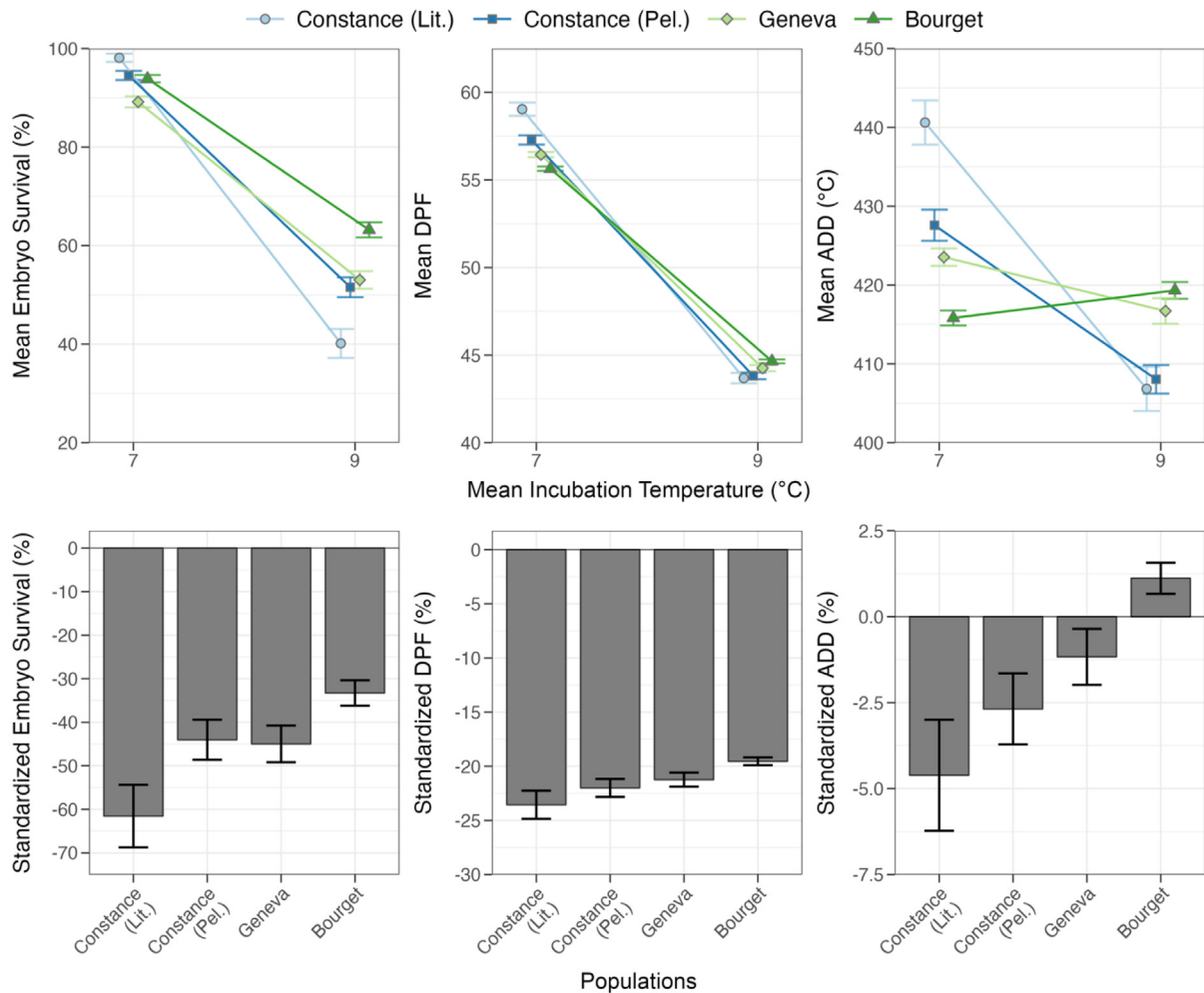


Fig. 4. Mean embryo survival (°C) and incubation duration from fertilization to hatch (days post-fertilization [DPF] and accumulated degree days [°C; ADD]) at each incubation temperature (°C; top row) and standardized temperature responses within each study group (%; bottom row) from European whitefish collected from lakes Constance (littoral and pelagic populations), Geneva, and Bourget. Error bars indicate standard error.

three peri-alpine lakes. This study revealed how environmental changes (*e.g.*, increasing water temperature) can negatively impact cold-water adapted fish species. All four tested European whitefish populations exhibited lower survival rates when exposed to warmer incubation conditions that were consistent with realistic future scenarios. Additionally, these peri-alpine populations displayed variable responses in incubation duration, indicating a level of phenotypic plasticity and notable interplay between genotype and environment. Furthermore, due to the modified (*i.e.*, shorter) hatching time under warmer conditions, hatching of larvae could be decoupled from the current conditions due to climate change. These climatological changes are likely to increase the spatiotemporal mismatch between larvae and suitably sized prey (Cushing, 1990; Straile *et al.*, 2007; Durant *et al.*, 2019).

The differences in the survival rates of embryos exposed to 7.0 and 9.0°C were high for all peri-alpine populations (–50.6% on average), which suggests that 9.0°C may be a threshold temperature for European whitefish populations along the southern extent of their distribution. Embryo survival in the littoral population from Constance was the highest at

7.0°C compared to other populations but the lowest at 9.0°C. The smaller number of families for Constance littoral compared to the Constance pelagic population ($n = 12$ vs. 36) makes the robustness of the data weaker and precludes deeper investigations on possible within lake population specific response to thermal stress. The fact that we could not sample more littoral gangfish is directly linked to population densities and harvest rates, which are below 50% of historical values (Baer *et al.*, 2023).

For the populations included in this study, the response to warmer water varied by a factor of almost two, as the standardized embryo survival for Constance littoral and Bourget populations was –61.6% and –33.3%, respectively. Contrasting reaction norms suggest genetic differentiation and adaptation could benefit certain populations, assuming parental effects were accounted for as a random effect in the modeling process. Bourget fish tolerated the same temperature increase better than Constance littoral, Constance pelagic, and Geneva. The assumption that the populations with the warmest reproductive natural thermal environment (*i.e.*, Geneva and Bourget) would have the highest survival at 9.0°C

was not clearly supported. Lake Bourget indeed had the highest survival at 9.0 °C but survival of embryos from Geneva at 9.0 °C was not better than those from Lake Constance. Why Bourget and Geneva embryos exhibited different survival rates when exposed to the warm treatment remains unclear, especially when their natural spawning thermal conditions are similar. The fact that Bourget embryos survived better to hatching in the warm treatment compared to Geneva and other populations (*i.e.*, they have a flatter norm of reaction) is a strong indication that they could be physiologically more adapted to warming. Their larger eggs (dry mass) provide a high energetic reserve which might be an advantage for embryo survival. However, the difference in egg dry mass between Constance pelagic and Constance littoral populations was not significant and thus could not alone explain the observed patterns. In addition, Karjalainen *et al.* (2016) stated that the positive relationship between age and the amount of energy allocated to eggs by spawning vendace (*Coregonus albula*, L.) did not affect the fertilization rate or embryo survival. Régnier *et al.* (2013) studied brown trout *Salmo trutta* L. embryos reared in experimental conditions and found egg size was not advantageous under thermal stress because relative oxygen demand increased with egg size, and oxygen availability decreased with temperature (Martin *et al.*, 2020). Moreover, Thorn and Morbey (2018) found that the contribution of egg size of Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) to the among-population variation in early life history traits decreased with temperature. They suggested that factors other than egg size (*e.g.*, genetic) are likely more important under thermal stress. Thus, the better performance of Bourget European whitefish under warm conditions could be related to genetic characteristics of the population more so than egg size.

In the peri-alpine lakes that were studied, populations have been affected by years of supportive breeding. In addition, introgressive hybridization between populations of various forms occurred due to habitat degradation and demographic decline (Vonlanthen *et al.*, 2012). This may have led to complex genetic changes in these populations and modified their potential for response to stressors such as temperature (Anneville *et al.*, 2015; Frei *et al.*, 2023).

Our survival estimates between both temperature treatments for all studied populations are much higher than the survival observed at 6.9 °C for a Finnish population of European whitefish from Lake Konnevesi (Stewart *et al.*, 2021). The mean survival rate of Konnevesi European whitefish *Coregonus lavaretus* was 30% at 6.9 °C and only 14% at 8.0 °C. Although the comparison of absolute survival is tenuous since survival in colder conditions was also lower (*i.e.*, 37% at 4.0 °C and 59% at 2.3 °C), they found a negative relationship between embryo survival and incubation temperatures starting at 2.0 °C, which corresponds to natural thermal conditions in Lake Konnevesi. Similarly, the incubation experiments of Cingi *et al.* (2010) demonstrated that incubation temperatures ≥ 7.0 °C increased the proportion of unfertilized and abnormally dividing eggs, deformed embryos, and subsequent mortality of Finnish anadromous Kemijoki and Kokemäenjoki *Coregonus lavaretus* populations. This suggests that the high incubation temperatures were more stressful for the northern European whitefish populations compared to peri-alpine lake populations and supports the hypothesis that

populations at the southern limit of the European whitefish distribution could be better adapted to higher temperatures compared to populations in the northern portion of the distribution.

Within treatments, the average incubation durations were similar. However, hatching was more precocious when embryos were exposed to warmer conditions and this shift in phenology could have consequences for larvae in the wild. In peri-alpine lakes, spring phytoplankton blooms are regulated by physical factors such as mixing depth (Sommer *et al.*, 1986). Under warmer climate scenarios, those physical factors could change dramatically, leading to different stratification dynamics in lakes resulting in shifts in the phenology of phytoplankton and zooplankton (Wahl and Peeters, 2014; Asch *et al.*, 2019). Therefore, the possibility exists for a decoupling of newly hatched larvae from their prey resources during a period when size selective processes impact survival (Straile *et al.*, 2007). Due to the fact that survival of larval European whitefish is known to be positively related to prey availability (Rellstab *et al.*, 2004), more than two weeks without sufficient acquisition of prey could lead to additional mortalities. Furthermore, a recent study (Roberts *et al.*, 2024) showed that contemporary declines of the European whitefish stock in Lake Constance could be caused in part by recent warm winters (Asse *et al.*, 2018). It is worth noting that although some European whitefish populations might exhibit adequate survival rates at higher temperatures, they could still succumb to elevated mortality rates later in their early life history due to the consequences associated with increased temperatures (*e.g.*, respiration, predation, disease, *etc.*).

We found contrasting responses to temperature for the four populations in terms of incubation duration. These different reaction norms may suggest physiological differences in vulnerability and/or response to warming incubation temperatures among populations. Interestingly, the Bourget population had the flattest reaction norm since it had the shortest incubation duration at 7.0 °C but had the longest incubation at 9.0 °C. Contrary to Lake Bourget, both populations from Lake Constance had a significant decrease in incubation duration from 7.0 to 9.0 °C (steeper reaction norm). Dissimilarity in incubation duration could be explained by differences in egg dry mass at fertilization, as larger eggs typically have a greater demand for yolk and faster development rates than smaller eggs (Hodson and Blunt, 1986; Kamler, 2008). However, the relative changes in incubation duration for lakes Bourget and Geneva were less pronounced than Lake Constance pelagic and littoral populations and suggests a different level of developmental plasticity could exist. In addition, European whitefish from Lake Bourget had larger eggs compared to Geneva but not compared to the Lake Constance pelagic and littoral populations, meaning that egg size alone could not explain the observed differences in terms of incubation duration. Incubation duration might also change as a response to environmental conditions such as hypoxia (Czerkies *et al.*, 2001), pathogens (Wedekind, 2002), or predation (Wedekind and Müller, 2005). There are indeed indications that incubation duration is not a pure indicator of developmental rate as hatching might occur at different stages (Valdimarsson *et al.*, 2002; Mitz *et al.*, 2019). Since yolk sac volume and larval size were not measured here it is not possible to assert how changes in incubation duration from warming incubation temperatures

could have impacted yolk-conversion efficiency and length-at-hatching. However, we can assume that the precocious hatching observed in the Constance population at 9.0°C indicated a stress response, as shown in the above cited studies. It is worth noting that in our study, the littoral population from Constance had a longer time to hatch in the 7.0°C treatment compared to pelagic population, which is consistent with the observations by Eckmann (1987). This author found European whitefish from Lake Constance had shorter incubation times compared to other coregonines and suggested faster development rates could be an adaptation to specific thermal environments. In the 9.0°C treatment, the difference of hatching timing between the two Constance populations decreased, which was consistent with Eckmann's (1987) findings.

The incubation duration in peri-alpine populations contrasted with what was observed in northern Lake Konnevesi where incubation was much longer at 6.9°C (*i.e.*, DPF=91, ADD=600; Stewart *et al.*, 2021). At 2.3°C, Konnevesi embryos needed > 6 months to hatch (DPF = 199, ADD = 374) suggesting they were physiologically adapted to efficiently develop at very low winter temperatures. European whitefish spawn in Lake Konnevesi in the beginning of November and hatching occurs at the end of April or in the beginning of May near when ice-out occurs (Karjalainen *et al.*, 2015). Winter temperatures in the shallow littoral zone, where the Konnevesi European whitefish spawn and eggs develop, can be <2.0°C for long periods. In the present study, embryos of peri-alpine populations were not exposed to such cold temperatures as we were mainly interested in investigating the response to warming above contemporary incubation temperatures. Employing a common garden approach with a wider range of incubation temperatures than what was used in the present study could provide additional insights. Expanding the incubation temperatures would also provide an opportunity to test the hypothesis of Metabolic Cold Adaptation (White *et al.*, 2012) that assumes populations adapted to cold environments have higher metabolic rates compared to populations in warmer environments. This hypothesis has mainly been tested at an inter-specific level and rarely at a lower taxonomic level (Lardies *et al.*, 2004; Tan *et al.*, 2021).

This study combined with the one from Stewart *et al.* (2021) shows, on a large-scale, that high and low latitude populations of European whitefish have highly contrasted responses to warming incubation conditions. At a more local scale, at low latitude, responses also differ between peri-alpine populations. The within- and among-population reaction norms observed in this study suggest European whitefish populations are likely adapted to local and regional thermal conditions. Knowledge of population-specific response to climate change could provide valuable information for resource managers (Meek *et al.*, 2023). For example, although incubating embryos in a cold environment may be attractive for obtaining higher production of fish for release, it may contribute to artificially selecting cold-water adapted genotypes that would not perform as well in the wild assuming a warming environment will continue to be a reality. Indeed, it has been shown that selection under artificial rearing conditions can occur rapidly (Christie *et al.*, 2012). Hatchery incubation conditions should therefore be diversified to

promote genetic diversity and adaptive potential of fish that are released. A more thorough analysis of the costs and benefits of such an approach would be required.

Our study supported the hypothesis that climate change could have detrimental effects on locally adapted European whitefish populations. In combination with other stressors (*e.g.*, invasive species; Rösch *et al.*, 2018; DeWeber *et al.*, 2022), climate change could lead some populations of European whitefish to the edge of extinction.

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Ethics statement

This experiment was designed in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. As this study focused on fish embryos and larvae that rely on endogenous feeding only, no special ethics permission was required. Permission for fish captures was granted by local authorities (DDT74 and DDT73).

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