Negative effects of parasite exposure and variable thermal stress on brown trout (*Salmo trutta*) under future climatic and hydropower production scenarios

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ARTICLE INFO

ABSTRACT

Future water temperature changes may have a profound impact on fish-parasite interactions. However, while the effect of temperature on fish, and particularly salmonids, is well-understood, its combined effects with parasitic exposure are not. Here, we use a multi-stage experimental approach to explore the impact of increased water temperatures consistent with persistent climate change-induced warming and extreme thermal fluctuations from hydropower (thermopeaking) on brown trout alevins and fry before and during exposure to *Saprolegnia parasitica*. Parasite exposure had the strongest and most significant effect on survival of both host life stages. The combination of parasite exposure, thermal pre-conditioning and the ongoing thermal regime had a weak but significant influence on alevin mortality. Both parasite-exposed alevin and fry experienced increased mortality when a constant increase in temperature was combined with intermittent thermal increases. The outcomes of this experimental approach provide the basis for future studies scaling up the potential impacts of temperatures and parasite exposure that key fish species may face in the wild. They also highlight the effects of anthropogenic changes on brown trout populations, as pressures on aquatic organisms are likely to intensify in future climate scenarios with increased hydropower development and thermopeaking, particularly in the presence of pathogens.

1. Introduction

Future climate change exacerbated by anthropogenic pressures threatens freshwater ecosystems worldwide [1–5]. While global models predict an increase in river temperatures [6] evidenced by long-term empirical studies of temperate streams [7–10], increasing energy demands are promoting hydropower developments [11], that in turn may lead to highly fluctuating thermal regimes with warm waters sporadically being released for power generation [12]. These extreme sub-daily thermal fluctuations, termed thermopeaking [13–15], generally lower water temperatures in spring and summer *e.g.* [16] and increase temperatures in the winter *e.g.* [17].

Changes in water temperature fundamentally impact aquatic system functioning, particularly the physiological condition of freshwater communities [18,19]. Of key concern is the effect of increased temperature on cold-water fish, such as brown trout (*Salmo trutta* Linnaeus, 1758) [20–24]. Like all ectotherms, an inability to regulate body temperature makes these fish highly vulnerable to temperature changes [25–27]. Brown trout are of key ecological and economic significance [28–

32], yet catch numbers have decreased significantly over the last 15 years in Europe *e.g.* [33]. Indeed, many salmonid populations are in decline, a trend likely to be exacerbated by increasing water temperatures and other local stressors [34]. The early life stages of brown trout, crucial for population recruitment, have the lowest thermal tolerance [20,35,36]. Optimal incubation temperatures for ontogenetic development range between 8 and 10 °C [37]. Lower and upper lethal limits for embryonic development are $\approx$ 1 °C and 14–16 °C, respectively [38,39], with an increased occurrence of deformities when subjected to temperatures below 3 °C and above 11 °C [40].

Another important biological risk factor for freshwater biota is infectious disease, which is also affected by thermal changes. The ubiquitous oomycete *Saprolegnia parasitica* has particularly devastating effects on salmonids worldwide [41,42]. This pathogen causes the disease saprolegniasis, characterised by patches of grey/white cotton-like mycelia on epidermal tissues, predominantly on the head or fins during the initial infection stages. These mycelia can subsequently spread to cover the entire body and cause impaired osmoregulation, which is the most frequent cause of death associated with this disease, but can also result in respiratory or general organ failure [41,43–45]. Saprolegniasis is one of

the most problematic infectious diseases in cultured salmonids in Japan [46], and it is responsible for an estimated annual economic loss of 10% from Scottish, Norwegian and Chilean salmon hatcheries and farms [47–49]. Saprolegniasis has also been increasingly associated with declines in wild salmon populations across England and Wales [44,50]. In the wild, the disease is most prevalent during the winter and spring when temperature changes expose fish to thermal stress; resulting in an impaired immune response, increased disease susceptibility, and higher predation of infected fish [41,43–45]. Therefore, climate-induced temperature changes could intensify the prevalence of *S. parasitica* due to increased thermal stress on fish hosts and faster progression of saprolegniasis in infected fish.

While the optimal thermal thresholds for growth and survival of brown trout early life stages are well established, and *S. parasitica* infections on salmonids have been well documented, the combined effects of thermal alterations alongside *S. parasitica* exposure on brown trout early life stages remain unexplored. Future climate change and hydropower production scenarios will most likely increase thermal stress on salmonids, potentially intensifying the prevalence of *S. parasitica* in freshwater systems and the progression of saprolegniasis in infected fish at different life stages. Consequently, fish and freshwater biodiversity conservation related to hydropower are considered a key challenge for the future [51]. In a multi-stage experiment, this study aims to test the hypothesis that the combined effects of (both continuous, due to climate change, and intermittent, due to thermopeaking) increased water temperatures and parasite exposure will negatively affect early development and increase the mortality of brown trout, notably alevins and fry, during winter. Acknowledging that natural systems present high variability, this experiment focuses on a set of potential scenarios modelling present and future climatic conditions in both unregulated and high-head hydropower regulated systems. Specific objectives include: (i) to establish the conditioning effects (pre-conditioning) of different thermal regimes, including continuous (climate-change induced) and intermittent (thermopeaking) increases in temperature; (ii) to investigate the combined effects of thermal pre-conditioning, ongoing continuous and intermittent increases in temperatures with an initial parasite exposure in alevins; (iii) to investigate the same combined effects during a second parasite exposure on fry.

2. Material and methods

2.1. Experimental approach

This study was designed as a three-stage experiment to assess alevin-to-fry growth and mortality of brown trout in a tank setup from 24 January to 18 March 2018 (53 days). In Stage 1 (day 1 to 18), alevins were assessed for growth and mortality. They were thermally pre-conditioned in an outdoor environment using four tanks representing four 'variable' thermal scenarios. Scenarios reflected present, and climate-change predicted stream temperatures combined with sub-daily thermal alterations simulating thermopeaking releases from hydropower (BASE, WARM, PEAK, W-P, Fig. 1). In Stage 2 (day 20 to 26), half of the pre-conditioned alevins were exposed to *S. parasitica*. In order to disentangle parasite-temperature interactions, alevin mortality was assessed by comparing the effect of parasite exposure during thermal pre-conditioning vs. ongoing thermal regimes. To do so, half of the control and parasite-exposed groups were maintained within their respective outdoor 'variable' ongoing thermal regimes, and the remaining half was moved to a 'constant' environment represented by stable thermal conditions recreated indoors. In Stage 3 (day 47–53), after the alevins from all treatments had absorbed their yolk sacs and become fry, the parasite treatment groups received a second *S. parasitica* challenge to examine the effect of additional exposure on fry with different temperature and parasite histories on brown trout mortality. Due to a malfunction of the temperature controls in one of the four 'variable' thermal conditions, only fish from the 'constant' environment of Stage 2 could be used in this final stage.

All practical work was conducted using a combination of indoor and outdoor facilities at Cardiff University, UK. Outdoor facilities allowed the simulation of the four thermal scenarios in the 'variable' environment. Indoor facilities, within the Cardiff University Aquarium, provided the environment to apply the 'constant' thermal conditions across treatments and enabled replication of the outdoor 'variable' setup.

2.2. Experimental setup and stages

2.2.1. Stage 1: thermal pre-conditioning

The four thermal scenarios established in the outdoor 'variable' environment were named BASE, WARM, PEAK and WARM-PEAK (W-P). Baseline water temperatures (BASE) were simply temperatures experienced in the outdoor facilities at Cardiff during the study period (between 24 January and 18 March 2018), typical of a temperate climate. The pre-conditioning period was carried out from days 1 to 18 (between 24 January and 11 February 2018). Warmer water temperature (WARM) was set at a continuous 3–4 °C higher than BASE, based on the predicted increase of UK water temperatures by 2050 [8,24]. Both BASE and WARM regimes were characterised by ambient variability. In order to examine thermopeaking conditions, intermittent rises of 2–3 °C in stream temperature (PEAK, *e.g.*, [14]) were applied above BASE, occurring between 16:00 and 17:00 h, corresponding to high energy demand periods in the UK [12,52]. The final thermal scenario (W-P) combined the 3–4 °C continual with the 2–3 °C intermittent increases in temperature (Table 1, Fig. 2).

Each of the four 'variable' thermal scenarios was recreated in a large lidded aquarium (64 L capacity, dimensions 37 W x 60.5D x 28H cm) filled with 50 L of aerated dechlorinated water. Aquarium heaters maintained the desired thermal regimes and were monitored by water temperature loggers (HOBO Pro-v2 onset®) at 15 min intervals throughout all experimental stages. Lids on top of the aquaria at $a \approx 45°$ angle enabled airflow with the external environment but prevented the entry of cold rainwater. Within each aquarium, four replicate containers (400 ml capacity, 12 W x 12D x 4H cm) were introduced, punctured with small holes (approx. 2 mm) to enable water flow-through. We assigned between 62 and 85 brown trout alevins to each container, hatched no later than four days before. Dead individuals were recorded daily and removed upon detection. At day 18, a sub-set of 60 individuals from each thermal scenario (15 individuals from each replicate container) were humanely euthanised by clove oil immersion [53], and photographed to measure fish size (fork length) using the software ImageJ v1.51j8, to estimate growth.

2.2.2. Stage 2: primary S. parasitica challenge on brown trout alevins

In the second stage (experiment days 20–26), both the effects of pre-conditioning and ongoing thermal regimes combined with parasite exposure on the mortality of 720 alevins were assessed in a non-replicated experiment. Half of the alevins in the outdoor tanks (two small containers from each) were kept in their respective 'variable' thermal conditions (Fig. 2). The other two containers were transferred to the indoor 'constant' environment that mirrored the outdoor 'variable' setup. All indoor containers were subject to equal 'constant' environmental temperatures, which were notably less variable than those in the outdoor aquaria (Table 1).

Each pair of containers in their respective 'variable' and 'constant' environments were further split into a parasite vs. control treatment. The number of fish in each of the pre-conditioned containers was standardised to $n = 45$. Prior to parasite exposure, each container was inserted into a larger non-perforated box (1.5 L capacity, dimensions 18.8 W x 18.8D x 11.7H cm) filled with aerated dechlorinated water and designed to prevent contact between treatments but maintain contact with the environmental water temperatures. On day 20, fish within the parasite treatment containers were challenged with 1 L of EA016 *S. parasitica* zoospores at a concentration of 3×10^5 L^{-1} for 24 h, following the standard procedure for infecting fish with this pathogen [54]. Briefly, this
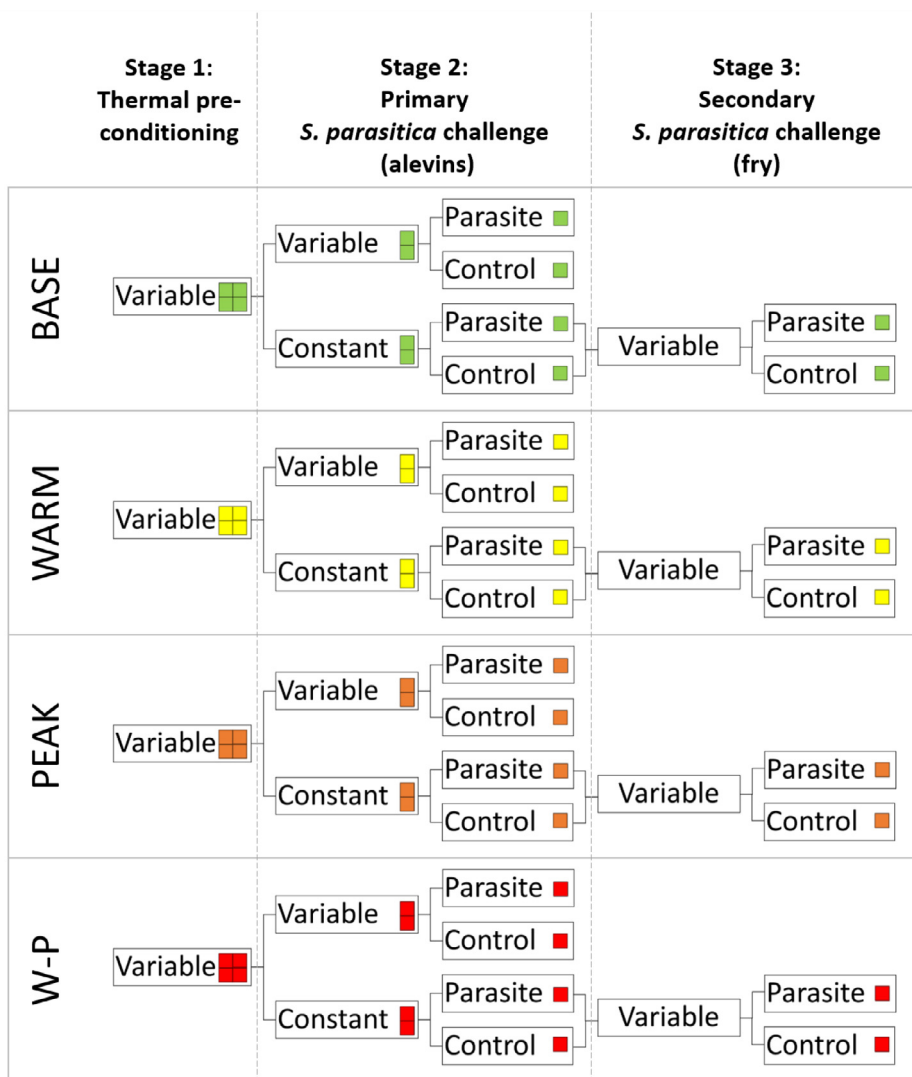
Fig. 1. Experimental stages of the study, showing both 'variable' and 'constant' environments, the four thermal scenarios and/or preconditioning in each (BASE, WARM, PEAK, and WARM-PEAK, W-P), and each parasite-exposed and control groups. Starting from 'variable'preconditioning, samples were then splitted into 'variable'(remaining in the outdoor tanks) and 'constant' (moved to indoor facilities) environments during Stage 2 (primary parasite challenge). In Stage 3, the remaining containers from the 'constant'enviornment were moved back to 'variable'outdoor conditions for the second and final parasite challenge.

Table 1

Mean (±SE) temperatures in both 'variable' (including BASE, WARM, PEAK and W-P scenarios) and 'constant' thermally regulated environments, for each experimental stage.

Experimental stage	Experimental temperatures (˚C)				Constant
	Variable				
	BASE	WARM	PEAK	W-P	
Stage 1	6.7 ± 0.04	10.3 ± 0.03	7.7 ± 0.04	10.6 ± 0.04	N/A
			9.9*±0.30*	12.7*±0.30*	
Stage 2	6.6 ± 0.10	10.3 ± 0.10	8.1 ± 0.10	11.2 ± 0.10	13.8 ± 0.10
			10.8*±0.30*	13.8*±0.30*	
Stage 3	9.0 ± 0.02	12.1 ± 0.02	10.3 ± 0.10	13.4 ± 0.10	N/A
			13.1*±0.10*	16.1*±0.10*	

* mean ± SE peaking temperatures in the PEAK and W-P treatments.

involved shaking each fish in a net for 30 s before being submerged in the spore solution. After this initial exposure period, the zoospore solution was removed and replaced with 1 L of dechlorinated water. The mortality rate of all the alevins in each of the 16 containers (8 outdoors and 8 indoors) was subsequently monitored every 2 h during the first 48 h from the point of initial parasite exposure, and then every 4–8 h until the cessation of the experiment on day 26. Any dead alevins were removed from their respective containers upon discovery. Several of these fish were randomly checked for the presence of *S.parasitica* under the microscope, and all of them were confirmed to be infected.

2.2.3. Stage 3: secondary S. parasitica challenge on brown trout fry

Upon termination of Stage 2, the remaining alevins in the 'constant' environment were maintained under the same conditions until their yolk sacs were totally absorbed. These fry were then fed ground trout pellets twice daily. Containers were checked daily for any dead individuals, which were removed. On day 47, fry within the parasite exposed treatment were re-challenged with EA016 *S. parasitica* zoospores (concentration 3×10^5 L^{-1} again for 24 h) in another non-replicated 6-day experiment. Both parasite exposed and control treatment groups ($n \approx 30$) were transferred back to their 'variable' respective thermal conditions (BASE,
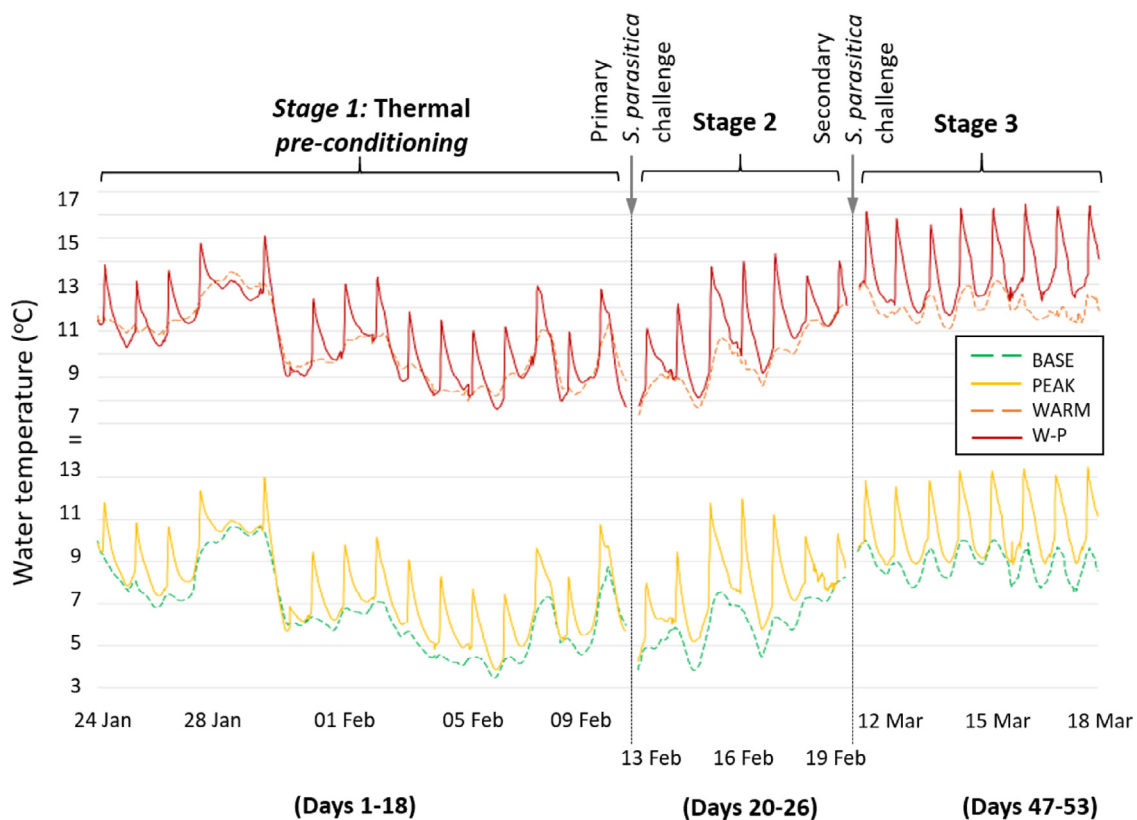
Fig. 2. Water temperatures (°C) for all experimental stages in the outdoor 'variable' thermal conditions including BASE, PEAK, WARM and WARM-PEAK (W-P) thermal scenarios.

WARM, PEAK, W-P) that they had been initially allocated to in experimental Stage 1, during thermal pre-conditioning (Fig. 2). Mortality of the fry was monitored at 9:00, 12:00, 15:00 and 18:00 daily until completion of experimental procedures on day 53. All other experimental procedures were kept the same as those from the initial challenge infection except for the additional feeding twice daily due to the absence of the yolk sac.

Water changes were conducted every 24 h, and water quality checks performed weekly on all treatments. The checks revealed no significant differences in pH, % dissolved O_2, electrical conductivity values between treatments throughout the experimental stages (One-way ANOVAs; $p > 0.05$ for all), ensuring optimal water quality conditions for brown trout were maintained throughout all experimental stages.

2.3. Fish and parasite origins and maintenance

All female brown trout diploid eggs were acquired from Northern Trout (Brown Well Fisheries Limited, UK). They were transferred to Cardiff University aquatic facilities in January 2018, post-eye stage. Eggs were maintained between 6 and 10 °C until they all hatched and became ready for the start of the experiment on day 1 (24 January 2018). All procedures and protocols were conducted under UK Home Office license (PPL 30/3424) following ARRIVE guidelines with approval by the Cardiff University Animal Ethics Committee.

The parasite, *Saprolegnia parasitica* isolate EA016, originated from a sea trout (*Salmo trutta*) in the River Dart, UK on 3 June 2016. Mycelia (approx. 4–5 cm^2) were extracted from the affected tissue using forceps and placed directly onto a potato dextrose agar (PDA, 39 g L^{-1}) plate, which was sealed with parafilm and transported to some parasite culturing facilities at Cardiff University. A stock culture was then plated onto fresh PDA plates monthly, according to Stewart et al. [54] . To produce zoospores, petri dishes containing ≈ 40 ml glucose-yeast broth (Glucose 10 g L^{-1}, Yeast Extract 2.5 g L^{-1}) were inoculated with three

5 mm diameter plugs of heathy white mycelia from the stock culture and were left to grow for 72 h at room temperature (≈ 20 °C). The resulting mycelial mats were washed with dechlorinated water in order to remove excess glucose-yeast broth and placed in a 50/50 mixture of dechlorinated water and aquarium water at 10 °C for 72 h to induce sporulation. Two 10 ml samples of the resulting zoospore solution were centrifuged at 4500 rpm for 10 min. The top 9 ml of solution was aspirated, zoospores in the remaining 1 ml were re-suspended via pipetting and enumerated using a haemocytometer. The concentration of the zoospore solution was then calculated and diluted to 3×10^5 L^{-1} with dechlorinated water for use in the challenge infections.

2.4. Statistical analyses

In Stage 2, the relationships between thermal pre-conditioning, growth (fork length) and mortality were modelled. Fork length was analysed with a simple linear model. Mortality (proportion of dead) was analysed using a Generalised Linear Model (GLM) with a binomial error distribution and a logit link function. There was no indication of overdispersion, so the simple binomial distribution was used. Model assumptions were assessed using residual plots (after [55]).

For the primary *S. parasitica* infection (Stage 2), mortality as a function of parasite exposure (parasite vs. control), thermal pre-conditioning during Stage 1, and the ongoing thermal regimes were analysed in a three-way orthogonal (crossed) fixed factor design, using a GLM. The mortality effect of the second *S. parasitica* infection (Stage 3) was assessed in a two-way crossed GLM of experimental thermal regime and parasite re-exposure. Both models (Stage 2 and 3) were fitted using a binomial distribution with logit link function and hypothesis tests were carried out using Type II Sums of Squares Likelihood Ratio tests, using the 'car::Anova' function in R [56]. In order to identify significant differences between treatments, *post-hoc* means tests with Tukey adjusted
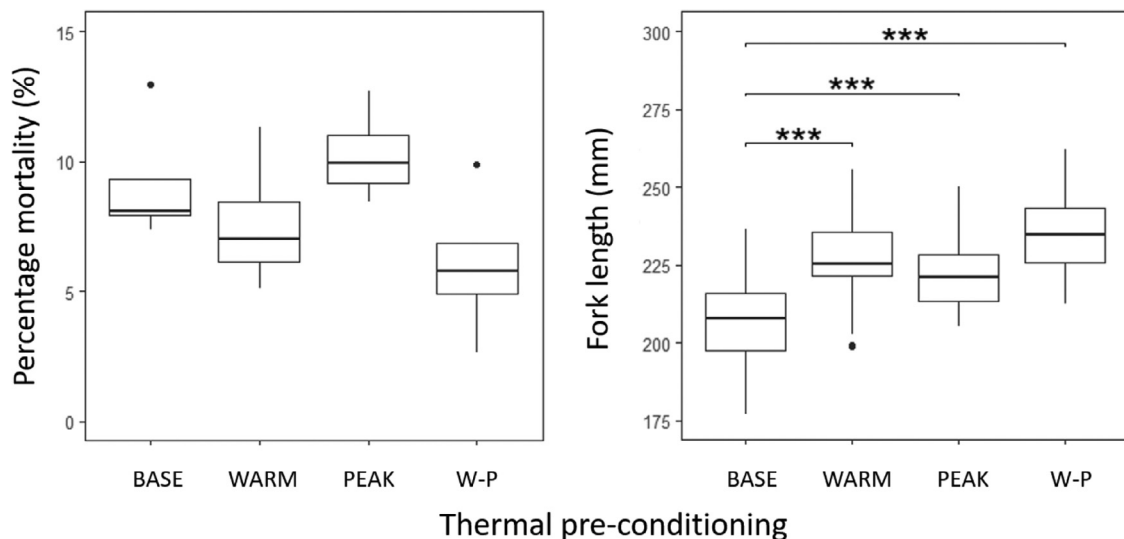
Fig. 3. Percentage of observed mortality (left) and final fork length (right) of brown trout alevins subject to the four thermal pre-conditioning scenarios in the 'variable' environment during Stage 1. The symbol '***' refers to significance level of $p < 0.001$.

Table 2

Likelihood ratio Analysis of Variance of alevin mortality with ongoing thermal regime, thermal pre-conditioning, parasite exposure and their interactions during experimental Stage 2.

	LR	Df	*p*-value	
Ongoing thermal regine	0.52	1	0.47297	
Thermal pre-conditioning	34.79	3	1.35E-07	***
Parasite exposure	61.46	1	4.52E-15	***
Ongoing thermal regime: Thermal pre-conditioning	28.91	3	2.34E-06	***
Ongoing thermal regime: Parasite exposure	2.97	1	0.0851	.
Thermal pre-conditioning: Parasite exposure	9.36	3	0.02488	*
Ongoing thermal regime: Thermal pre-conditioning: Parasite exposure	8.92	3	0.03035	*

Signif. codes: *** <0.001, * <0.05, . <0.1, >0.1.

p-values (to account for multiple comparison) using the 'lsmeans' package [57] were carried out.

All analyses were conducted using R statistical software v3.5.1 [58], and significance was accepted at $p < 0.05$.

3. Results

In Stage 1, thermal pre-conditioning under different scenarios in the 'variable' environment did not impact fish mortality (proportion dead; Likelihood ratio χ^2 4.26, df = 3, $p = 0.235$), but did significantly affect growth (fork length; Likelihood ratio χ^2 168.93; df = 3, $p < 0.0001$). All warming regimes resulted in fish growth above that in the baseline temperature, with PEAK, WARM and W-P conditions resulting in 6, 9 and 11 % significant increases respectively in body length compared to BASE (Fig. 3). There were, however, no significant differences in growth between the warming regimes.

In Stage 2, parasite exposure was the dominant cause of mortality across all treatments (Table 2) after the primary *S. parasitica* challenge. There was a weak but significant three-way interaction between pre-conditioning, ongoing temperature regime and parasite challenge, indicating they reciprocally influence each other in their effect on alevin mortality (Table 2). This three-way interaction appeared to be driven in part by low mortality in non-exposed treatments (Fig. 4a), which was not associated with any particular ongoing temperature regime or pre-conditioning. In contrast, high mortality was maintained in the W-P treatment, particularly in the parasite-exposed containers (Fig. 4a). Two-way interactions indicated significant effects of combined ongoing thermal regimes and thermal pre-conditioning on mortality. How-

Table 3

Likelihood ratio Analysis of Variance of alevin mortality with ongoing thermal regime, parasite exposure and their interactions during experimental Stage 3.

	LR	Df	p-value	
Ongoing thermal regime	65.91	3	3.20E-14	***
Parasite exposure	46.91	1	7.43E-12	***
Ongoing thermal regime: Parasite exposure	3.11	3	0.3754	

Signif. codes: *** <0.001, >0.1.

ever, only thermal pre-conditioning appeared as a significant effect on its own (Table 1). *Post-hoc* tests confirmed that fish within parasite exposed treatments in 'variable' scenarios experienced significantly higher mortality in W-P (53.3%, $p < 0.0001$), WARM (13.3%; $p < 0.05$) and PEAK (4.4%; $p < 0.0001$) compared to BASE (0%, $p < 0.0001$) (Fig. 4a). In contrast, however, for 'constant' scenarios, parasite exposed fish experienced no significant differences in mortality (BASE 26.7%; WARM 8.9%; PEAK 4.4%; and W-P 17.8%; $P > 0.05$; Fig. 4a).

In Stage 3, the effects of secondary parasite exposure and temperature regime operated independently of each other on fry mortality rates (Table 3). Parasite exposure resulted in increased mortality across all treatments (Fig. 4b), regardless of the ongoing thermal regime (BASE 0 to 20%, WARM 5 to 25.6%, PEAK 5 to 21.1% and W-P 28.6 to 85.7%). However, within thermal regimes, *post-hoc* tests identified that such increase was only significant between BASE and W-P, and mortality was most pronounced in this ongoing thermal regime.

a. Stage 2: Primary *S. parasitica* challenge

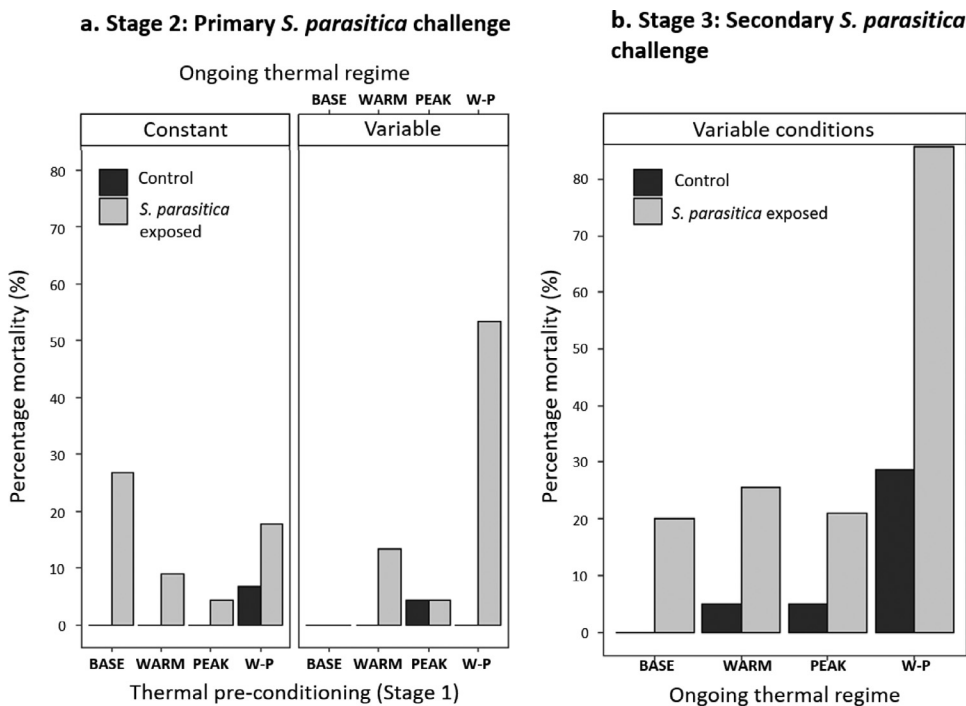**b. Stage 3: Secondary *S. parasitica* challenge**

Fig. 4. Percentage observed mortality of brown trout (a) alevins from the primary *Saprolegnia parasitica* challenge (Stage 2) within the 'constant' (left) and 'variable' (right) ongoing thermal regimes, and respective thermal pre-conditioning from Stage 1; (b) fry for each of the ongoing 'variable'thermal regimes (BASE, WARM, PEAK, W-P), from the secondary *Saprolegnia parasitica* challenge (Stage 3).

4. Discussion

This study, to the best of our knowledge, is the first to indicate how the combined effects of parasite exposure, constant and intermittent ongoing and pre-conditioning warming can impact brown trout growth and survival. Parasite exposure had the strongest and most significant effects on both alevin and fry survival, with weak but still significant effects when parasite exposure was combined with warmer and variable thermal pre-conditioning and ongoing thermal regimes. Whilst the robustness of individual findings of the experiments cannot be overstressed due to non-replication, all subsequent experiments of the study point in the same direction that the negative effects of parasite exposure on brown trout can be enhanced when additional stressors such as continuous and intermittent warming play a role.

During thermal pre-conditioning (Stage 1), survival of alevins within the combined continuous and intermittent thermal increases (W-P) was similar, but these fish grew larger than those in the other scenarios. Upon exposure to *S. parasitica* during Stage 2, alevins experienced increased mortality, particularly those exposed to the W-P ongoing thermal regime. Faster egg-to-fry development resulting from increased water temperatures has been long reported in salmonids [59,60], with comparable predicted results from intermittent water increases due to thermopeaking *e.g.* [61]. Although Ojanguren and Braña [37] observed smaller sizes and deformities in hatched embryos and alevins subject to increased water temperature, these were not observed in the present study. Despite growing larger, the alevins in W-P were exposed to an arguably higher level of thermal stress for 18 days above 11 °C, [40] than the other scenarios, which could have impacted their immune development and increased their susceptibility to parasites *e.g.* [62], explaining the higher mortality. Another explanation for mortalities in Stage 2 could be that the ongoing thermal conditions outrode the pre-conditioning effect at the time of exposure: in fact, only when alevins in Stage 2 remained within the W-P at the time of *S. parasitica* exposure did high mortality occur. This explanation aligns with previous works by Stewart et al. [54], which concluded that temperature at the specific time of *S. parasitica* exposure influences infection and immunity of three-spined sticklebacks (*Gasterosteus aculeatus*). The outcomes of Stage 2, therefore, emphasise the importance of accounting for both continuous

(*e.g.* due to climate change) and intermittent (*e.g.* due to thermopeaking) increases in water temperature interacting with parasite exposure for the assessment of brown trout alevin mortality.

In Stage 3, while the independent effects of parasite exposure and ongoing thermal regime were strong and significant on fry mortality, the combination of both was not significant. Differences between stages could be attributed to higher temperatures during Stage 2. Although fry have higher thermal tolerances than alevin brown trout [20], temperature increases within W-P scenarios were significantly higher in Stage 3 (mean 13.4 ± 0.1 °C with peaking 16.1 ± 0.1 °C; Table 1), than in Stage 2 (mean 11.2 ± 0.1 °C with peaking 13.8 ± 0.3 °C; Table 1), hinting that the thermal stress of the W-P regime alone could have been lethal.

In natural freshwater environments, *Saprolegnia* is considered ubiquitous and mostly infects wounded or otherwise immune-compromised animals [49]. Despite knowing that many fish die from this disease in aquaculture settings, quantifying the infection amongst wild fish is not easy. The potential impact of freshwater aquaculture on wild fish populations is controversial [63,64]. Transfer of *Saprolegnia* from captive fish to the surrounding environment is theoretically possible (*e.g.* [49], cited in [65]), but as yet, little research is available on the spillover and spillback of *Saprolegnia* between fish farms and natural fish populations.

In the context of a predicted warming climate and increased demand for renewable energy, hydropower developments causing hydropeaking will be promoted in the future [11], resulting in major challenges for fish and freshwater biodiversity conservation related to hydropower [51]. Both continuous and intermittent increases in river water temperatures are likely to become more common. Equally, infectious diseases are predicted to be affected in future climatic scenarios [66,67]. Thermal stress increases the susceptibility of salmonids to pathogens, including *S. parasitica* [44,68]. In addition, the effect of increased water temperatures needs to be considered in conjunction with other stressors, and in particular aquatic habitat degradation when developing management plans for brown trout populations in future climatic scenarios [69]. With prevention always being better than cure for parasites, river managers should consider climate change adaptation tools to buffer the effect of increased temperatures (*e.g.* targeted riparian vegetation plantation) and increase environmental flow regulations during hydropower

licensing *e.g.* reduce water level fluctuations during critical life stage periods, [70] as possible mitigation measures.

Climate change-induced declines in brown trout populations are likely to intensify with increased implementation of hydropower energy sources and the presence of infectious diseases. With the recognition that there are still many open questions to help advance river management strategies (*e.g.* immunological and wider genetic response of fish to temperature and parasite exposure), this study provides a first basis to scale up the potential impacts of two key stressors that brown trout faces in the wild. The outcomes of this study suggest that any assessment of the combined effects of temperature and parasite exposure in fish should consider both specific early life stages and thermal regimes, including continuous and intermittent thermal increases.

5. Conclusions

This study confirms that the combined effects of varying thermal regimes as a result of climate warming and hydropower production, and parasite exposure will increase the mortality of brown trout alevins and fry. It highlights the importance of contextualising the impact of multiple pressures in freshwater ecosystems. A realistic assessment of the combined effects of temperature and parasite exposures in fish should not only to consider a constant warming of water temperatures as a result of climate change, but also fluctuating thermal increases as a consequence of hydropower operations. Climate change-induced declines in brown trout populations are likely to intensify with the increased implementation of hydropower energy sources, and the presence of infectious diseases. Our results highlight the need for effective river management strategies in future warming scenarios aimed at protecting economically and environmentally valuable species and maintaining freshwater biodiversity.

Acknowledgments

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