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RESEARCH ARTICLE

Acclimation temperature and parasite infection drive metabolic changes in a freshwater fish at different biological scales

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Abstract

1. Environmental stressors such as elevated temperature and parasite infection can impact individual energy metabolism. However, organismal responses to co-occurring stressors and their effects across biological scales remain unexplored despite the importance of integrative studies for accurately predicting the resilience of natural populations in changing environments.
2. Using wild-caught, naturally parasitized pumpkinseed sunfish, *Lepomis gibbosus*, we quantified changes in cellular and whole-organism metabolism in response to temperature and parasite infection. We acclimated pumpkinseeds for 3 weeks at 20°C, 25°C or 30°C before measuring whole-organism oxygen uptake ($\dot{M}O_2$) using intermittent flow-respirometry to quantify maximal and standard metabolic rates (MMR and SMR, respectively) and aerobic scope (AS). We also measured the maximal activity of enzymes [citrate synthase (CS), respiratory complexes I+III and IV of the electron transport system and lactate dehydrogenase (LDH)] linked with cellular bioenergetics in fish heart, brain, spleen and gills using spectrophotometry.
3. We found no interactions between acclimation temperatures and parasite intensity on cellular or whole-organism metabolism. However, both stressors were independently related to fish metabolism, with differing impacts across biological scales.
4. Whereas MMR increased with acclimation temperature, this was not mirrored by increasing SMR or decreasing AS, suggesting thermal compensation across acclimation temperatures at the whole-organism level.
5. On a cellular level, acclimation responses were similar across organs, with maximal activity of all enzymes decreasing with increasing acclimation temperature. However, LDH activity remained higher than aerobic enzyme activities (CS, ETS complexes I+III and IV) across acclimation temperatures and organs, especially in gills, where LDH activity drastically increased at 30°C. This may indicate a stronger reliance on anaerobic metabolism to sustain whole-organism metabolic performance at high temperatures.

6. Fish with greater trematode infection had lower MMR. There were no relationships between parasite intensity and SMR, AS or maximal enzyme activity.
7. Our work shows that co-occurring stressors have distinct impacts on fish metabolism: parasites are primarily related to whole-organism metabolism while temperature impacts metabolism across biological scales. This highlights that interactions among co-occurring stressors are important for ecological realism and accurate predictions regarding population resilience to environmental changes.

KEYWORDS
cellular metabolism, ectotherms, infection, physiology, thermal acclimation

1 | INTRODUCTION

Warming water temperatures are threatening freshwater ecosystems globally (Hassan et al., 2020). Ectotherms, such as freshwater fishes, are sensitive to these shifts in ambient temperature. Warmer waters can alter the rate of cellular and whole-organism level processes, ultimately affecting an individual's capacity to perform in their environment (Guderley & St-Pierre, 2002; Schulte, 2015). To counteract the direct effects of temperature, fishes can thermally acclimate through physiological adjustments (e.g. changes in enzyme activities, organ morphology, oxygen uptake) to enable sufficient ATP production for the maintenance of metabolic performance across thermal environments (Schulte, 2015). Although the physiological responses underlying thermal acclimation are well-studied in some species and habitats, gaps in our understanding remain (Jutfelt et al., 2024).

Most studies investigating metabolism during acclimation concentrate either on changes at the organismal or sub-organismal levels. However, evidence indicates that metabolic responses might vary across levels of biological organisation (Iverson et al., 2020). Thus, there is an urgent need for research exploring metabolic rate measurements at different scales to better understand how ectotherms cope with variations in their environment. At the whole-organism level, elevated temperatures can increase an organism's standard metabolic rate (SMR, i.e. the minimum energy required to maintain basal physiological demands at a given temperature) and maximum metabolic rate (MMR, i.e. the maximum rate of aerobic energy expenditure). Variations in SMR and MMR directly affect an organism's aerobic scope (AS), calculated as the difference between SMR and MMR, which represents an organism's capacity to aerobically perform fitness-enhancing activities that require energy, such as growth, digestion and reproduction (Fry, 1971).

At the sub-cellular level, temperature acclimation involves quantitative changes in the production and activity of enzymes (Somero, 2004). In response to elevated temperature, the activity levels of aerobic and anaerobic metabolic enzymes, such as citrate synthase (CS) and lactate dehydrogenase (LDH), which fishes use to produce energy via glycolysis, may be altered to meet the increased energy demands associated with metabolic adjustments triggered by temperature changes (Grim et al., 2010; Ressel et al., 2022; Soengas & Aldegunde, 2002). Enzymes related to the electron transport system complexes I+III and complex IV (cytochrome *c* oxidase; CCO) are also subjected to similar changes due to their role in oxidative phosphorylation (OXPHOS), a merging point where metabolic pathways interact to support energy production (Blier et al., 2014). For instance, studies on warm-acclimated fishes found that rainbow trout (*Oncorhynchus mykiss*) decrease CCO activity as acclimation temperature increases and common killifish (*Fundulus heteroclitus*) acclimated to high temperatures (33°C) have altered electron transport system performance (Chung et al., 2017; Kraffe et al., 2007). Variation in reaction rates can also occur among organs in response to thermal acclimation (Chung et al., 2017; Cominassi et al., 2022). For example, in the skeletal muscle of three-spine stickleback (*Gasterosteus aculeatus*), the maximal activity of CS and LDH was equivalent among acclimation groups (5°C, 12°C, and 20°C) (Cominassi et al., 2022). However, stickleback acclimated at 20°C showed higher maximal activity of CS and LDH in the liver compared to fish acclimated at 5°C and 12°C. Despite mounting evidence suggesting organ-specific responses to acclimation, studies looking for general patterns across many organs remain scarce. Such studies are especially important for work on wild animals, where organs may differ in their response to acclimation because of interacting effects with biotic stressors.

Among biotic stressors, parasites are a critical part of all ecosystems, but are often an overlooked ecological component despite their pervasive effects on host performance (Chrétien et al., 2023; McElroy & de Buron, 2014). Parasite infections can alter host metabolic rates, organ mass and the availability of metabolic substrates (Nadler et al., 2021; Ryberg et al., 2020). Furthermore, recent studies on wild-caught parasitized pumpkinseed sunfish (*Lepomis gibbosus*) have revealed substantial variability in metabolic enzyme activity across different organs (Mélançon et al., 2023; Sabbagh et al., 2024). Parasites may also influence individual acclimatory responses to temperature. For instance, the bacterial pathogen, *Pasteuria ramosa* reduces the heat tolerance of *Daphnia magna* hosts (Hector et al., 2024). To date, no studies on wild animals have included parasites in investigating the physiological responses to thermal acclimation across different biological scales. Therefore, whether and how parasitism relates to an organism's metabolic response to temperature acclimation remains an open question.

Here, we explored how thermal acclimation and parasitism affect the metabolism of pumpkinseed sunfish at different levels of biological organisation (cellular and whole-organism). Pumpkinseeds host several endoparasite species, including cestodes, nematodes and trematodes (e.g. *Uvulifer* sp., *Apophallus* sp., i.e. black-spot disease) (Margolis & Arthur, 1979). Our study addressed the following questions: (1) Does thermal acclimation and/or parasites alter whole-organism metabolic rates? If organismal metabolic rates increase with increasing temperature in response to modified enzyme kinetics, we predict that organisms will show higher metabolic rates at higher temperatures to support increased energy demands. A similar prediction is made for parasites if parasite intensity causes an increase in maintenance cost caused by parasite-induced damage. Otherwise, if parasites impact metabolically active tissues, we predict a decrease in whole-organism metabolism as parasite intensity increases. (2) Does metabolic enzyme activity vary in response to thermal acclimation and/or parasites? If, in response to temperature and parasite, there are changes in cellular membranes (e.g. fluidity; integrity) (Daniel et al., 2009), we predict that enzymes will show a decreased activity. (3) If so, are there any organ-specific patterns in enzymatic activity related to temperature and/or infection? Given that temperature and parasites affect organs and metabolic enzymes in different ways (Cominassi et al., 2022; Mélançon et al., 2023), we predict organ response to vary according to its metabolic function.

2 | MATERIALS AND METHODS

2.1 | Fish collection and husbandry

In June 2022, we captured 120 wild pumpkinseed sunfish in Lake Cromwell located near the Université de Montréal's Station de Biologie des Laurentides (Canada, 45.98898° N, −74.00013° W). Fish were captured with baited minnow traps placed in the lake's littoral zone. Selected fish were transferred in opaque containers filled with lake water aerated by a transportable air bubbler. Upon arrival at the SBL laboratory housing facilities (15 min walk), fish were placed in a saltwater bath (3 g/L) for 7 days to reduce the risk of fungal and bacterial infections. All salt bath tanks were 40 L (10–15 fish per tank), with water maintained at 18°C (i.e. water temperature at capture). Water was aerated, changed and siphoned twice a day to maintain water quality. Post-quarantine, fish were weighed, measured and uniquely tagged with visible elastomer implants (VIE; Northwest Marine Technology) on both sides of the dorsal fin with a 29-gauge needle. Fish were distributed among three 600 L flow-through holding tanks (215 × 60 × 60 cm, length × width × height) separated into three sections. Fish were randomly assorted into groups of 10–16 fish per section, with a total of 40 fish per holding tank. In each section, artificial plants and PVC refuges were used for environmental enrichment. Water entering the flow-through tanks was sourced from a nearby lake (Lake Croche; 45.99003° N, −74.00567° W) with a water profile similar to Lake Cromwell (Vigneault, 2024). Water was particle-filtered and UV-sterilised before entering the tanks. The light photoperiod followed the summer light–dark cycle (14L:10D). Fish were fed ad libitum daily with bloodworms.

All procedures carried in this study were approved by the Université de Montréal Comité de Déontologie de l'Expérimentation sur les Animau animal ethics committee (Permit number 22-025) with fishing permits granted by the Ministère des Forêts, de la Faune et des Parcs (permit number: SEG#2022-05-16-1971-15-S-P).

2.2 | Acclimation treatments

Environmentally relevant acclimation temperatures were selected based on water temperature data collected from June to September 2021 with a temperature logger (HOBO Pendant MX 2201, USA) placed at a 1-meter depth in the littoral zone of Lake Cromwell (Figure S1): 20°C (early summer temperature), 25°C (mid-summer temperature) and 30°C (climate warming projection), which is 2°C higher than the highest temperature recorded during the summer of 2021 (Figure S1). We increased water temperature in holding tanks 24 h after the tagging procedure (1.5°C day $^{-1}$). Temperature was maintained with heating (1500 and 2000 W heaters, GESAIL) and cooling systems (EK20 immersion cooler, Thermo ScientificTM, USA) connected to a temperature controller (ITC-308S, Inkbird) (Figure S2). We also monitored the water temperature twice a day with a digital thermometer (Hanna HI98509 *Checktemp 1* Digital Thermometer). Each tank had a constant inflow of aerated water with a replacement rate ranging from 4.8 to 22.8 L/h. Air bubblers and submersible pumps (ECO-396, EcoPlus®, China) were placed throughout the tank to ensure water mixing and enhance air saturation. Fish had similar body sizes across acclimation temperatures (mean ± SD Total length = 8.96 ± 0.89 cm at 20°C, 8.42 ± 2.23 at 25°C and 8.5 ± 0.67 at 30°C) and were left to acclimate for at least 3 weeks to allow thermal compensation (Dent & Lutterschmidt, 2003).

2.3 | Whole-organism metabolic rates

We measured the fish's oxygen uptake rate ($\dot{M}O_2$; mg O_2 h^{-1}) using intermittent-flow respirometry. We followed the guidelines described by Killen et al. (2021). Each day, we measured between 8 and 12 fish from the same acclimation temperature in individual acrylic respirometry chambers placed in 80 L water-controlled baths. Detailed information regarding the water-recirculation system and temperature control can be found in Table S1. Each respirometry chamber was opaque, with a top viewing window to minimise disturbances. A Firesting fibre optic oxygen meter (Firesting 4-channel oxygen meter, PyroScience GmbH, Aschem, Germany) located in the recirculation loop measured the dissolved oxygen concentration every 3 s.

Fish were fasted for 24 h before the start of the trial. Each trial started between 15:00 and 18:00, with an estimate of the maximum metabolic rate (Peak $\dot{M}O_{2recovery}$; hereafter MMR). Each fish was placed in a circular arena (31 cm diameter) filled with 8 L of

water from the holding tank. Fish were manually chased for 3 min and exposed to air for 1 min before being transferred to the respirometry chamber (Roche et al., 2013). $\dot{M}O_2$ was continuously measured for ~15 min to estimate MMR, defined as the upper limit of the ability of the fish to perform aerobic metabolism. Afterwards, fish remained in the chamber between 19 and 22 h to estimate their standard metabolic rate ($\dot{M}O_{2min}$; hereafter SMR), defined as the minimum energy required to maintain basal physiological demands.

MMR was analysed using the respR package in R (Harianto et al., 2019). We used the rolling regression method with a 2-min window width over ~15 min to determine the highest rate of $\dot{M}O_2$ (Prinzing et al., 2021). We discarded the first minute of data after the respirometers were sealed to allow proper mixing of the water in the chambers. Fish's SMR estimates were analysed using the FishResp package in R (Morozov et al., 2019). We included all overnight measurements taken from the point where $\dot{M}O_2$ stabilised (~6/7 h). SMR was calculated as the mean of the lowest 20th percentile with a minimum R^2 for each slope set at 0.95 (Chabot et al., 2016). We then subtracted SMR from MMR to calculate the aerobic scope (AS). We measured background respiration on empty chambers for three 10-minute cycles at the trial's start and end. We subtracted averaged values from all fish respiration measurements, assuming a linear increase in microbial respiration. To limit bacterial proliferation, we cleaned the all apparatus with hydrogen peroxide (H_2O_2) and warm water every second day and left them to dry in sunlight. At the end of each trial, we removed fish from the chamber and euthanized them with an overdose of 10% eugenol (4 mL L^{-1} water). Fish were immediately measured, weighed to the nearest gram, and frozen at −18°C until organ sampling and parasite screening.

2.4 | Tissue sample preparation and parasite screening

Fish heart, brain, spleen and gills were dissected on ice to prevent cellular degradation (Stemi DV_4, Zeiss, Germany). Each organ was weighed (MSE225S, Sartorius Weighing Company, Germany), diluted at 20 times their wet weight with a buffer solution (100 mM potassium phosphate, 20 mM EDTA, pH 8.0; modified protocol from Hunter-Manseau et al., 2019), homogenised with a polytron (PT1200) and immediately stored at −80°C.

Following organ removal, we screened for parasites that had infected the fish in the lake before capture. For each fish, we counted first the total number of encysted black spots (metacercaria) present on the body, fins, muscles and gills on both sides of the fish. We found that 100% of the fish were infected by black spot but with varying intensity across acclimation temperatures (20°C: median = 333, range = 26–1057; 25°C: median = 368, range = 13–1127 and 30°C: median = 259, range = 9–1314) (Figure 1a). Fish digestive tract, liver and gonads were dissected to screen for other endoparasites. Infection by bass tapeworms (*Proteocephalus ambloplitis*) was

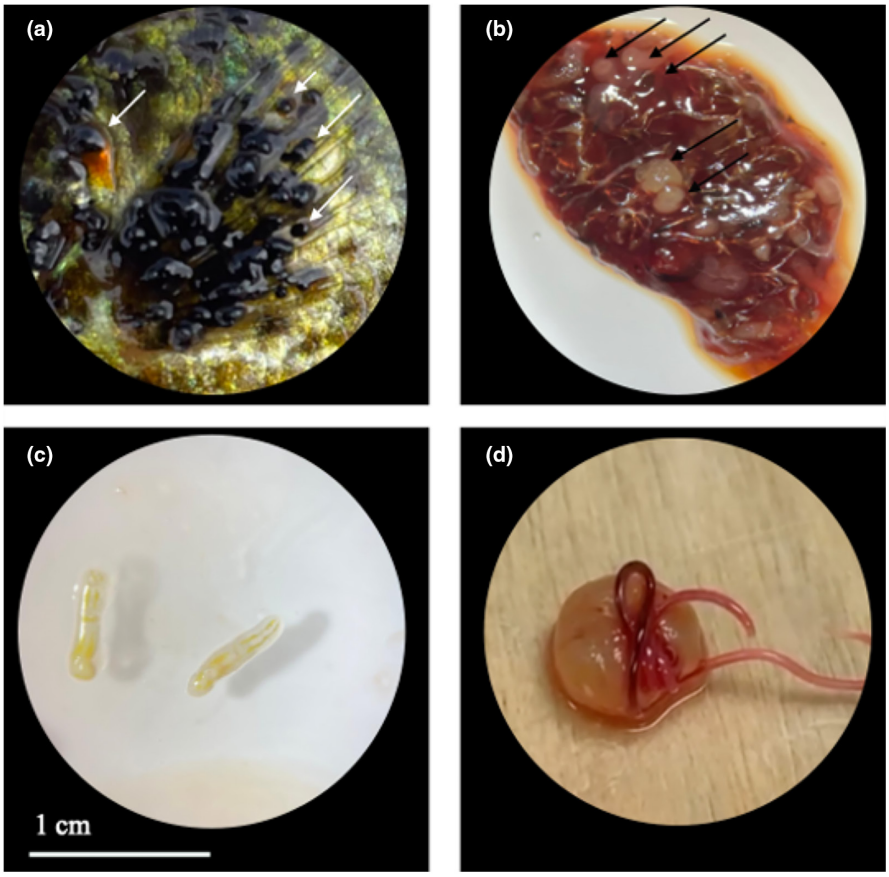

FIGURE 1 Pictures of the four parasite species found in sampled pumpkinseeds: (a) Black spot trematodes (*Uvulifer* sp., *Apophallus* sp.) shown on the pectoral fin of a pumpkinseed sunfish and pointed at by white arrows. Each black dot represents an individual parasite. (b) Bass tapeworms (*Proteocephalus ambloplitis*) encysted in the liver and pointed at by black arrows. (c) Yellow grub trematodes (*Clinostomum marginatum*). (d) Nematode larvae emerging from its eggshell. Scale (bottom left) applies to all panels of the figure.

found in the liver of all fish held at 25°C and 30°C (median = 43, range = 2–301 and median = 52, range = 1–301 respectively) while 99% of the fish held at 20°C were infected (median: 28, range: 0–213) (Figure 1b). Forty fish were also infected by yellow grub trematodes (*Clinostomum marginatum*) found encysted in muscles, close to the heart, in the cranium and encysted in the operculum and gill filaments (prevalence of 42.5% at 20°C and 25°C (median = 0, range: 0–14 and median = 0, range = 0–9 respectively) and 17.5% at 30°C (median = 0, range: 0–6)) (Figure 1c). We excluded arches and filaments where yellow grub and black spot trematodes were found in our tissue analyses to avoid parasite contamination in enzyme activity measurements. We also found nematode larvae in the body cavity of two fish acclimated at 25°C and four fish acclimated at 30°C (5% and 10% prevalence, respectively with for both median = 0 and range = 0–1) (Figure 1d). These parasites are hereafter grouped under the term internal parasites. Finally, we corrected each fish's mass by the combined parasites' mass (Lagrue & Poulin, 2015). We used the mass calculation for bass tapeworm and yellow grub determined by Guitard et al. (2022) and weighed each nematode individually. The fish mass correction did not account for black spots due to their negligible weight (<0.000001 g). For one fish acclimated at 25°C and one fish acclimated at 30°C, we were not able to precisely quantify the internal parasite intensity due to the extremely high parasite aggregation in their livers. For both fish, we attributed the same number of cestodes as the highest number counted in our sample (301 cestodes), which is an underestimate of the parasite intensity in these individuals.

2.5 | Mitochondrial enzymatic activity

We measured enzymatic activities on four key organs (heart, brain, spleen and gills). The cardiovascular system was selected because water temperature is known to strongly affect its mitochondrial functions (Chung et al., 2017), while little is known about how parasites may impact these relationships (but see Mélançon et al., 2023). We selected the spleen (lymphoid organ) for its critical role in immune activation in fish infected by helminth parasites (Zapata et al., 2006). Finally, we selected the brain because parasites and temperature can both influence cellular activity (Chung et al., 2017; Nadler et al., 2021).

We measured enzyme activity via spectrophotometry using a microplate reader (Mithras LB940 microplate reader, Berthold Technologies, Germany) and its associated software (MikroWin 2010, Labsis Laborsysteme, Germany). Protocols were adapted from Thibault et al. (1997), Hunter-Manseau et al. (2019) and Mélançon et al. (2023). Each measurement was performed in duplicate and in a temperature-controlled room set at the acclimation temperature of the fish. Enzymatic activities were normalised for protein concentration (mg mL^{-1}), determined with the bicinchoninic acid assay (BCA) method (Smith et al., 1985). Due to the limited amount of tissues for some fish and organs, we were not able to measure all enzymatic activities in all organs. A detailed description of each assay protocol is provided as Supporting Information (see Appendix S1; Table S2) and details on replication are shown in Table 1.

2.6 | Data analysis

All data were analysed using R v.4.3.1 (R Core Team, 2022). First, we used linear mixed effects models to test whether acclimation temperature and parasite intensity were related to a fish's whole-organism metabolic traits (MMR, SMR, and AS). In all three models, we log-transformed metabolic variables (response variables) while acclimation temperature, internal parasites, black spot and log-transformed mass (parasite-corrected fish mass) were included as fixed effects and experimental day as a random effect. We included an interaction between each parasite group and acclimation temperature. Candidate models were selected based on Akaike's information criterion (AIC) (Burnham et al., 2011). In all cases, the final model included the fixed effects of acclimation temperature, black spot intensity, internal parasite intensity and fish mass as well as the interaction between acclimation temperature and internal parasite. We tested variables present in the final models for both collinearity and variance inflation (VIF; car package). We used post hoc tests for pairwise comparisons between the means of acclimation temperature groups (*emmeans* package; Lenth, 2018). A visual inspection of the diagnostic plots ensured that all assumptions were met for each selected model. Second, we used a multivariate linear regression model as our measurements of enzyme activity are highly correlated due to the fact that we took multiple measurements within each organ. We built one model per enzyme and included a matrix of organs (heart, brain, spleen, gills) bound as a multivariate response variable. For each organ, we log transformed enzyme activity. Acclimation temperature, internal parasite, black spot and the interaction between each parasite group and acclimation temperature were included as fixed effects. We used the mvIC package (Hoffman, 2022) for model selection. Final models included only the fixed effects of acclimation temperature, internal parasite and blackspot intensity for each enzyme normalised for protein content.

3 | RESULTS

3.1 | Whole-organism metabolic rate

There was no significant interaction between acclimation temperatures and internal parasite count on MMR, SMR or AS (Table S3). Acclimation temperature positively affected MMR ($F_{2,112} = 17.832$, $p < 0.001$) with MMR increasing between 20°C and 30°C and between 25°C and 30°C (Figure 2A; Table S3). Acclimation temperatures also had an effect on SMR ($F_{2,112} = 22.745$, $p < 0.001$; Table S3), which significantly increased from to 20°C to 25°C and subsequently decreased from 25°C to 30°C, resulting in no detectable variation in SMR between 20°C and 30°C (Figure 2A; Table S3). These changes in MMR and SMR translated into

TABLE 1 Replication statement for the experimental design examining how parasite and acclimation temperature affects fish metabolism at different biological scales.

	Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Whole-organism metabolism	Individuals	Individuals assayed at their acclimation temperature (20°C, 25°C or 30°C)	The level of replication was 40 individuals per acclimation temperature
Cellular-metabolism	Individuals	Organs (heart, brain, spleen and gills) assayed at the acclimation temperature fish were acclimated at	The level of replication varied per organs and temperature due to limited amount of tissues (for detailed information Table S2)

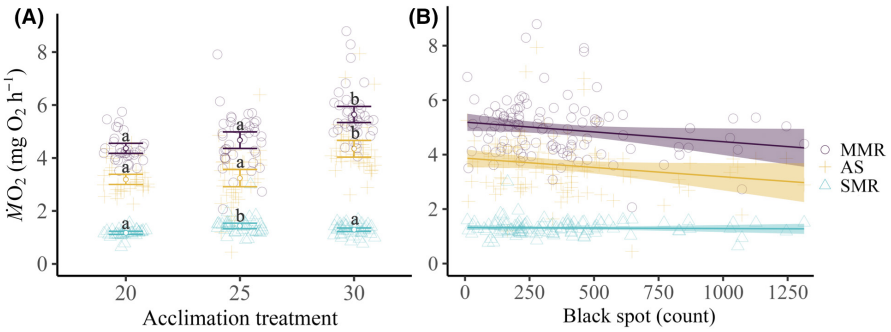

FIGURE 2 Whole-organism metabolic rates ($\dot{M}O_2$) as a function of (A) acclimation temperature (B) and black spot counts in pumpkinseed sunfish ($n=120$). Circles (purple) represent maximum metabolic rate (MMR), triangles (blue) represent standard metabolic rate (SMR), and crosses (yellow) represent aerobic scope (AS). (A) For the relationship between metabolic rates (MMR, SMR, AS) and acclimation temperature, white points and error bars represent each acclimation temperature's mean and confidence interval. Different letters denote significant differences across acclimation temperatures ($p<0.05$). (B) Lines represent linear regressions for each metabolic trait as a function of parasite intensity. The shaded areas represent the upper and lower 95% confidence intervals. For visual representation, all metabolic rates were standardised to the mean fish mass (11.46 g) using a log–log scale and taking the residuals expressed in relation to the average mean body mass. Individual body mass is included as a co-factor in the statistical analysis.

significant differences in AS in relation to acclimation temperature ($F_{2,112}=14.143$, $p<0.001$). Fish acclimated at 30°C had significantly higher AS compared to both lower temperatures (Figure 2A; Table S3). Internal parasite intensity was not related to any of the three metabolic traits (MMR: $F_{1,112}=0.114$, $p=0.734$; SMR: $F_{1,112}=1.988$, $p=0.158$; AS: $F_{1,112}=0.006$, $p=0.935$). Black spot count negatively correlated with MMR ($F_{1,112}=4.564$, $p=0.032$) but not with SMR ($F_{1,112}=1.122$, $p=0.289$) and AS ($F_{1,112}=2.426$, $p=0.119$) (Figure 2B). All three metabolic traits were positively related to fish body mass (Table S3; Figure S3).

3.2 | Mitochondrial enzymatic activity

Acclimation temperature altered the maximal activity of enzymes related to aerobic metabolism (CS: $F_{2,80}=8.782$, $p<0.001$; ETS: $F_{2,89}=10.780$, $p<0.001$; CCO: $F_{2,80}=9.343$, $p<0.001$) and anaerobic metabolism (LDH: $F_{2,101}=18.351$, $p<0.001$). For all enzymes, we observed organ-specific patterns in response to acclimation temperature (Figures 3 and 4).

All three aerobic enzymes showed no relationship between maximal heart activity and acclimation temperature (Figures 3A–D and 4; Tables S4, S6 and S7), while heart LDH anaerobic enzyme activity was altered in response to acclimation temperature ($F_{2,101}=3.093$, $p=0.049$). There was a significant difference in mean LDH activity between 20°C and 30°C only ($t=-2.171$; $p=0.026$) (Figure 3E).

Acclimation temperature altered brain CS, CCO and ETS maximal activity (Tables S4, S6 and S7). Maximal activity in CS and ETS decreased significantly between 20°C and 30°C (CS: $t=-2.907$; $p<0.001$ and ETS: $t=-4.154$; $p<0.001$) and between 25°C and 30°C (CS: $t=-2.000$, $p=0.044$; ETS: $t=-5.987$, $p<0.001$), but not between 20°C and 25°C (CS: $t=-0.928$; $p=0.500$ and ETS: $t=1.806$; $p=0.077$) (Figures 3B and 4B). CCO maximal activity decreased significantly between 20°C and 25°C ($t=-6.086$, $p<0.001$) and between 20°C and 30°C ($t=-5.628$, $p<0.001$) but not between 25°C and 30°C ($t=0.191$, $p=0.970$; Figure 3F). Anaerobic enzyme LDH showed no difference in activity among acclimation temperatures in the brain (Figure 3F; Table S5).

Spleen ETS maximal activity was significantly altered by acclimation temperature with a significantly lower activity at 20°C compared to both 25°C and 30°C (Figure 4D; Table S6). For spleen LDH maximal activity, there were significant differences among all acclimation treatments (20°C–25°C: $t=-4.818$; 20°C–30°C: $t=-7.607$; 25°C–30°C: $t=-2.959$; all $p<0.001$; Figure 3H) with activity decreasing at higher temperatures treatments (Figure 3H; Table S5). For spleen CS, there were differences in the maximum activities between groups 20°C and 25°C ($t=-5.058$; $p<0.001$) and between 20°C and 30°C ($t=-6.061$; $p<0.001$), but not between 25°C and 30°C ($t=-1.476$; $p=0.128$; Figure 3D).

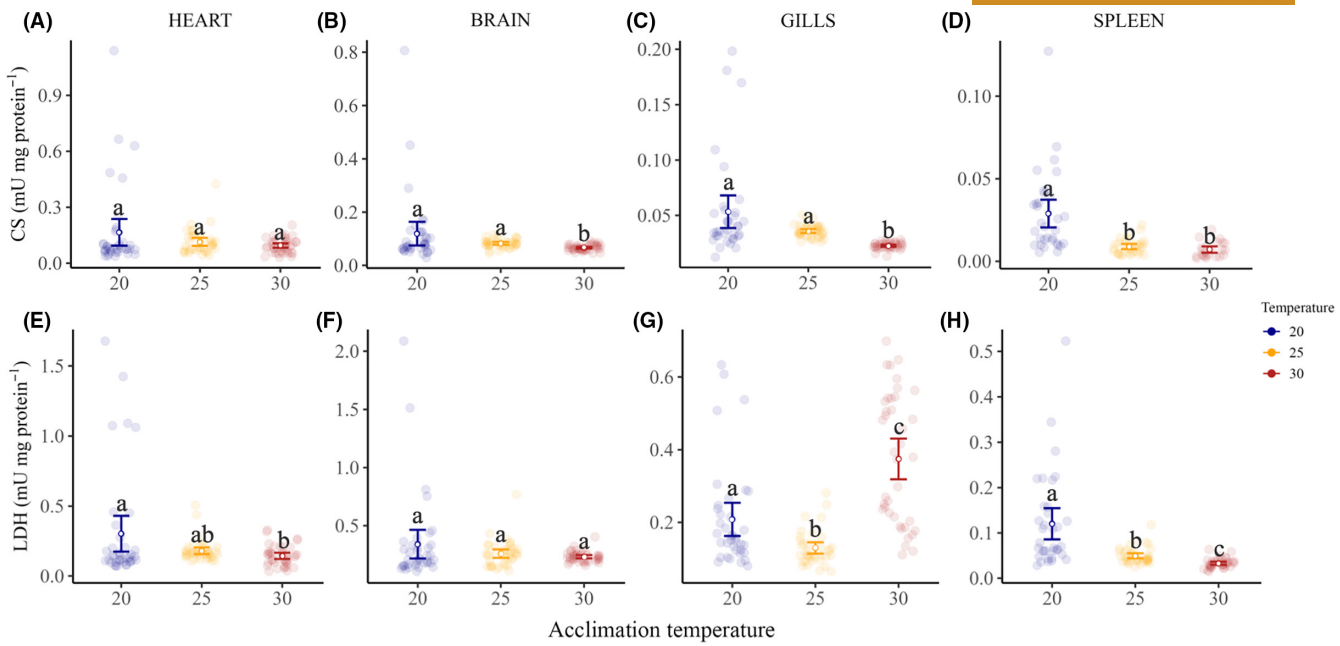

FIGURE 3 Maximal activity of citrate synthase (CS) and lactate dehydrogenase (LDH) of heart (A, E), brain (B, F), gills (C, G) and spleen (D, H) tissues sampled from pumpkinseed sunfish acclimated at three temperatures. Each panel's error bars represent confidence intervals and white points represent means. Each coloured point represents the enzyme activity normalised for protein content of a single fish tested at its acclimation temperature, with blue coloured points representing fish acclimated and tested at 20°C, orange at 25°C and red at 30°C. Lowercase letters on top of the error bars represent the significant differences.

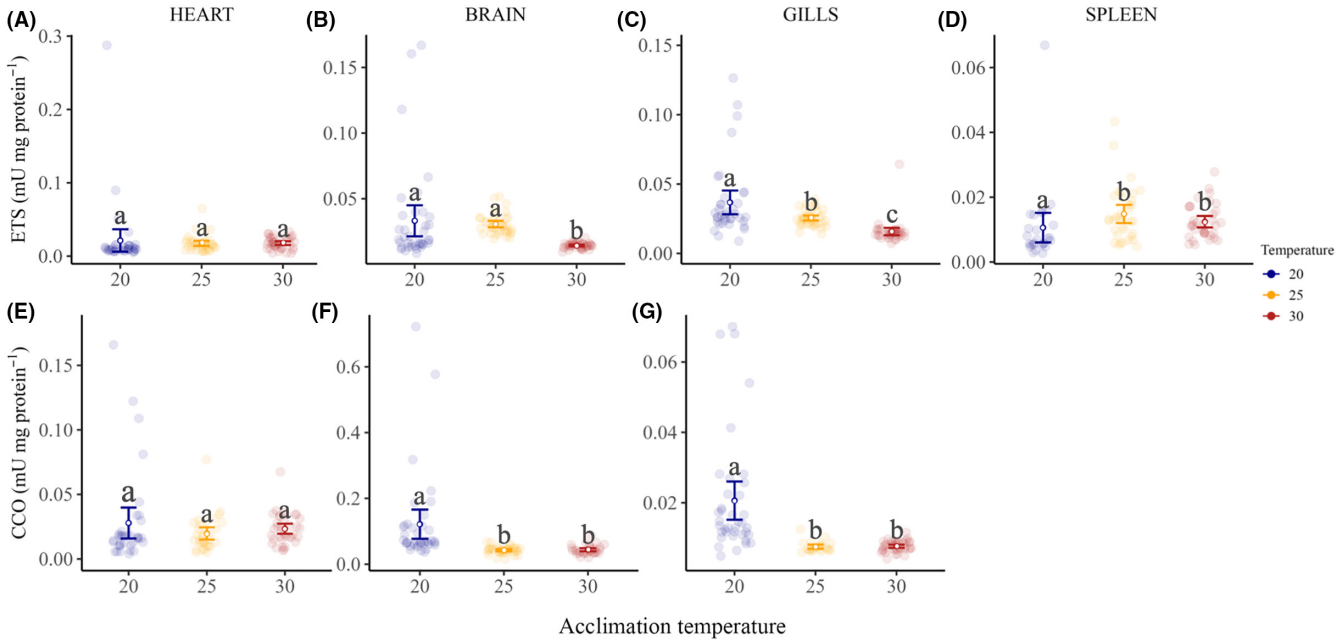

FIGURE 4 Maximal activity of electron transport system (ETS) and cytochrome *c* oxidase (CCO) of heart (A, E), brain (B, F), gills (C, G) and spleen (D) tissues sampled from pumpkinseed sunfish acclimated at three temperatures. Each panel's error bars represent confidence intervals, and white points represent means. Each coloured point represents the enzyme activity normalised for protein content of a single fish tested at its acclimation temperature, with blue coloured points representing fish acclimated and tested at 20°C, orange at 25°C and red at 30°C. Lowercase letters on top of the error bars represent the significant differences.

Acclimation temperature significantly affected gill CS, ETS and CCO maximal activity (all enzymes: $p < 0.001$; Tables S4, S6 and S7). ETS and CCO enzyme activities were significantly different between 20°C and 25°C (ETS: $t = -2.012$ and CCO: $t = -5.781$; $p < 0.05$ for both) and between 20°C and 30°C (ETS: $t = -6.809$ and CCO: $t = -6.650$; $p < 0.001$ for both) but not between 25°C

and 30°C (ETS: $t = -4.904$, $p = 0.646$; CCO: $t = 0.079$, $p = 0.994$; Figure 4C,G). Gill CS activity was significantly higher in 20°C acclimated fish than in 30°C acclimated fish ($t = -6.302$; $p < 0.001$), but there were no significant differences in the maximal CS activities between 20°C and 25°C ($t = -1.911$; $p = 0.057$), or between 25°C and 30°C ($t = -4.476$; $p = 0.994$; Figure 3C). Gill LDH maximal activity was also altered by acclimation temperature with higher activity in 20°C than in 25°C acclimated fish ($t = -2.705$; $p = 0.003$). The maximal activity in gill LDH was also significantly higher in 30°C acclimated fish compared to both 20°C and 25°C acclimated fish (20°C–30°C: $t = 4.589$; 25°C–30°C: $t = 7.329$; all $p < 0.001$; Figure 3G).

Despite a positive trend, internal parasite intensity was not significantly related to gill LDH maximal activity ($F_{1,101} = 3.665$; $p = 0.058$; Table S5). Internal parasite intensity was not significantly related to maximal enzyme activity in CS, ETS, CCO and LDH in all organs. Similar results were found for black spot intensity (see Tables S4–S7).

4 | DISCUSSION

Our objectives were to determine the effects of acclimation temperature, parasite infection and their interaction on host fish metabolism at the cellular and whole-organism levels. While we found no evidence of interacting effects between acclimation temperature and parasite intensity, thermally acclimated fish showed whole-organism thermal compensation where fish MMR increased across acclimation temperatures but not SMR, resulting in increased AS. Black spot infection showed a different pattern, with MMR being negatively correlated with increasing black spot intensity, while there was no detectable link between black spot intensity and SMR or AS. Moreover, we found no evidence of altered cellular metabolism (enzymatic activities) in response to parasite intensity. Acclimation temperature had a significant effect on enzyme activity with a higher activity of enzymes linked to anaerobic metabolism (LDH) compared to ones linked with aerobic energy production (CS, ETS, CCO). Overall, these results emphasise that parasites and temperature, both essential elements of the biotic and abiotic environment, can have differing effects on components of the metabolic phenotypes of fish at different biological scales.

4.1 | Physiological consequences of parasite infection

We found no evidence for a relationship between SMR and blackspot infection (Figure 2B), which aligns with other studies suggesting that established trematode infection has no measurable maintenance costs to hosts (du Toit et al., 2024; Guitard et al., 2022; Nadler et al., 2021). However, fish in our study were naturally infected, and thus, we cannot establish a causal link between parasite intensity and the metabolic traits measured. Interestingly, Thambithurai et al. (2022) studied the relationship between metabolic traits and parasite density in three interconnected lakes varying in infection prevalence and intensity. They found a positive relationship between SMR and parasite density where individuals with no or low infection levels displayed lower SMR. This suggests that there may be a threshold of infection above which impacts on host metabolism are apparent. Indeed, Lemly and Esch (1984) found impacts on bluegill sunfish body condition and overwinter survival when black spot infection exceeded 50 cysts. By comparison, we had a median number of 305 black spots on fish in our study, suggesting that our heavily infected population may indeed be experiencing parasite-induced alterations to metabolic traits that are decoupled from individual infection intensity. Contrary to SMR, there was a negative relationship between black spot infection and MMR (Figure 2B). Fish with low MMR may be more susceptible to infection since this trait is associated with reduced swimming capacity (Norin & Clark, 2016). Infection can induce lethargy in hosts as immune activation increases energy demands (Levet et al., 2024; Lopes et al., 2021). Thus, infection itself may cause decreased metabolic performance as a result of altered energy allocation. A recent study on pumpkinseed suggests that individual movement behaviours are both predictors of infection susceptibility and are altered following infection, highlighting the complicated nature of host–parasite effects on behaviour and physiology (Gradito et al., 2024). Further work quantifying metabolic traits pre- and post-natural infection is needed to establish causality and determine adaptive changes of metabolic phenotype in response to infection.

Internal parasite intensity was not related to any of the metabolic traits measured at the whole-organism level (Table S3). The level of infestation in our study (median: 37 parasites per fish) exceeds the intensity observed previously in similar-sized pumpkinseed from the same population (median: 15 parasites per fish), where metabolic rates were found to decrease with increasing parasite intensity (Guitard et al., 2022). This discrepancy may be explained by the amount of time fish spent in the laboratory environment (3–5 days vs. 1 month in this study). Indeed, physiological differences between wild and captive animals are well-known (Turko et al., 2023). Any physiological effects induced by infection may have been masked in our fish by the time they were tested. Therefore, it is possible that our results diverged from previous studies because the host's energy demands were altered in the holding conditions. Further investigations should be made to determine how host energy demands change over time in captive parasitized fish.

Similar to results described in Mélançon et al. (2023), we observed no differences in maximal enzyme activity in relation to either black spots or internal parasite intensity (Tables S4–S7). However, some parasites have been shown to impact cellular metabolism: brain-infecting trematode *Euhaplorchis californiensis* alter California killifish *Fundulus parvipinnis* neuronal signalling through an effect on LDH levels in the brain (Nadler et al., 2021). Recent work on several populations of pumpkinseed from lakes with different infection prevalence and intensity found that cestode density was related to increased maximal LDH activity in the liver, the location of

the infection (Sabbagh et al., 2024). Interestingly, the directionality of the differences observed varies with fish populations which included an uninfected fish population. Here, we included neither the liver nor the muscles (both sites of parasite infection) in our study to avoid the risk of measuring enzyme activity coming from the parasites. However, a recently developed protocol testing enzyme activity before and after the removal of parasites from infected organs showed no evidence for contamination in fish enzyme activity from parasite tissue (Pepin et al., unpublished data). Therefore, a future direction could be to integrate measurements of organs where parasite infection is located as the cellular response may differ in those organs because of inflammation, tissue dysfunction and necrosis caused by parasite presence.

4.2 | Aerobic performance across acclimation temperatures

Aerobic scope (AS) significantly increased across acclimation temperature as a result of increased MMR with no detectable changes in minimum energy demands (SMR) (Figure 2A). Such results indicate that fish were able to compensate across thermal regimes. A recent study demonstrated that the critical thermal limit of pumpkinseed from the same population as the one studied here averaged 35.58°C, 38.66°C and 40.65°C after 3 weeks of acclimation at 20°C, 25°C, and 30°C respectively (De Bonville et al., 2024). Thus, the metabolic traits were measured on our fish at temperatures well below their critical thermal limit. This may explain why at temperatures above their natural thermal range (30°C), they were able to counteract the direct effect of elevated temperature on metabolism and sustain aerobic performance (Figure 2A). Chronic exposure to elevated temperature can cause a state of physiological stress which ultimately compromises fish fitness and survival (Alfonso et al., 2021). Thus, it is possible that the thermal compensation observed in our study may come at the cost of decreased growth rate or reproductive success despite fish being well-below their critical thermal limit. Due to the potential implication for fish population dynamics, future work should consider integrating chronic exposure to elevated temperature with co-occurring environmental stressors to better understand physiological changes that impair survival in nature.

Sub-organismal levels of biological organisation are more directly influenced by thermodynamic laws, and our results found evidence for altered maximal activity for enzymes related to aerobic pathways, namely CS, ETS, and CCO (Figures 3A–D and 4A–G). Long-term exposure to acclimation temperatures can reduce enzyme activities in the tricarboxylic acid (TCA) cycle, alter glycolysis, and lower enzyme sensitivity to temperature change (Ekström et al., 2017; Pichaud et al., 2019). CS is part of the TCA cycle, which is a central aerobic pathway for producing ATP, while CCO is assumed to be a key regulator of electron flow and thus to control OXPHOS capacity in ectotherms (Blier et al., 2014). As acclimation temperature increased, mitochondrial density decreased in the brain, spleen and gills, reducing the number of mitochondria available to carry out OXPHOS. Downregulation of enzyme activity in response to increasing temperature may indicate organisms' capacity for metabolic reorganisation by optimising energy production to sustain energetic demands as observed at the whole-organism level (Figure 2A). It can also be a sign of reduced efficiency as temperature can alter substrate availability and, thus, the catalysing rate of enzymes. Furthermore, it may reflect a shift in metabolic pathways away from aerobic metabolism and towards alternative energy-producing pathways suggesting that acclimation causes a shift in the way energy is produced.

Fish held at 30°C had significantly higher gill LDH activity compared to lower acclimation temperatures, suggesting a strong reliance on anaerobic metabolism to produce energy at elevated temperatures. Gills remodelling is an acclimation strategy occurring in response to temperature and oxygen variations (Bowden et al., 2014; Johansen et al., 2021). For instance, prolonged exposure to elevated temperatures and lower oxygen availability in the environment may cause gills' physiological adjustments with changes in oxygen diffusion across the lamellae and in the amount of interlamellar mass which ultimately modifies the total surface of respiratory lamellae gills (Bowden et al., 2014). Such adaptation can lead to prolonged increases in LDH activity. For example, several weeks are needed for yellowtail fusilier (*Caesio cuning*) to return to LDH activity levels similar to pre-acclimation ones (Johansen et al., 2021). Organs have a time-dependent response to acclimation and may adjust to acclimation temperature at a different pace (Bouchard & Guderley, 2003). Future work on thermal acclimation should consider this time-course aspect if we want to fully comprehend how organisms adapt and adjust to thermal variation in nature.

5 | CONCLUSIONS

Our findings suggest that temperature and parasite infection impact the metabolism of pumpkinseed sunfish, with varying effects at different biological levels. Acclimation temperatures affected metabolic rate estimates, with aerobic scope maintained due to increased maximum metabolic rate (MMR) without significant changes in standard metabolic rate (SMR). This suggests fish can sustain aerobic performance even at temperatures above their natural range, which may be advantageous in the context of global climate change. Altered maximal activity of all enzymes in response to acclimation temperature indicated that metabolic changes at sub-organismal levels may have contributed to thermal compensation at the whole-organism level. In contrast, parasite infection was not related to enzyme activity, while increased black spot intensities were related to reduced MMR and AS, suggesting compromised aerobic capacity in parasitized fish. Despite no interaction between stressors, our findings illustrate that temperature and parasite infection can each disrupt metabolism, affecting fish performance. This highlights the ecological importance of understanding how multiple environmental stressors independently and collectively affect freshwater organisms.

AUTHOR CONTRIBUTIONS

Marie Levet, Shaun S. Killen and Sandra A. Binning conceived the ideas. Marie Levet, Shaun S. Killen, Sandra A. Binning, Stefano Bettinazzi and Sophie Breton designed the methodology. Marie Levet collected the data. Marie Levet extracted the data with help from Vincent Mélançon. Marie Levet analysed the data with help from Sandra A. Binning and Shaun S. Killen. Sandra A. Binning and Sophie Breton provided equipment. Marie Levet and Sandra A. Binning drafted the manuscript. All authors contributed to the manuscript's final draft and approved publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

This study's data and analytical code are available to at https://figshare.com/s/2c1df214c152a6b98f8b.

STATEMENT ON INCLUSION

Our study brought together researchers from multiple countries and included participants at various career stages, as we believed diverse perspectives and expertise were beneficial for the construction of our project. We also prioritised inclusiveness by ensuring the active participation of underrepresented groups in science and by engaging with the public while at the field sites. We dedicated time to explain our research in the spirit of promoting broader community engagement and took this opportunity to bring awareness about challenges faced by freshwater ecosystems.

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REFERENCES

Alfonso, S., Gesto, M., & Sadoul, B. (2021). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, *98*(6), 1496–1508. https://doi.org/10.1111/jfb.14599

Blier, P. U., Lemieux, H., & Pichaud, N. (2014). Holding our breath in our modern world: Will mitochondria keep the pace with climate changes? *Canadian Journal of Zoology*, *92*(7), 591–601. https://doi.org/10.1139/cjz-2013-0183

Bouchard, P., & Guderley, H. (2003). Time course of the response of mitochondria from oxidative muscle during thermal acclimation of rainbow trout, Oncorhynchus mykiss. *Journal of Experimental Biology*, *206*(19), 3455–3465. https://doi.org/10.1242/jeb.00578

Bowden, A. J., Gardiner, N. M., Couturier, C. S., Stecyk, J. A. W., Nilsson, G. E., Munday, P. L., & Rummer, J. L. (2014). Alterations in gill structure in tropical reef fishes as a result of elevated temperatures. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *175*, 64–71. https://doi.org/10.1016/j.cbpa.2014.05.011

Burnham, K. P., Anderson, D. R., & Huyvaert, K. P. (2011). AIC model selection and multimodel inference in behavioral ecology: Some background, observations, and comparisons. *Behavioral Ecology and Sociobiology*, *65*(1), 23–35. https://doi.org/10.1007/s00265-010-1029-6

Chabot, D., Steffensen, J. F., & Farrell, A. P. (2016). The determination of standard metabolic rate in fishes. *Journal of Fish Biology*, *88*(1), 81–121. https://doi.org/10.1111/jfb.12845

Chrétien, E., De Bonville, J., Guitard, J., Binning, S. A., Melis, É., Kack, A., Côté, A., Gradito, M., Papillon, A., Thelamon, V., Levet, M., & Barou-Dagues, M. (2023). Few studies of wild animal performance account for parasite infections: A systematic review. *Journal of Animal Ecology*, *92*(4), 794–806. https://doi.org/10.1111/1365-2656.13864

Chung, D. J., Bryant, H. J., & Schulte, P. M. (2017). Thermal acclimation and subspecies-specific effects on heart and brain mitochondrial performance in a eurythermal teleost (Fundulus heteroclitus). *Journal of Experimental Biology*, *220*(8), 1459–1471. https://doi.org/10.1242/jeb.151217

Cominassi, L., Ressel, K. N., Brooking, A. A., Marbacher, P., Ransdell-Green, E. C., & O'Brien, K. M. (2022). Metabolic rate increases with acclimation temperature and is associated with mitochondrial function in some tissues of threespine stickleback. *Journal of Experimental Biology*, *225*(21), jeb244659. https://doi.org/10.1242/jeb.244659

Daniel, R. M., Peterson, M. E., Danson, M. J., Price, N. C., Kelly, S. M., Monk, C. R., Weinberg, C. S., Oudshoorn, M. L., & Lee, C. K. (2009). The molecular basis of the effect of temperature on enzyme activity. *Biochemical Journal*, *425*(2), 353–360. https://doi.org/10.1042/BJ20091254

De Bonville, J., Côté, A., & Binning, S. A. (2024). Thermal tolerance and survival are modulated by a natural gradient of infection in differentially acclimated hosts. Conservation. *Physiology*, *12*(1), coae015. https://doi.org/10.1093/conphys/coae015

Dent, L., & Lutterschmidt, W. I. (2003). Comparative thermal physiology of two sympatric sunfishes (Centrarchidae: Perciformes) with a discussion of microhabitat utilization. *Journal of Thermal Biology*, *28*(1), 67–74. https://doi.org/10.1016/S0306-4565(02)00038-4

du Toit, H. D., Rishworth, G. M., Strydom, N. A., & Welman, S. (2024). High levels of metacercarial infestation (family: Diplostomidae) do not affect host energetics and swimming performance in the epaulette goby (*Coryogalops sordidus*, Gobiidae). *Journal of Fish Biology*, *104*(4), 1165–1179. https://doi.org/10.1111/jfb.15657

Ekström, A., Sandblom, E., Blier, P. U., Dupont Cyr, B.-A., Brijs, J., & Pichaud, N. (2017). Thermal sensitivity and phenotypic plasticity of cardiac mitochondrial metabolism in European perch, *Perca*

fluviatilis. *Journal of Experimental Biology*, *220*(3), 386–396. https://doi.org/10.1242/jeb.150698

Fry, F. E. J. (1971). 1—The effect of environmental factors on the physiology of fish. In W. S. Hoar & D. J. Randall (Eds.), *Fish physiology* (Vol. *6*, pp. 1–98). Academic Press. https://doi.org/10.1016/S1546-5098(08)60146-6

Gradito, M., Dubois, F., Noble, D. W. A., & Binning, S. A. (2024). Double trouble: Host behaviour influences and is influenced by co-infection with parasites. *Animal Behaviour*, *215*, 31–44. https://doi.org/10.1016/j.anbehav.2024.06.016

Grim, J. M., Miles, D. R. B., & Crockett, E. L. (2010). Temperature acclimation alters oxidative capacities and composition of membrane lipids without influencing activities of enzymatic antioxidants or susceptibility to lipid peroxidation in fish muscle. *Journal of Experimental Biology*, *213*(3), 445–452. https://doi.org/10.1242/jeb.036939

Guderley, H., & St-Pierre, J. (2002). Going with the flow or life in the fast lane: Contrasting mitochondrial responses to thermal change. *Journal of Experimental Biology*, *205*(15), 2237–2249. https://doi.org/10.1242/jeb.205.15.2237

Guitard, J. J., Chrétien, E., De Bonville, J., Roche, D. G., Boisclair, D., & Binning, S. A. (2022). Increased parasite load is associated with reduced metabolic rates and escape responsiveness in pumpkinseed sunfish. *Journal of Experimental Biology*, *225*(15), jeb243160. https://doi.org/10.1242/jeb.243160

Harianto, J., Carey, N., & Byrne, M. (2019). respR—An R package for the manipulation and analysis of respirometry data. *Methods in Ecology and Evolution*, *10*(6), 912–920. https://doi.org/10.1111/2041-210X.13162

Hassan, B., Qadri, H., Ali, M. N., Khan, N. A., & Yatoo, A. M. (2020). Impact of climate change on freshwater ecosystem and its sustainable management. In H. Qadri, R. A. Bhat, M. A. Mehmood, & G. H. Dar (Eds.), *Fresh water pollution dynamics and remediation* (pp. 105–121). Springer Singapore. https://doi.org/10.1007/978-981-13-8277-2_7

Hector, T. E., Shocket, M. S., Sgrò, C. M., & Hall, M. D. (2024). Acclimation to warmer temperatures can protect host populations from both further heat stress and the potential invasion of pathogens. *Global Change Biology*, *30*(6), e17341. https://doi.org/10.1111/gcb.17341

Hoffman, G. (2022). *mvIC: Multivariate Information Criteria to identify important predictors in high dimensional multivariate regression*. https://github.com/GabrielHoffman/mvIC

Hunter-Manseau, F., Desrosiers, V., Le François, N. R., Dufresne, F., Detrich, H. W., Nozais, C., & Blier, P. U. (2019). From Africa to Antarctica: Exploring the metabolism of fish heart mitochondria across a wide thermal range. *Frontiers in Physiology*, *10*, 1220. https://doi.org/10.3389/fphys.2019.01220

Iverson, E. N. K., Nix, R., Abebe, A., & Havird, J. C. (2020). Thermal responses differ across levels of biological organization. *Integrative and Comparative Biology*, *60*(2), 361–374. https://doi.org/10.1093/icb/icaa052

Johansen, J. L., Nadler, L. E., Habary, A., Bowden, A. J., & Rummer, J. (2021). Thermal acclimation of tropical coral reef fishes to global heat waves. *eLife*, *10*, e59162. https://doi.org/10.7554/eLife.59162

Jutfelt, F., Ern, R., Leeuwis, R., & Clark, T. (2024). *Effects of climate warming*. https://doi.org/10.1016/B978-0-323-90801-6.00183-X

Killen, S. S., Christensen, E. A. F., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A., Munson, A., Nati, J. J. H., Papatheodoulou, M., & McKenzie, D. J. (2021). Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. *Journal of Experimental Biology*, *224*(18), jeb242522. https://doi.org/10.1242/jeb.242522

Kraffe, E., Marty, Y., & Guderley, H. (2007). Changes in mitochondrial oxidative capacities during thermal acclimation of rainbow trout Oncorhynchus mykiss: Roles of membrane proteins, phospholipids and their fatty acid compositions. *Journal of Experimental Biology*, *210*(1), 149–165. https://doi.org/10.1242/jeb.02628

Lagrue, C., & Poulin, R. (2015). Measuring fish body condition with or without parasites: Does it matter? *Journal of Fish Biology*, *87*(4), 836–847. https://doi.org/10.1111/jfb.12749

Lemly, A. D., & Esch, G. W. (1984). Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: Ecological implications. *The Journal of Parasitology*, *70*(4), 475–492. https://doi.org/10.2307/3281395

Lenth, R. (2018). *Emmeans: Estimated marginal means, aka least-squares means* [computer software]. https://CRAN.R-project.org/package=emmeans

Levet, M., Roche, D. G., Killen, S. S., Colosio, S., Bshary, R., Miest, J. J., & Binning, S. A. (2024). Energetic costs of mounting an immune response in a coral reef damselfish (*Pomacentrus amboinensis*). *Canadian Journal of Zoology*, *102*(4), 380–392. https://doi.org/10.1139/cjz-2023-0033

Lopes, P. C., French, S. S., Woodhams, D. C., & Binning, S. A. (2021). Sickness behaviors across vertebrate taxa: Proximate and ultimate mechanisms. *Journal of Experimental Biology*, *224*(9), jeb225847. https://doi.org/10.1242/jeb.225847

Margolis, L., & Arthur, J. (1979). Synopsis of the parasites of fishes of Canada. Bulletin of the Fisheries Research Board of Canada, No. 199, vi + 269 pp. CABI Databases.

McElroy, E. J., & de Buron, I. (2014). Host performance as a target of manipulation by parasites: A meta-analysis. *Journal of Parasitology*, *100*(4), 399–410. https://doi.org/10.1645/13-488.1

Mélançon, V., Breton, S., Bettinazzi, S., Levet, M., & Binning, S. A. (2023). Mitochondrial enzyme activities and body condition of naturally infected sunfish (*Lepomis gibbosus*). *Physiological and Biochemical Zoology*, *96*(4), 247–259. https://doi.org/10.1086/725209

Morozov, S., McCairns, R. J. S., & Merilä, J. (2019). FishResp: R package and GUI application for analysis of aquatic respirometry data. *Conservation Physiology*, *7*(1), coz003. https://doi.org/10.1093/conphys/coz003

Nadler, L. E., Bengston, E., Eliason, E. J., Hassibi, C., Helland-Riise, S. H., Johansen, I. B., Kwan, G. T., Tresguerres, M., Turner, A. V., Weinersmith, K. L., Øverli, Ø., & Hechinger, R. F. (2021). A brain-infecting parasite impacts host metabolism both during exposure and after infection is established. *Functional Ecology*, *35*(1), 105–116. https://doi.org/10.1111/1365-2435.13695

Norin, T., & Clark, T. D. (2016). Measurement and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology*, *88*(1), 122–151. https://doi.org/10.1111/jfb.12796

Pichaud, N., Ekström, A., Breton, S., Sundström, F., Rowinski, P., Blier, P. U., & Sandblom, E. (2019). Cardiac mitochondrial plasticity and thermal sensitivity in a fish inhabiting an artificially heated ecosystem. *Scientific Reports*, *9*(1), 17832. https://doi.org/10.1038/s41598-019-54165-3

Prinzing, T. S., Zhang, Y., Wegner, N. C., & Dulvy, N. K. (2021). Analytical methods matter too: Establishing a framework for estimating maximum metabolic rate for fishes. *Ecology and Evolution*, *11*(15), 9987–10003. https://doi.org/10.1002/ece3.7732

R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Ressel, K. N., Cominassi, L., Sarrimanolis, J., & O'Brien, K. M. (2022). Aerobic scope is not maintained at low temperature and is associated with cardiac aerobic capacity in the three-spined stickleback *Gasterosteus aculeatus*. *Journal of Fish Biology*, *100*(2), 444–453. https://doi.org/10.1111/jfb.14955

Roche, D. G., Binning, S. A., Bosiger, Y., Johansen, J. L., & Rummer, J. L. (2013). Finding the best estimates of metabolic rates in a coral reef fish. *Journal of Experimental Biology*, *216*(11), 2103–2110. https://doi.org/10.1242/jeb.082925

Ryberg, M. P., Skov, P. V., Vendramin, N., Buchmann, K., Nielsen, A., & Behrens, J. W. (2020). Physiological condition of Eastern Baltic cod, *Gadus morhua*, infected with the parasitic nematode *Contracaecum osculatum*. *Conservation Physiology*, *8*(1), coaa093. https://doi.org/10.1093/conphys/coaa093

Sabbagh, S., Mélançon, V., Breton, S., & Binning, S. A. (2024). Differences in body condition and hepatic cellular metabolism among distinct populations of cestode infected pumpkinseed sunfish (*Lepomis gibbosus*). *Canadian Journal of Zoology*, *102*, 847–858. https://doi.org/10.1139/cjz-2023-0169

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, *218*(12), 1856–1866. https://doi.org/10.1242/jeb.118851

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., & Klenk, D. C. (1985). Measurement of protein using bicinchoninic acid. *Analytical Biochemistry*, *150*(1), 76–85. https://doi.org/10.1016/0003-2697(85)90442-7

Soengas, J. L., & Aldegunde, M. (2002). Energy metabolism of fish brain. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *131*(3), 271–296. doi:10.1016/S1096-4959(02)00022-2

Somero, G. N. (2004). Adaptation of enzymes to temperature: Searching for basic "strategies." Special issue dedicated to the memory of Peter W. Hochachka (1937–2002). *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*, *139*(3), 321–333. https://doi.org/10.1016/j.cbpc.2004.05.003

Thambithurai, D., Lanthier, I., Contant, E., Killen, S. S., & Binning, S. A. (2022). Fish vulnerability to capture by trapping is modulated by individual parasite density. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1989), 20221956. https://doi.org/10.1098/rspb.2022.1956

Thibault, M., Blier, P. U., & Guderley, H. (1997). Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, *16*(2), 139–155. https://doi.org/10.1007/BF00004671

Turko, A. J., Firth, B. L., Craig, P. M., Eliason, E. J., Raby, G. D., & Borowiec, B. G. (2023). Physiological differences between wild and captive animals: A century-old dilemma. *Journal of Experimental Biology*, *226*(23), jeb246037. https://doi.org/10.1242/jeb.246037

Vigneault, J. (2024). *Écologie spatiale de la maladie du point noir chez les communautés de poissons dulcicoles* [*Mémoire*]. Université de Montréal.

Zapata, A., Diez, B., Cejalvo, T., Gutiérrez-de Frías, C., & Cortés, A. (2006). Ontogeny of the immune system of fish. *Reviews in Fish Immunology*, *20*(2), 126–136. https://doi.org/10.1016/j.fsi.2004.09.005

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Enzyme activity preparation and measurements.

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