Mitochondrial Enzyme Activities and Body Condition of Naturally Infected Sunfish (*Lepomis gibbosus*)

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ABSTRACT

Parasites can affect host behavior, cognition, locomotion, body condition, and many other physiological traits. Changes to host aerobic metabolism may be responsible for these parasite-induced performance alterations. Whole-organism metabolic rate is underpinned by cellular energy metabolism driven most prominently by mitochondria. However, few studies have explored how mitochondrial enzymatic activity relates to body condition and parasite infection, despite it being a putative site for metabolic disruptions related to health status. We studied correlations among natural parasite infection, host body condition, and activity of key mitochondrial enzymes in target organs from wild-caught pumpkinseed sunfish (*Lepomis gibbosus*) to better understand the cellular responses of fish hosts to endoparasite infection. Enzymatic activities in the gills, spleen, and brain of infected fish were not significantly related to parasite infection or host body condition. However, the activity of cytochrome c oxidase, an enzyme involved in oxidative phosphorylation, in fish hearts was higher in individuals with a lower body condition. Activities of citrate synthase, electron transport system (complexes I and III), and carnitine palmitoyltransferase were also significantly different among organ types. These results provide preliminary information regarding the likely mitochondrial pathways affecting host body condition, the maintenance energetic requirements of different organs, and the organs' specific dependency on particular mitochondrial pathways. These results help pave the way for future studies on the effects of parasite infection on mitochondrial metabolism.

Keywords: aerobic metabolism, black spot disease, Centrarchidae, endoparasite, cellular metabolism, energetic trade-offs, host-parasite interactions, oxidative phosphorylation.

Introduction

Most wild animals are infected with parasites (Dobson et al. 2008). Parasites are organisms living in or on another species (the host) that they exploit for resources (shelter and/or energy; Lewin 1982). However, quantifying parasite-induced damages and/or costs to hosts is often difficult since host-parasite interactions are complex, with costs to hosts being dependent on the parasite life stage, infection intensity, season, and/or presence of concomitant environmental stressors (Eure 1976; Lemly and Esch 1984; Poulin 1992; Araújo et al. 2003; Leung and Poulin 2008). Coinfection with multiple parasite species can also make it difficult to attribute parasite-induced effects (Pedersen and Fenton 2007; Timi and Poulin 2020). In addition, parasites are often much smaller than their host, making infection difficult to detect and thus easy for researchers to overlook (Marcogliese 2004). This oversight can be problematic, considering that many parasites have important effects on host physiology and behavior (Lagrue and Poulin 2015; Timi and Poulin 2020). For instance, body condition (the relationship between an animal's mass and body size) is used in many taxa as a proxy of individual health status (Jakob et al. 1996). However, when infected individuals are weighed, the mass of their parasites is inevitably included in that measure. This can lead to an overestimate of an individual's body condition in heavily infected individuals if host mass is not corrected for parasite biomass (Lagrue and Poulin 2015; Timi and Poulin 2020). Thus, considering parasite infection in ecophysiological studies is critical to ensure accurate estimates of many traits of interest.

Host performance capacity (i.e., the ability of an organism to execute fitness-enhancing behaviors) has recently been identified as a target of direct or indirect manipulation by parasites (Poulin 2010; McElroy and de Buron 2014; Binning et al. 2017; Timi and Poulin 2020). For example, bridled monocle bream

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(*Scolopsis bilineata*) infected by an ectoparasitic isopod (*Anilocra nemipteri*) experience decreased aerobic capacity and maximum swimming speed compared with uninfected fish (Binning et al. 2013). Similarly, pumpkinseed sunfish (*Lepomis gibbosus*) heavily infected with bass tapeworms (*Proteocephalus ambloplitis*) display reduced responsiveness, standard metabolic rates, and maximum metabolic rates, suggesting that parasites may have an effect on host aerobic physiology and escape performance (Guitard et al. 2022; Thambithurai et al. 2022). Parasite infection can also increase host energy demand by triggering the costly activation of the host immune system or by causing tissue damage or other parasite-induced pathologies (Ots et al. 2001; Freitak et al. 2003). For example, house sparrows (*Passer domesticus*) injected with lipopolysaccharide (an endotoxin that induces an immune response in most vertebrates) from *Escherichia coli* show decreased activity, feeding rates, and reproductive success (Bonneaud et al. 2003). Similarly, nestling Mediterranean blue tits (*Parus caeruleus* L.) infected with *Protocalliphora* ectoparasites experience decreased aerobic competence (Simon et al. 2004), and white cabbage butterflies (*Pieris brassicae* L.) implanted with nylon to mimic a parasitic infection have increased metabolic rates (Freitak et al. 2003). Despite the growing body of work linking infection to altered energetics, the mechanisms underlying altered host metabolic activities during infection are not well understood.

In addition to energetics, host body condition tends to be negatively related to parasite intensity (Lemly and Esch 1984; Sánchez et al. 2018). Body condition reflects the amount of accumulated energy reserves in the form of lipids and/or proteins in an organism (Brosset et al. 2015*a*, 2015*b*). Thus, individuals with a lower condition index likely have lower energy reserves than conspecifics with a higher condition index. For instance, intracellular parasites like *Toxoplasma gondii* can exploit the host lipophagy machinery to access the fatty acids necessary to boost their own proliferation, at the expenses of host lipid reserve and body condition (Charron and Sibley 2002; Caffaro and Boothroyd 2011; Pernas et al. 2018). Similarly, in female springbok (*Antidorcas marsupialis*) and female horses (*Equus ferus*), body condition is negatively correlated with increasing parasite loads (Turner et al. 2012; Debeffe et al. 2016). A negative correlation between infection and body condition also occurs in juvenile bluegill sunfish (*Lepomis macrochirus*) infected by trematodes (*Uvulifer ambloplitis*; Lemly and Esch 1984) and in icefish (*Chionodraco hamatus*) infected with flatworms (helminths; Santoro et al. 2013). The altered body condition and metabolic rate observed in infected hosts may be linked, but once again, the underlying mechanisms are not well explored in the literature.

The study of these underlying mechanisms starts with mitochondria, key regulators of cellular energy metabolism. These organelles produce most of the cells' energy (ATP) through a process known as oxidative phosphorylation (OXPHOS). Mitochondria are known to be affected by intracellular parasites (Syn et al. 2017; Lentini et al. 2018; Escoll et al. 2019). For example, a perturbation of mitochondrial host functions (i.e., fragmented morphology, increased ROS production, and decreased expression of OXPHOS proteins) has been observed following infection by *T. gondii* (Syn et al. 2017). It has also been shown that host mitochondria can mount a metabolic defense against the intracellular parasite *T. gondii* by fusing together to limit its uptake of fatty acids, therefore limiting its growth rate (Pernas et al. 2018). However, how mitochondria respond to extracellular parasites is less well understood. Some extracellular parasites seem to also affect host mitochondria. For example, *Giardia duodenalis*, a parasite of the intestine, increases cell apoptosis through the modulation of host mitochondrial membrane potential and ROS production (Liu et al. 2020). Mitochondrial OXPHOS is one of the primary users of oxygen (Capaldi 1990; Nath and Villadsen 2015), and some studies suggest that there is a lowered OXPHOS activity in response to parasite infection (Mills et al. 2017; Escoll et al. 2019; Hunter-Manseau et al. 2019). For example, when mounting an immune response to infection, metabolism sometimes shifts toward the anaerobic glycolytic pathway (Escoll et al. 2019; Hortová-Kohoutková et al. 2021). Mitochondria can also change their fuel of choice in response to different stressors (Stanley et al. 2014). For example, the above-cited metabolic defense against *T. gondii* involves fusion and fatty acid uptake by host mitochondria, suggesting an increase in lipid metabolism activity (Pernas et al. 2018). The metabolism of lipids, precisely fatty acid oxidation, may thus increase with parasite infection. This could also explain why host body condition tends to be lower when individuals have a higher parasite intensity, since a higher lipid metabolism activity decreases the amount of stored lipids.

Physiological studies should also consider different organ systems because the systems may have varying responses to stressors, including parasite infection. For example, the brain is known for its high energetic demand (Magistretti and Allaman 2013), and it uses most of the glucose, amino acids, and monocarboxylates available to an organism. The brain uses primarily OXPHOS and the tricarboxylic acid (TCA) cycle mitochondrial metabolic pathway, which can vary in response to environmental stressors (Soengas and Aldegunde 2002). Some evidence suggests that infection with the trematode parasite *Euhaplorchis californiensis* in the brains of California killifish (*Fundulus parvipinnis*) affects brain enzyme activities. In this case, a decrease was observed for both lactate dehydrogenase activity and citrate synthase activity, but it was not significant for the latter (Nadler et al. 2021). In comparison, the spleen is a small organ primarily used in immune responses, iron recycling (hematopoiesis), and blood filtration (Fänge and Nilsson 1985). The normal energetic demand of this organ is not high, but it can greatly increase when an immune response is initiated (Bronte and Pittet 2013). The spleen uses various metabolic pathways, depending on the immune cells needed to respond to a specific infection (Bronte and Pittet 2013) and the stage of the infection (Mills et al. 2017; Escoll et al. 2019; Hortová-Kohoutková et al. 2021). In fishes, both the heart and the gills are important for the circulation and delivery of oxygen throughout the body (Laurent et al. 1983). The energetic demand of the heart and the gills may also vary in response to stress, since oxygen consumption increases when individuals experience stressful situations (Wendelaar Bonga 1997); this variability may be linked to the enzymatic activities of these organs (Rees et al. 2022). For example, gilthead seabream (*Sparus aurata*) exposed to cortisol, a stress hormone, show different gill enzymatic

activities and mitochondrial pathway prioritization (Laiz-Carrión et al. 2002). Fish hearts use several metabolic pathways but primarily rely on lipid oxidation and OXPHOS (Hunter-Manseau et al. 2019). For gills, carbohydrate metabolism is more common (Tseng and Hwang 2008). Parasites may also indirectly affect enzymatic activity in gills and other tissues by inducing the host stress response via the hypothalamo-pituitary-interrenal axis (O'Dwyer et al. 2020). Finally, parasites may affect host nutrient circulation, damage organs, and/or limit locomotor performance, all of which may affect an organ's cellular metabolism, but these relationships have not been well explored to date.

From the literature above, it is clear that parasites affect host physiology, but many results are contradictory. A better knowledge of the underlying mechanisms could help explain this variance and ultimately lead to a better understanding of parasite effects on host physiology and energetics (Robar et al. 2011). In this perspective, the objective of this study is to measure the mitochondrial metabolism of a freshwater fish naturally parasitized with helminth endoparasites and to explore relationships among infection intensity, enzymatic activity, and body condition in four key organs. To achieve this objective, we used pumpkinseed sunfish (*L. gibbosus*), a small common freshwater fish native to eastern North America, as a model system. In lakes around the Laurentian region in Quebec, pumpkinseed sunfish are often the second intermediate host of several parasitic helminths, including the cestode *Proteocephalus ambloplitis* (bass tapeworm) and the trematode *Clinostomum marginatum* (yellow grub). Pumpkinseed sunfish are also the second intermediate hosts of the trematodes *Uvulifer* sp. and *Apophallus* sp., which form melanized cysts that are visible on a fish's body surface and fins and can be easily quantified. This visible sign of parasitism, referred to colloquially as black spot disease, allowed us to select fish across a gradient of infection upon capture. These parasites have complex life cycles, involving gastropods or copepods, fish, and piscivorous birds or fish as hosts. Trematode infection in fish occurs when the parasite cercariae are released from a gastropod and penetrate the muscles and fins of fish. The cercariae develop into metacercariae, forming a cyst that is melanized by the host immune system and leading to a visible black spot (Fischer and Freeman 1969; Berra and Au 1978). Once encysted, trematode metacercariae are metabolically inactive. However, heavy infections have been associated with altered behavior, reduced body condition and lipid content, and increased overwinter mortality in sunfish, suggesting a high energetic cost of infection (Lemly and Esch 1984; Timi and Poulin 2020). Cestode infection occurs when fish consume infected copepods. A cestode's final hosts are other centrarchid fishes, including smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*M. salmoides*) that become infected after ingesting infected sunfish. In sunfish, cestodes are found in the digestive tract and the abdominal cavity (more precisely, in the liver and the spleen). There, they cause substantial lesions and possible organ failure. Thus, they are suspected to have a large effect on the metabolism of fishes by affecting the amount of nutrients received by the host and perhaps indirectly affecting mitochondria (Hugghins 1959; Margolis and Arthur 1979). Given this parasite-induced host damage, we hypothesize that the activity of key enzymes involved in OXPHOS, fatty acid metabolism, and the TCA cycle (table S1) are related to parasite intensity in naturally infected sunfish. Because metabolic pathways may be upregulated or downregulated depending on the context, we do not have specific predictions regarding the direction of the relationships in this system.

Methods

Fish Sampling

Pumpkinseed sunfish (*Lepomis gibbosus*) were collected using baited minnow traps from Lake Cromwell near the Station de biologie des Laurentides in Quebec (lat. 45.98861, long. −74.00585). Traps were set for 30–60 min before being pulled in and screened for fish. In total, 22 fish measuring between 90 and 120 mm in total length were collected across a gradient of visible black spot intensity (approximately, 0–50 black spots represented a low infection level, 51–100 black spots represented a medium infection level, and 101 or more black spots represented a high infection level; fig. 1), which was assessed by the researcher (V. Mélançon) upon capture and later confirmed by the quantification of black spots on dead specimen in the lab. Selected fish were immediately euthanized in an overdose of 10% eugenol (clove oil) solution (4 mL L^{-1} water) and packed on ice in coolers. Fish that were not selected because they fell outside of the targeted size range or infection level were immediately released. Fish still on ice for no longer than 4 h were quickly brought to the Station de biologie des Laurentides following sampling and frozen at −20°C before they were transported to the Université de Montréal's Complexe des sciences laboratory facilities in refrigerated coolers for dissection and enzymatic assays.

Fish Dissection and Body Condition Calculation

Each specimen was thawed and dissected on ice to maintain the enzymatic capacities of the tissues. First, fish total length was measured using a 0–150-mm digital caliper. The total wet weight was measured using an electronic balance (MSE225S, Sartorius Weighing Company, Goettingen, Germany). Second, heart, spleen, and brain were extracted and kept on ice in 5-mL cryogenic tubes. Gill filaments were dissected out on ice using a dissecting microscope (Stemi DV4, Zeiss, Oberkochen, Germany) and also kept on ice in cryogenic tubes once dissected. Third, fish were screened for parasites. Cestodes were extracted out of the fish and counted. Trematodes causing black spot disease were counted on only the left side of fish to avoid double counting parasites on unpaired fins, and then the amount was doubled. Previous work suggests that there is no significant difference in the number of parasites found on the left versus right side of the fish (S. A. Binning, unpublished data). Last, the corrected weight of fish (i.e., fish mass excluding cestode parasites) was measured by weighing fish and tissues once all cestodes were removed. All tissues were screened at least twice to ensure that residual parasite tissues were removed. The weight of individual black spot cysts was negligible and thus was not corrected for. The corrected fish mass (i.e., fish wet weight mass excluding

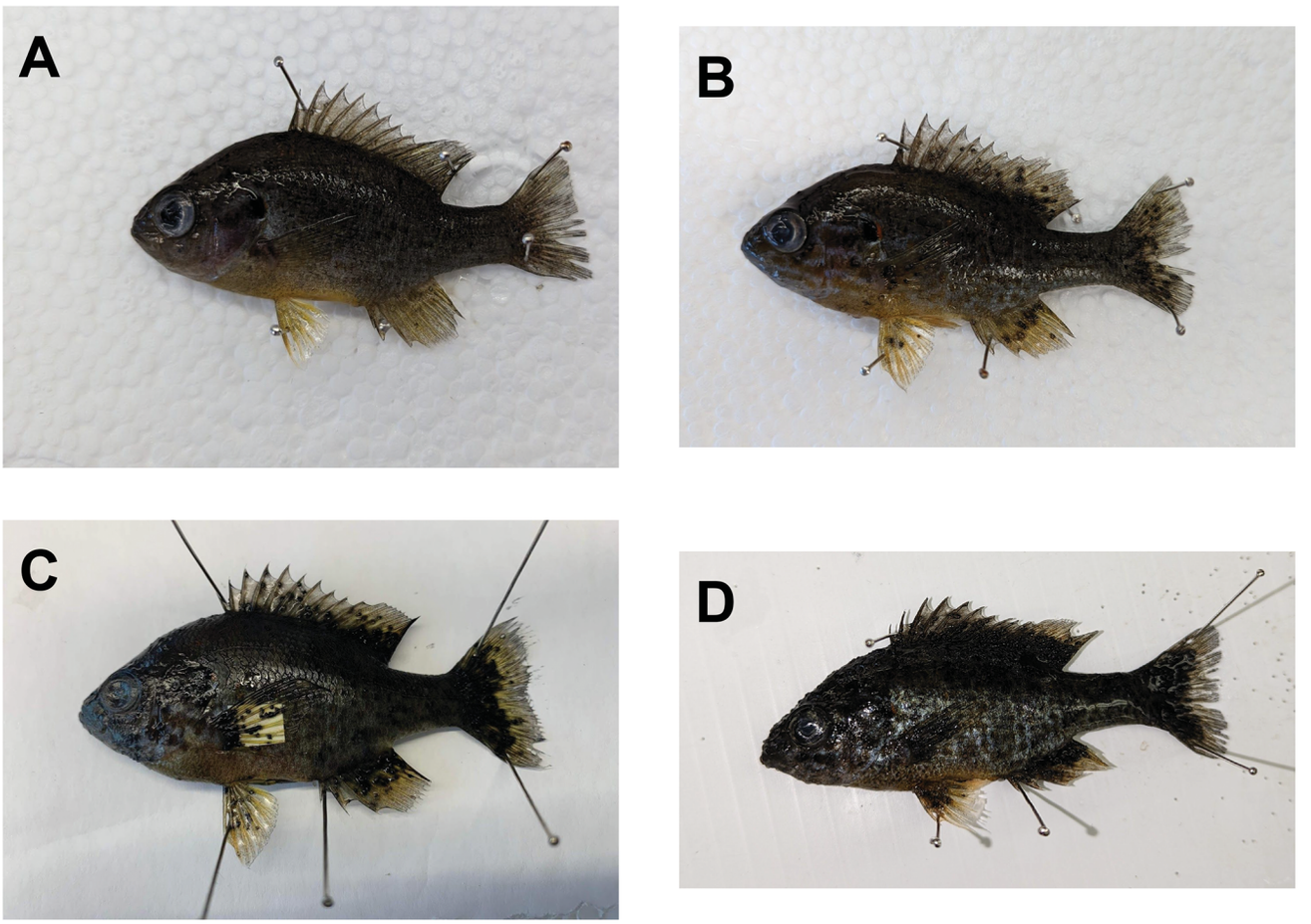

Figure 1. Examples of infected fish. Pictures of fish during dissection, representing the following visual gradient: low infection (*A*), medium infection (*B*), and high infection (*C, D*). Photograph credits: V. Mélançon, M. Levet, and J. De Bonville.

cestode wet weight) was used to calculate the density of both types of parasites (trematodes and cestodes) for each fish by dividing the number of parasites in each fish by the corrected mass (Lagrue and Poulin 2015).

Le Cren's (1951) body condition index was calculated for each fish. This index uses the log relation between the corrected mass and the total length of the fish to estimate a slope and an intercept for the population. These values are then used to measure the body condition of each individual that is centered around the population mean value of 1 (Le Cren 1951; Wuenschel et al. 2019).

Enzymatic Assays

Mitochondrial enzymatic activities were measured along a natural gradient of infection with the goal of exploring possible correlations between mitochondrial metabolism and parasite infection. These analyses were performed on brain, heart, gills, and spleen because of their relevance in various physiological systems. Enzymes were selected to assess multiple mitochondrial pathways (more precisely, OXPHOS, TCA cycle, and lipid metabolism) with the goal of measuring possible relationships between parasite infection and these metabolic processes.

Once the organs were extracted from the fish, they were diluted 20 times their wet weight in a 5-mL cryogenic tube with 100 mM potassium phosphate buffer, 20 mM EDTA, pH 8.0 (modified protocol from Hunter-Manseau et al. 2019). Then, they were homogenized using a polytron (PT1200 E) three times for 5 s with a 30-s rest time on ice between each step. The homogenized samples were stored at −80°C.

Enzymatic assays were performed at room temperature (25°C) in 96-well plates by using a microplate reader (Mithras LB 940, Berthold Technologies, Bad Wildbad, Germany). Cytochrome c oxidase (CCO) and electron transport system (ETS; complexes I and III) were chosen because of their importance in OXPHOS (Mills et al. 2017; Ryan et al. 2021). To explore the relationships between parasitism and lipid metabolism, we selected carnitine palmitoyltransferase (CPT) because of its role in transferring a carnitine group to fatty acids, thus allowing their entry into the mitochondrial matrix (Schulz 1991; Hiltunen et al. 2010; Kastaniotis et al. 2017). An enzyme from the TCA cycle, citrate

synthase (CS), was also chosen as a quantitative enzyme marker of mitochondria content because it is also indicative of the aerobic capacity (Wiegand and Remington 1986; Mills et al. 2017; Ryan et al. 2021).

All assays were run in duplicate. Enzyme activities were estimated using the Beer-Lambert law (Thibault et al. 1997), corrected for the dilution factor, and normalized for the amount of proteins in each sample. All the reagents came from Sigma-Aldrich Chemicals (St. Louis, MO).

Enzymatic Activity

Cytochrome c oxidase (Enzyme Commission [EC] 7.1.1.9). CCO activity was measured at 550 nm over 5 min following the oxidation of cytochrome c ($\varepsilon = 18.5\ mM^{-1}\ cm^{-1}$) using 100 mM potassium phosphate buffer containing 0.05 mM oxidized cytochrome c from equine heart, 4.5 mM sodium dithionate, and 0.03% Triton X-100, pH 8.0. Control reactions were performed by adding 10 mM sodium azide to the sample, and background activities were performed by omitting sodium dithionate and then deducted from the activity of the assay. The reaction solution was bubbled for 5 min, and the absorbance ratio of 550:565 nm was determined. If the ratio was higher than 9, the solution was used (Thibault et al. 1997; Hunter-Manseau et al. 2019).

Electron transport system (EC 7.1.1.2 and 7.1.1.8). ETS activity (combined mitochondrial complexes I and III activities) was assessed at 490 nm over 6 min by measuring the reduction of p-iodonitrotetrazolium violet ($\varepsilon = 15.9\ mM^{-1}\ cm^{-1}$) using 100 mM potassium phosphate buffer with 2 mM p-iodonitrotetrazolium violet and 0.85 mM NADH, pH 8.5. Background and specificity of the reaction were calculated by running a control without tissues and a background without NADH (Hunter-Manseau et al. 2019).

Carnitine palmitoyltransferase (EC 2.3.1.21). CPT activity was assessed at 412 nm over 5 min by measuring the conversion of 5,5′dithiobis-2-nitrobenzoic acid (DTNB) into 2-nitro-5-thiobenzoic acid (TNB; $\varepsilon = 14.15\ mM^{-1}\ cm^{-1}$) using 75 mM Tris-HCl buffer, 1.5 mM EDTA with 0.25 mM DTNB, 0.035 mM palmitoyl-CoA, and 2 mM L-carnitine, pH 8.0. Control reactions were performed by omitting the sample (Thibault et al. 1997).

Citrate synthase (EC 2.3.3.1). CS activity was measured at 412 nm following the conversion of DTNB into TNB ($\varepsilon = 14.15\ mM^{-1}\ cm^{-1}$) over 6 min. The reaction solution consisted of 100 mM imidazole-HCl buffer with 0.1 mM DTNB, 0.1 mM acetyl-CoA, and 0.15 mM oxaloacetate, pH 8.0. A background without oxaloacetate was first performed followed by the final assay (Hunter-Manseau et al. 2019). Enzyme activities were normalized by total protein content ($mg\ mL^{-1}$) measured through the bicinchoninic acid method (Smith et al. 1985) and were expressed as U mg^{-1} protein, where U represents 1 μmol of substrate transformed into product in 1 min.

Statistical Analyses and Measures

Parasites and host body condition. To explore the relationship between parasite infection and host body condition, we used generalized linear models (GLM) with a gamma error distribution to meet model assumptions. The following two models were tested: (1) host body condition as a function of cestode density and (2) host body condition as a function of trematode density. Both types of parasites have different ecologies and likely have different effects on their hosts. Their intensities were also not strongly correlated in our sample ($R^2 = 0.35$; fig. S1). However, we considered them separately in our analyses.

Parasites and enzymatic activity. To explore the relationship between parasite infection and enzymatic activity, we used linear mixed models. Response variables were Box-Cox transformed to meet model assumptions. For each of the four enzymes tested (CCO, CS, ETS, CPT), the following two models were run: (1) enzymatic activity as a function of cestode density and (2) enzymatic activity as a function of trematode density. In both models, organ type (heart, spleen, gills, brain) was included as a fixed factor, and body condition was included as a covariable. Full models included a three-way interaction between parasite density, organ type, and body condition, with fish ID included as a random effect. Simplified models were selected based on their corrected Akaike information criterion (AICc)—the model with the lowest AICc was kept. At first, models 1 and 2 were tested within the same model without interaction between these two three-way interaction models for each enzyme. The AICc showed that this model was not the most appropriate for all enzymes and was therefore discarded. For CS, CCO, and CPT, the selected model included only body condition, organ type, and their interaction as factors. For ETS, the model with cestode density, organ type, and their interaction was chosen. When interactions between factors and covariables were not significant, interactions were removed from the model. All statistics were performed using the lmer, glm, and ggplot functions in R version 4.1.0 (R Development Core Team 2022).

Results

Parasites and Host Body Condition

Despite the quantity of parasites found on some individuals, no significant effect of parasites on body condition was found. Fish collected in this study had a mean total length of 103 mm (minimum–maximum ± SE = 91.9–121.5 ± 8.9 mm). Mean fish wet weight was 21.5 g (15.4–36.4 ± 5.3 g), and the mean parasite mass-adjusted wet weight was 20.6 g (14.7–35.6 ± 5.4 g). Parasites thus represented a mean of 4.7% of total fish weight (0.8%–7.6% ± 0.7%). They were mostly found in the liver and, in some cases, appeared to cause significant damage to the integrity of liver tissues, which prevented us from using this organ for enzyme activities. Fish body condition index ranged from 0.82 to 1.19. Trematode density ranged from 0.06 to 29.4 trematodes g^{-1} (mean ± SE = 10.4 ± 14.2 trematodes g^{-1}). Individuals carried as few as two trematodes and as many as 518 trematodes (median = 10.1 trematodes). For cestodes, parasite density was lower with a minimum of 0.3 cestodes g^{-1} and a maximum of 11.1 cestodes g^{-1} (mean ± SE = 2.8 ± 1.5 cestodes g^{-1}). The cestode intensity ranged from 4 to 210 cestodes per fish (median = 2.1 cestodes).

Even though there appeared to be a slight negative correlation between fish parasite density and host body condition for both types of infections (fig. S2), this effect was not statistically significant (cestodes, $R^2 = 0.03$; trematodes, $R^2 = 0.11$; cestodes and trematodes, $P > 0.1$).

Parasites and Host Enzymatic Activity

There was no significant relationship between parasite infection and CCO enzyme activity, despite the appearance of positive and negative trends with trematode and cestode infections, respectively (figs. 2*A*, 2*B*; table S2). In comparison, the model containing host body condition and organ type revealed a significant interaction between the two factors ($F = 3.00$, $P = 0.038$); increased body condition was correlated with a decrease in CCO activity for the heart ($t = -2.27$, $P = 0.027$; fig. 2*C*) but not for other organs.

As for CCO, parasites did not significantly affect CS enzyme activity; although, a positive trend was observed with trematode density, and a negative trend was observed with cestode density (figs. 3*A*, 3*B*). There were no significance relationships found in models that included parasite density and organ type (table S2), and there was no significant interaction between body condition and organs ($P = 0.42$) in the model including both factors (fig. 3). A significant difference among organs was detected ($P < 2 \times 10^{-16}$) in the model including body condition and organ type without interactions. These differences are between the spleen (0.013 ± 0.00071 U mg^{-1} protein) and the brain (0.063 ± 0.003 U mg^{-1} protein; $P < 2 \times 10^{-16}$), between the heart (0.087 ± 0.0051 U mg^{-1} protein) and the gills (0.026 ± 0.0014 U mg^{-1} protein; $P < 2 \times 10^{-16}$), between the spleen and the heart ($P < 2 \times 10^{-16}$), between the brain and the gills ($P < 2 \times 10^{-16}$), between the heart and the brain ($P < 3.52 \times 10^{-12}$), and between the gills and the spleen ($P < 1.04 \times 10^{-12}$; fig. 4*A*).

CPT also followed the trends observed for CCO and CS when looking at parasite densities (fig. 5*A*, 5*B*), and again, none of the previously mentioned models including parasite density were found to be significant (table S2), but negative trends were present for body condition ($P = 0.062$; fig. 5). Such as in the case of CS, there were significant differences among organ types ($P < 2 \times 10^{-16}$), with the brain having a higher enzymatic activity (0.0034 ± 0.00021 U mg^{-1} protein) compared to the gills (0.0016 ± 0.000046 U mg^{-1} protein; $P = 4.41 \times 10^{-14}$), the heart (0.0016 ± 0.000010 U mg^{-1} protein; $P = 3.83 \times 10^{-15}$), and the spleen (0.0013 ± 0.00010 U mg^{-1} protein; $P < 2 \times 10^{-16}$). Also, the spleen had a significantly different CPT activity compared to the gills ($P = 0.0087$) and the heart ($P = 0.041$; fig. 4*B*). These results were obtained using the statistical model including only body condition and organ type without their interaction.

For ETS, there were no significant relationships among any of the tested factors and enzymatic activities for models with

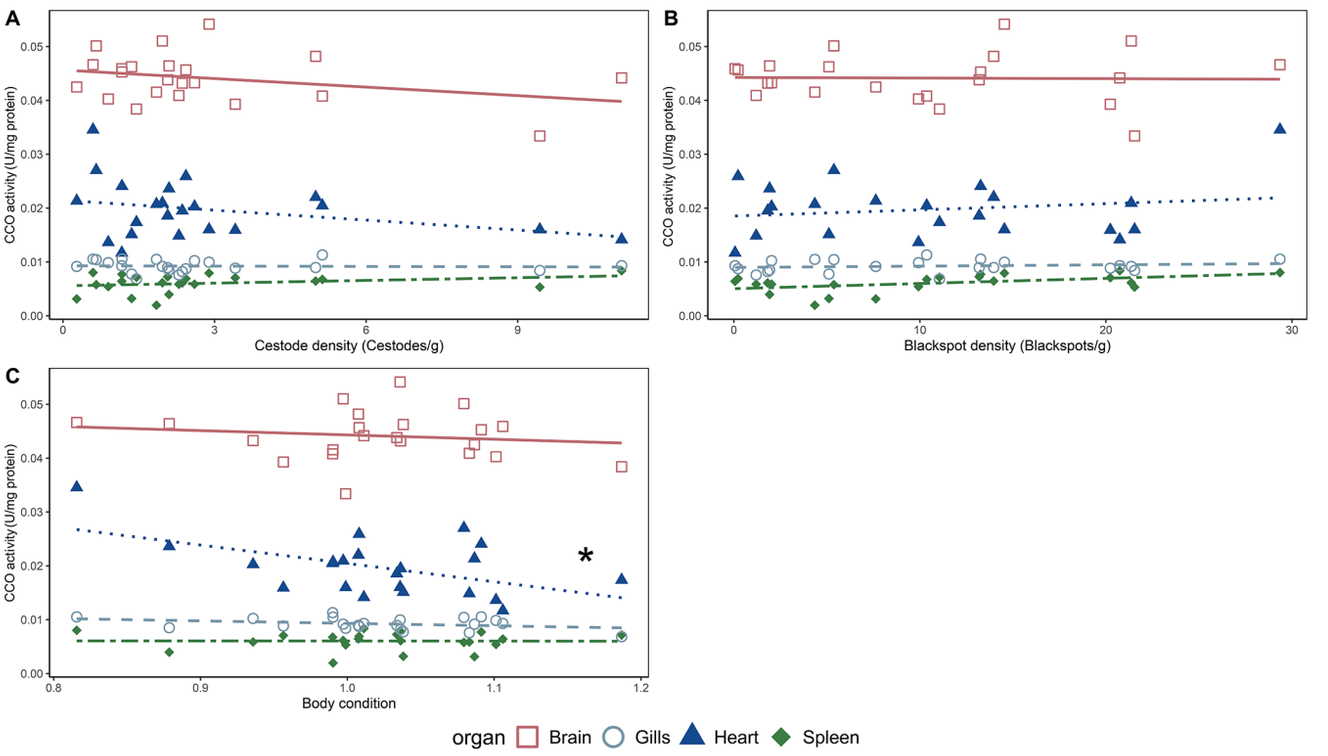

Figure 2. Relationships between parasite infections and enzymatic activity of cytochrome c oxidase (CCO). Least squares linear regressions of the enzymatic activity (U mg^{-1} protein) of CCO as a function of cestode density (*A*), black spot density (*B*), and body condition (*C*). Enzymatic activity of the heart in relation to body condition was found to be significant (*$P < 0.05$).

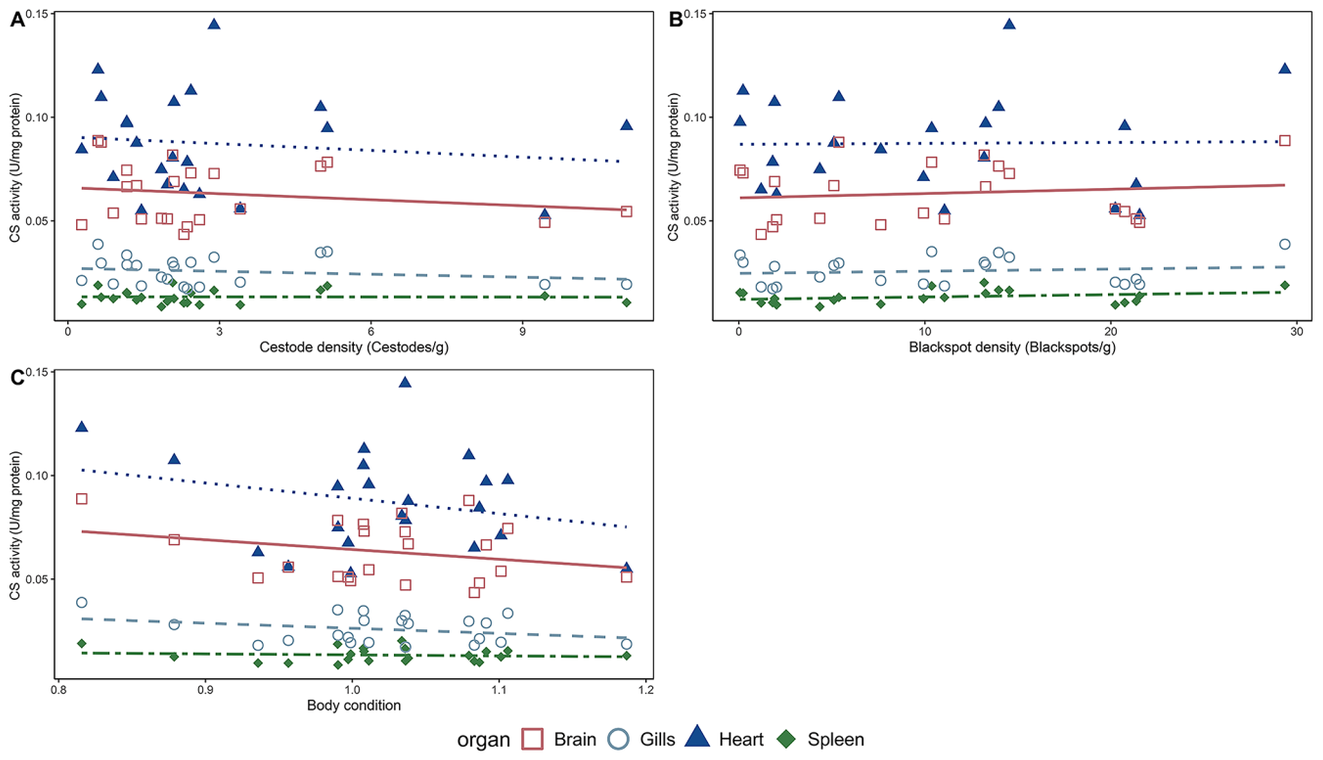

Figure 3. Relationships between parasite infections and enzymatic activity of citrate synthase (CS). Least squares linear regressions of the enzymatic activity (U mg^{-1} protein) of CS as a function of cestode density (*A*), black spot density (*B*), and body condition (*C*).

either cestode or trematode density (table S2; fig. 6), but just like CCO, CS, and CPT, ETS activity tended to be negatively correlated with cestode density and positively correlated with trematode density. Significant differences ($P = 0.00078$) among organ types were found in the model including cestode density and organ type without interactions. Cestode density approached significance ($P = 0.093$), and the ETS activity of the gills (0.011 ± 0.00065 U mg^{-1} protein) was found to be significantly different from the brain activity (0.0082 ± 0.00041 U mg^{-1} protein; $P = 0.00051$) and the spleen activity (0.0084 ± 0.00082 U mg^{-1} protein; $P = 0.00031$; fig. 4*C*). Trends for ETS were different than those for CCO, CS, and CPT. Indeed, brain and heart were negatively linked to body condition, but gills and spleen showed a slight positive correlation to body condition.

Since differences in enzymatic activities among organs seemed to be present for all four enzymes measured, we used a Kruskal-Wallis test to compare the protein content among organs to ensure differences among organs were not driving these trends. We found that protein contents were not significantly different among organs ($\chi^2 = 3.6969$, $P = 0.2961$; fig. S3). Brains had the lowest protein content at 97.8 mg g^{-1} tissue (minimum–maximum ± SE = 74.5–121.2 ± 26.0 mg g^{-1} tissue) followed by gills at 98.9 mg g^{-1} tissue (77.2–122.5 ± 20.0 mg g^{-1} tissue), hearts at 101.7 mg g^{-1} tissue (52.0–126.9 ± 53.8 mg g^{-1} tissue), and spleens at 107.5 mg g^{-1} tissue (77.3–177.6 ± 100.5 mg g^{-1} tissue).

Discussion

The goal of this study was to explore the mitochondrial metabolism of parasitized *Lepomis gibbosus*. We found no significant relationships between parasite density and host mitochondrial enzymatic activity in any of the tested enzymes in the four organs that we studied. However, there was a significant negative relationship between body condition and CCO activity of the heart. Indeed, CCO activity of the heart was higher when an individual had a lower body condition. The activity of CS varied significantly among organs, with brain activity and heart activity being significantly higher than spleen activity, and heart activity also being higher than gill activity. The activities of ETS and CPT were also found to be significantly different between organs; the gills and the brain had the highest activity, respectively.

Parasites and Host Body Condition

We found no correlation between parasite density and host body condition, despite a negative trend with both types of parasites (fig. S2). Although parasites tend to be negatively related to host body condition, some studies have also found positive relationships or no relationships between these variables (Sánchez et al. 2018). Our results slightly contrast with those reported by Lemly and Esch (1984), who found a significant negative relationship between body condition and infection with similar trematode parasites (*Uvulifer ambloplitis*) in bluegill sunfish (*Lepomis*

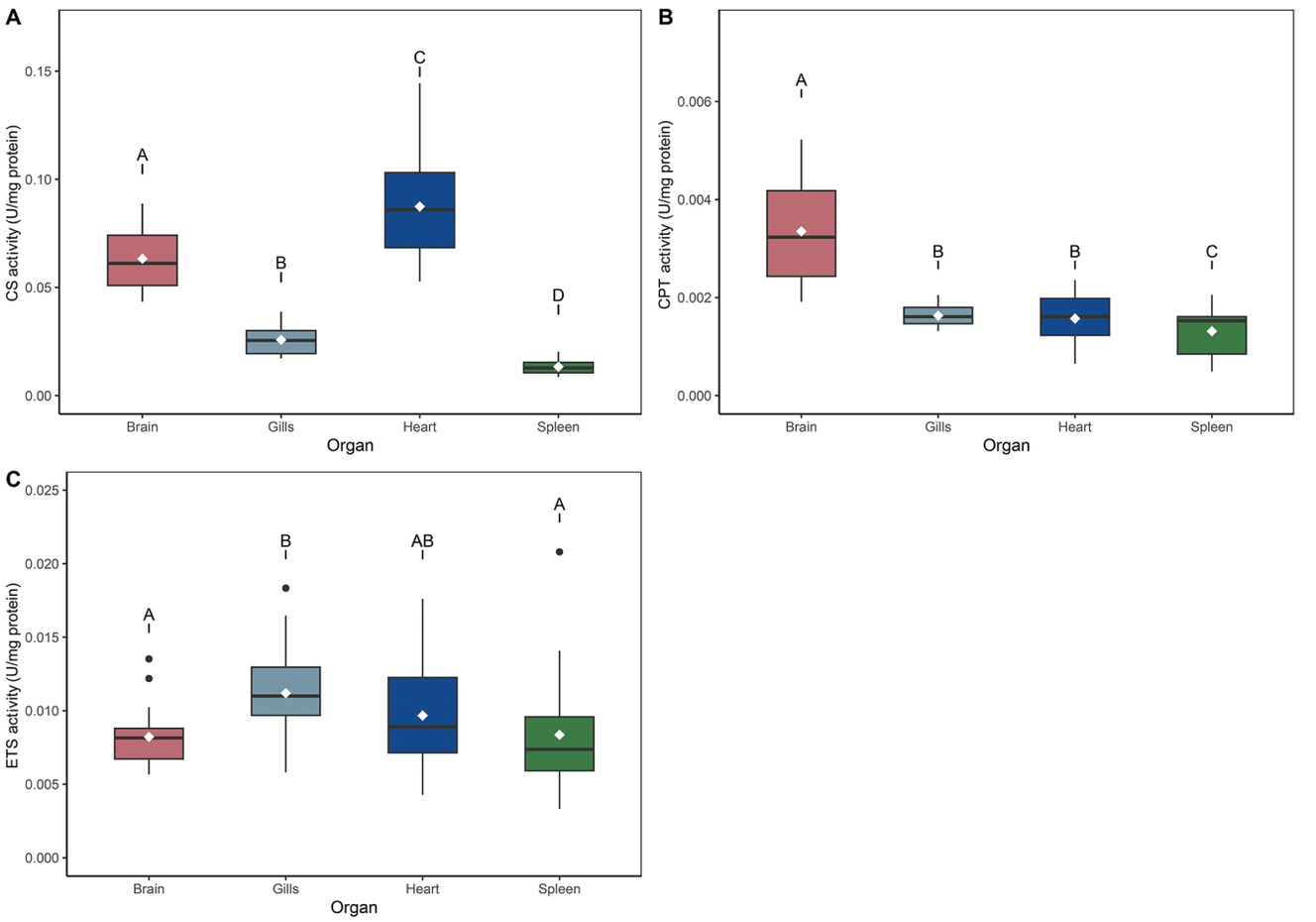

Figure 4. Differences in the enzymatic activity among organs. Boxplots represent differences in citrate synthase (CS; U mg^{-1} protein; *A*), carnitine palmitoyltransferase (CPT; U mg^{-1} protein; *B*), and electron transport system (ETS; U mg^{-1} protein; *C*) activities among all selected organs. Boxplots show the interquartile range, with the median represented as the line between the 25th and 75th percentiles. The vertical lines indicate minimum and maximum values, with points outside of these lines being potential outliers. The diamonds represent the mean enzymatic activity of each organ. Uppercase letters on top of the boxplots represent the significant differences.

macrochirus). This discrepancy may be because Lemly and Esch (1984) explored these relationships in a semicontrolled experimental setup (cages placed in a lake), as opposed to our wild-caught fish. In our case, it is impossible to know when parasite infections occurred in hosts and thus whether fish were experiencing acute stages of infection that are more likely to be associated with increased energetic costs (Lemly and Esch 1984). Given that the cestodes that we studied are known to damage the liver, an important glycogen reserve, we expected to find a significant negative relationship between parasite infection and host body condition. Heavy damage to the liver may result in a loss of function and in host fish resorting to other stored metabolic substrates (Santoro et al. 2013). For example, Slavik et al. (2017) found that *Cyprinus carpio* infected by glochidia of the Chinese pond mussel (*Sinanodonta Anodonta woodiana*) had an altered energy metabolism that could not be explained by variations of movement activities but by biochemical alterations of the liver and other organs. Although it would have been interesting to measure liver enzymatic activities directly, this is difficult because the liver tissue was often so heavily damaged in our population of fish that uninfected tissue was difficult to discriminate and collect properly with our dissection technique. The biomass of parasites was also very large in some individuals (mean = ~5% of fish mass, maximum = ~8% of fish mass), reaffirming the importance of correcting fish mass for parasite biomass (Lagrue and Poulin 2015; Timi and Poulin 2020). Protocols to collect intact liver tissue from this study system are currently being developed and offer an exciting avenue for future research (V. Mélançon, unpublished data). Also, while we could visually assess infection with black spot disease before collection, infection with cestodes could be determined only upon dissection. Since parasites tend to aggregate in hosts (Shaw and Dobson 1995), our collection protocol made it difficult to sample many fish heavily infected with cestodes.

Parasites and Host Mitochondrial Enzymatic Activity

Although no statistically significant relationship was detected, cestode density tended to be negatively related to mitochondrial

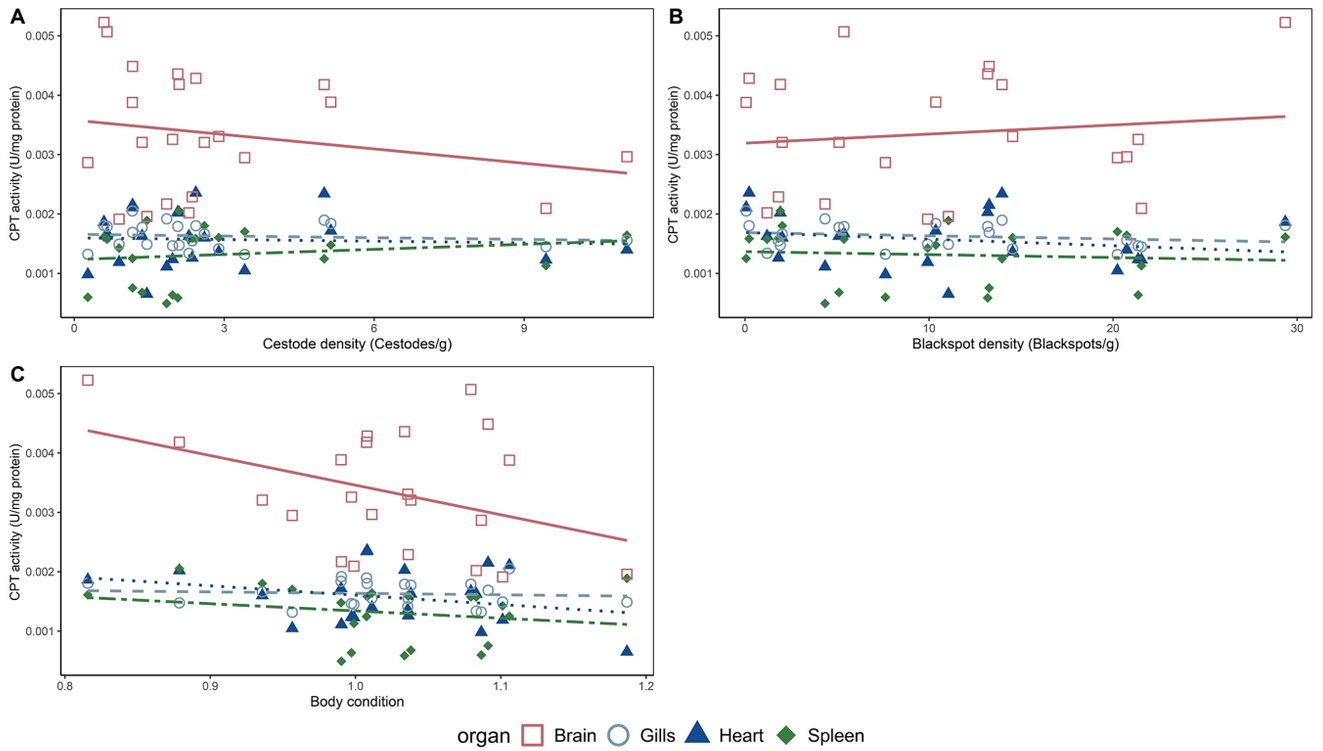

Figure 5. Relationships between parasite infections and enzymatic activity of carnitine palmitoyltransferase (CPT). Least squares linear regressions of the enzymatic activity (U mg^{-1} protein) of CPT as a function of cestode density (*A*), black spot density (*B*), and body condition (*C*).

enzymatic activity, whereas trematode density tended to be positively related to mitochondrial enzymatic activity (figs. 1, 2, 3*A*, 3*B*). These trends are interesting and merit further investigation. For instance, cestodes are metabolically active while in the host and may have a bigger impact on aerobic capacity, swimming speed, and other fitness-related traits, as they cause organ damage and consume nutrients from the host to continually support their own growth during their time in the host. Indeed, Guitard et al. (2022) found a negative correlation between host fish standard metabolic rate and cestode infection, suggesting that reduced metabolic activity during heavy infection possibly occurred as a result of parasite-induced damage to this metabolically active organ. In contrast, the impact of trematode infection on host metabolism may be limited to a brief period in time during the process of encystment when the host immune response is activated (Lemly and Esch 1984). Thambithurai et al (2022) found a positive relationship between host standard metabolic rate and black spot density, which suggests increased mitochondrial activity as well. Experimental infection with trematodes could help capture the acute phase of this infection when metabolic activities of hosts are likely the most affected.

Enzymatic Activity and Host Body Condition

Our results showed that CCO activity was correlated with a decreasing body condition index but in only one organ—the heart (fig. 2*C*). CCO is the mitochondrial enzyme involved in the transfer of electrons to oxygen, the final electron carrier in aerobic cellular respiration. CCO is believed to be a key component for mitochondrial oxidative metabolism (Capaldi 1990; Lemieux and Blier 2022). Higher enzymatic activity in the heart means increased overall capacity for energy production and muscular activity. Since the heart is responsible for the circulation of the blood throughout the body, increased energy production may lead to increased blood circulation to tissues. It is possible that fish in poor body condition experience increased nutrient demand and thus have greater heart activity. Alternatively, heart morphology may be modified by infection. In cold environments or in food-deprived states, fish hearts can undergo hypertrophy or hyperplasia to counter the decreased contractility caused by the environment temperature that affects the stroke volume of hearts (Gamperl and Farrell 2004). A similar response may occur following the biotic stress of parasite infection (Holmstrup et al. 2010). This could explain why an individual with a lower body condition index has a higher heart CCO activity. Including heart somatic index (i.e., heart mass expressed as a percentage of body wet mass) or another metric of heart morphology would be useful in the future to quantify this phenomenon and to confirm the hypertrophy or hyperplasia hypothesis in response to a parasite infection (Driedzic et al. 1996). In general, we tend to see a nonsignificant negative trend between enzymatic activity and

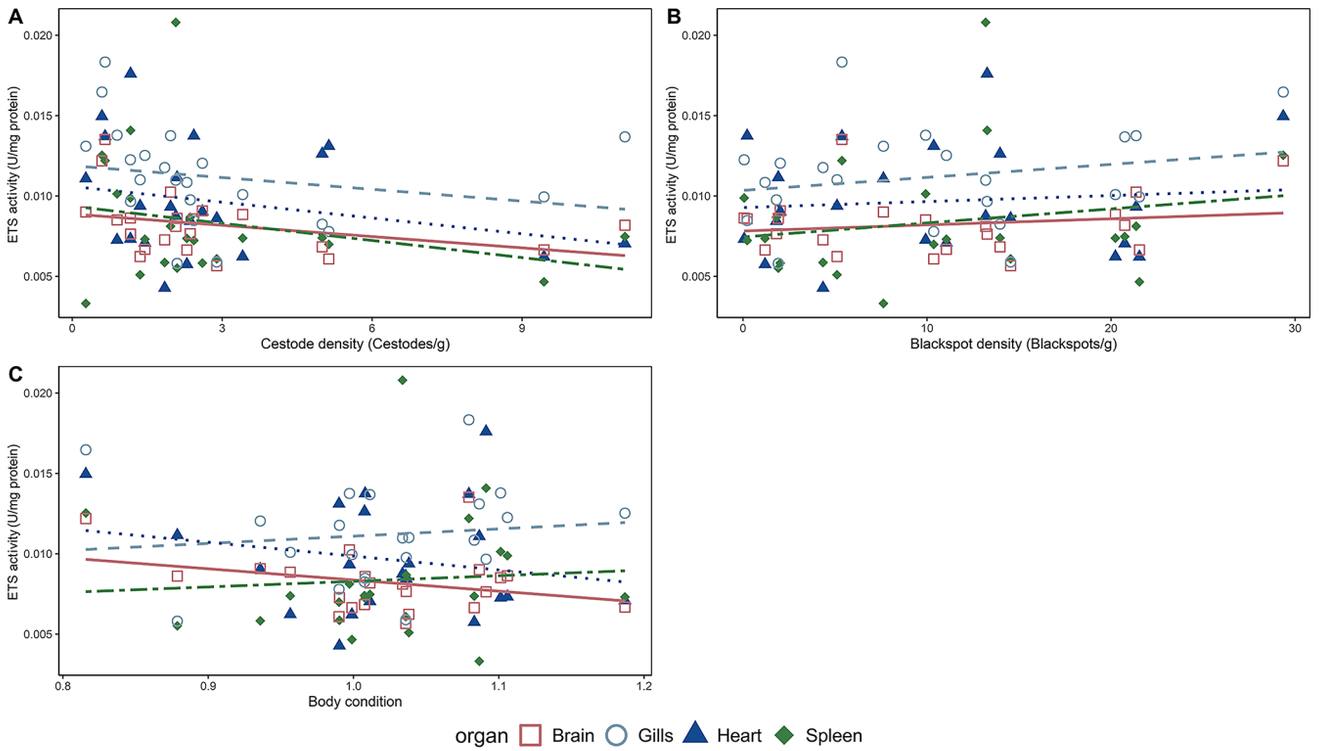

Figure 6. Relationships between parasite infections and enzymatic activity of the electron transport system (ETS; combined activity of complexes I and III). Least squares linear regressions of the enzymatic activity (U mg^{-1} protein) of the ETS as a function of cestode density (*A*), black spot density (*B*), and body condition (*C*).

body condition for all organs. Some of these trends are close to being significant, especially in the case of CPT, justifying increasing the sample size to clarify these trends.

Enzymatic Activity and Organ Type

Although activities of most tested enzymes did not change in response to parasite infection or body condition, they all differed significantly between organs. One notable example is CS, an enzyme of the TCA cycle widely used as a proxy of mitochondria density. The heart and the brain had significantly higher CS activities than other organs, possibly indicating a higher mitochondria density (fig. 4*A*). This coincides with the fact that the heart and the brain are two highly energy-dependent organs. Increased TCA activity and/or mitochondrial density leads to more reducing equivalents feeding the OXPHOS pathway, which may further increase ATP production. The heart has a high baseline energetic demand (Laurent et al. 1983). By undergoing hypertrophy or hyperplasia in response to physiological stress induced by parasite infections, it is likely that hearts require an increase in CS activity and/or in the number of mitochondria. These relationships should be further explored. Another interesting observation was CPT activity, which was highest in the brain (fig. 4*B*). This organ has stored lipids that can be used by neuronal cells to produce ATP (Soengas and Aldegunde 2002). By having a higher CPT activity, it shows that the brain potentially tends to consume more of its lipid reserve than other organs, possibly to compensate for a lack of substrates coming from the digestive system—a consequence of parasitism. The observed differences among organs cannot be explained by differences in their protein content (fig. S3). Thus, differences in enzymatic activities are more likely to be linked to organ pathway preferences/utilization than to protein content.

The spleen is a small organ with a low energetic demand when it is not mounting an immune reaction, and we found low activity in this organ for all tested enzymes. More research is needed to compare the enzymatic activity of spleen from fish with active immune systems. A study comparing fish injected with an immune stimulant, such as lipopolysaccharide, and/or fish infected experimentally could help correlate an immune response to parasite infections.

Conclusion

When studying natural populations, multiple biotic and abiotic factors should be considered (Holmstrup et al. 2010). Parasite infections are important biotic stressors that are more overlooked than abiotic factors (e.g., temperature, pH, hypoxia), despite their potential physiological costs. Thus, overlooking parasitic infection in physiology studies can be problematic (Suzuki et al. 2014; Timi and Poulin 2020). Our study represents

the first attempt to link parasite infection with changes in mitochondrial bioenergetics in four different organs of freshwater fish characterized by a gradient of parasite infection. Our results suggest that CCO activity in the heart might be linked to body condition and that CS, ETS, and CPT activities differ significantly between organs. Even though there were no significant relationships between fish parasite load and mitochondrial metabolism, interesting trends help pave the way toward a clearer understanding of cellular mechanisms underlying metabolic alterations experienced during infection, something not currently well explored in the literature. Additional sampling to obtain a broader range of cestode infection would help reveal trends across ecologically relevant infection gradients. Further studies should continue to pursue these questions across a greater range of organs (e.g., liver, muscle, gonads) and enzymes (e.g., enzymes of the glycolytic pathway, such as lactate dehydrogenase, and antioxidant enzymes, such as catalase and glutathione peroxidase) to deepen our mechanistic understanding of the role of parasites on host physiology in natural systems. Combining enzyme activities with other metabolism measures (e.g., metabolic rates, aerobic scope) might also indicate whether these analyses fail to show some effect of parasites on organisms. It would also help link whole-organism metabolism to cellular metabolism and establish possible underlying mechanisms.

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Literature Cited

Araújo A., A.M. Jansen, F. Bouchet, K. Reinhard, and L.F. Ferreira. 2003. Parasitism, the diversity of life, and paleoparasitology. Mem Inst Oswaldo Cruz 98:5–11.

Berra T.M. and R.-J. Au. 1978. Incidence of black spot disease in fishes in Cedar Fork Creek, Ohio. Ohio J Sci 78:318.

Binning S.A., D.G. Roche, and C. Layton. 2013. Ectoparasites increase swimming costs in a coral reef fish. Biol Lett 9:20120927.

Binning S.A., A.K. Shaw, and D.G. Roche. 2017. Parasites and host performance: incorporating infection into our understanding of animal movement. Integr Comp Biol 57:267–280.

Bonneaud C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the cost of mounting an immune response. Am Nat 161:367–379.

Bronte V. and M.J. Pittet. 2013. The spleen in local and systemic regulation of immunity. Immunity 39:806–818.

Brosset P., J.-M. Fromentin, F. Ménard, F. Pernet, J.-H. Bourdeix, J.-L. Bigot, E. Van Beveren, M.A.P. Roda, S. Choy, and C. Saraux. 2015*a*. Measurement and analysis of small pelagic fish condition: a suitable method for rapid evaluation in the field. J Exp Mar Biol Ecol 462:90–97.

Brosset P., F. Ménard, J.-M. Fromentin, S. Bonhommeau, C. Ulses, J.-H. Bourdeix, J.-L. Bigot, E. Van Beveren, D. Roos, and C. Saraux. 2015*b*. Influence of environmental variability and age on the body condition of small pelagic fish in the Gulf of Lions. Mar Ecol Prog Ser 529:219–231.

Caffaro C.E. and J.C. Boothroyd. 2011. Evidence for host cells as the major contributor of lipids in the intravacuolar network of *Toxoplasma*-infected cells. Eukaryot Cell 10:1095–1099.

Capaldi R.A. 1990. Structure and function of cytochrome c oxidase. Annu Rev Biochem 59:569–596.

Charron A.J. and L.D. Sibley. 2002. Host cells: mobilizable lipid resources for the intracellular parasite *Toxoplasma gondii*. J Cell Sci 115:3049–3059.

Debeffe L., P.D. Mcloughlin, S.A. Medill, K. Stewart, D. Andres, T. Shury, B. Wagner, E. Jenkins, J.S. Gilleard, and J. Poissant. 2016. Negative covariance between parasite load and body condition in a population of feral horses. Parasitology 143:983–997.

Dobson A., K.D. Lafferty, A.M. Kuris, R.F. Hechinger, and W. Jetz. 2008. Homage to Linnaeus: how many parasites? how many hosts? Proc Natl Acad Sci USA 105:11482–11489.

Driedzic W.R., J.R. Bailey, and D.H. Sephton. 1996. Cardiac adaptations to low temperature in non-polar teleost fish. J Exp Zool 275:186–195.

Escoll P., L. Platon, and C. Buchrieser. 2019. Roles of mitochondrial respiratory complexes during infection. Immunometabolism 1:e190011.

Eure H. 1976. Seasonal abundance of *Proteocephalus ambloplitis* (Cestoidea: Proteocephalidea) from largemouth bass living in a heated reservoir. Parasitology 73:205–212.

Fänge R. and S. Nilsson. 1985. The fish spleen: structure and function. Experientia 41:152–158.

Fischer H. and R.S. Freeman. 1969. Penetration of parenteral plerocercoids of *Proteocephalus ambloplitis* (Leidy) into the gut of smallmouth bass. J Parasitol 55:766–774.

Freitak D., I. Ots, A. Vanatoa, and P. Hörak. 2003. Immune response is energetically costly in white cabbage butterfly pupae. Proc R Soc B 270:S220–S222.

Gamperl A.K. and A. Farrell. 2004. Cardiac plasticity in fishes: environmental influences and intraspecific differences. J Exp Biol 207:2539–2550.

Guitard J.J., E. Chrétien, J. De Bonville, D.G. Roche, D. Boisclair, and S.A. Binning. 2022. Increased parasite load is associated with reduced metabolic rates and escape responsiveness in pumpkinseed sunfish. J Exp Biol 225:jeb243160.

Hiltunen J.K., K.J. Autio, M.S. Schonauer, V.A.S. Kursu, C.L. Dieckmann, and A.J. Kastaniotis. 2010. Mitochondrial fatty acid synthesis and respiration. Biochim Biophys Acta 1797: 1195–1202.

Holmstrup M., A.-M. Bindesbøl, G.J. Oostingh, A. Duschl, V. Scheil, H.-R. Köhler, S. Loureiro, A.M. Soares, A.L. Ferreira, and C. Kienle. 2010. Interactions between effects of environmental chemicals and natural stressors: a review. Sci Total Environ 408:3746–3762.

Hortová-Kohoutková M., P. Lázničková, and J. Frič. 2021. How immune-cell fate and function are determined by metabolic pathway choice. BioEssays 43:2000067.

Hugghins E.J. 1959. Parasites of fishes in South Dakota. SD Agric Exp Stn Bull 484.

Hunter-Manseau F., V. Desrosiers, N.R. Le Francois, F. Dufresne, H.W. Detrich III, C. Nozais, and P.U. Blier. 2019. From Africa to Antarctica: exploring the metabolism of fish heart mitochondria across a wide thermal range. Front Physiol 10:1220.

Jakob E.M., S.D. Marshall, and G.W. Uetz. 1996. Estimating fitness: a comparison of body condition indices. Oikos 77:61–67.

Kastaniotis A.J., K.J. Autio, J.M. Keratar, G. Monteuuis, A.M. Makela, R.R. Nair, L.P. Pietikainen, A. Shvetsova, Z. Chen, and J.K. Hiltunen. 2017. Mitochondrial fatty acid synthesis, fatty acids and mitochondrial physiology. Biochim Biophys Acta 1862:39–48.

Lagrue C. and R. Poulin. 2015. Measuring fish body condition with or without parasites: does it matter? J Fish Biol 87:836–847.

Laiz-Carrión R., S. Sangiao-Alvarellos, J.M. Guzmán, M.P. Martín del Río, J.M. Míguez, J.L. Soengas, and J.M. Mancera. 2002. Energy metabolism in fish tissues related to osmoregulation and cortisol action. Fish Physiol Biochem 27:179–188.

Laurent P., S. Holmgren, and S. Nilsson. 1983. Nervous and humoral control of the fish heart: structure and function. Comp Biochem Physiol 76:525–542.

Le Cren E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J Anim Ecol 20:201.

Lemieux H. and P.U. Blier. 2022. Exploring thermal sensitivities and adaptations of oxidative phosphorylation pathways. Metabolites 12:360.

Lemly A.D. and G.W. Esch. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. J Parasitol 70:475–492.

Lentini G., N. Dos Santos Pacheco, and B.A. Burleigh. 2018. Targeting host mitochondria: a role for the *Trypanosoma cruzi* amastigote flagellum. Cell Microbiol 20:e12807.

Leung T.L. and R. Poulin. 2008. Parasitism, commensalism, and mutualism: exploring the many shades of symbioses. Vie Milieu 58:107–115.

Lewin R. 1982. Symbiosis and parasitism—definitions and evaluations. BioScience 32:254–260.

Liu L., R. Fang, Z. Wei, J. Wu, X. Li, and W. Li. 2020. *Giardia duodenalis* induces apoptosis in intestinal epithelial cells via reactive oxygen species-mediated mitochondrial pathway in vitro. Pathogens 9:693.

Magistretti P. and I. Allaman. 2013. Brain energy metabolism. Pp. 1591–1620 in D.F. Pfaff, ed. Neuroscience in the 21st century: from basic to clinical. Springer, New York.

Marcogliese D.J. 2004. Parasites: small players with crucial roles in the ecological theater. EcoHealth 1:151–164.

Margolis L. and J.R. Arthur. 1979. Synopsis of the parasites of fishes of Canada. Bull Fish Res Board Can 199:1–269.

McElroy E.J. and I. de Buron. 2014. Host performance as a target of manipulation by parasites: a meta-analysis. J Parasitol 100:399–410.

Mills E.L., B. Kelly, and L.A.J. O'Neill. 2017. Mitochondria are the powerhouses of immunity. Nat Immunol 18:488–498.

Nadler L.E., E. Bengston, E.J. Eliason, C. Hassibi, S.H. Helland-Riise, I.B. Johansen, G.T. Kwan, M. Tresguerres, A.V. Turner, and K.L. Weinersmith. 2021. A brain-infecting parasite impacts host metabolism both during exposure and after infection is established. Funct Ecol 35:105–116.

Nath S. and J. Villadsen. 2015. Oxidative phosphorylation revisited. Biotechnol Bioeng 112:429–437.

O'Dwyer K., F. Dargent, M.R. Forbes, and J. Koprivnikar. 2020. Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: a meta-analysis. J Anim Ecol 89:519–529.

Ots I., A.B. Kerimov, E.V. Ivankina, T.A. Ilyina, and P. Hõrak. 2001. Immune challenge affects basal metabolic activity in wintering great tits. Proc R Soc B 268:1175–1181.

Pedersen A.B. and A. Fenton. 2007. Emphasizing the ecology in parasite community ecology. Trends Ecol Evol 22:133–139.

Pernas L., C. Bean, J.C. Boothroyd, and L. Scorrano. 2018. Mitochondria restrict growth of the intracellular parasite *Toxoplasma gondii* by limiting its uptake of fatty acids. Cell Metab 27:886–897.

Poulin R. 1992. Toxic pollution and parasitism in freshwater fish. Parasitol Today 8:58–61.

———. 2010. Parasite manipulation of host behavior: an update and frequently asked questions. Pp. 151–186 in H.J. Brockmann, ed. Advances in the study of behavior. Elsevier, Amsterdam.

R Development Core Team. 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Rees B.B., J.E. Reemeyer, and B.A. Irving. 2022. Interindividual variation in maximum aerobic metabolism varies with gill morphology and myocardial bioenergetics in Gulf killifish. J Exp Biol 225:jeb243680.

Robar N., D.L. Murray, and G. Burness. 2011. Effects of parasites on host energy expenditure: the resting metabolic rate stalemate. Can J Zool 89:1146–1155.

Ryan D.G., C. Frezza, and L.A. O'Neill. 2021. TCA cycle signalling and the evolution of eukaryotes. Curr Opin Biotechnol 68:72–88.

Sánchez C.A., D.J. Becker, C.S. Teitelbaum, P. Barriga, L.M. Brown, A.A. Majewska, R.J. Hall, and S. Altizer. 2018. On the

relationship between body condition and parasite infection in wildlife: a review and meta-analysis. Ecol Lett 21:1869–1884.

Santoro M., S. Mattiucci, T. Work, R. Cimmaruta, V. Nardi, P. Cipriani, B. Bellisario, and G. Nascetti. 2013. Parasitic infection by larval helminths in Antarctic fishes: pathological changes and impact on the host body condition index. Dis Aquat Organ 105:139–148.

Schulz H. 1991. Beta oxidation of fatty acids. Biochim Biophys Acta 1081:109–120.

Shaw D. and A. Dobson. 1995. Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology 111:S111–S133.

Simon A., D. Thomas, J. Blondel, P. Perret, and M.M. Lambrechts. 2004. Physiological ecology of Mediterranean blue tits (*Parus caeruleus* L.): effects of ectoparasites (*Protocalliphora* spp.) and food abundance on metabolic capacity of nestlings. Physiol Biochem Zool 77:492–501.

Slavík O., P. Horký, K. Douda, J. Velíšek, J. Kolářová, and P. Lepič. 2017. Parasite-induced increases in the energy costs of movement of host freshwater fish. Physiol Behav 171:127–134.

Smith P.E., R.I. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Olson, and D. Klenk. 1985. Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85.

Soengas J.L. and M. Aldegunde. 2002. Energy metabolism of fish brain. Comp Biochem Physiol 131:271–296.

Stanley I.A., S.M. Ribeiro, A. Giménez-Cassina, E. Norberg, and N.N. Danial. 2014. Changing appetites: the adaptive advantages of fuel choice. Trends Cell Biol 24:118–127.

Suzuki N., R.M. Rivero, V. Shulaev, E. Blumwald, and R. Mittler. 2014. Abiotic and biotic stress combinations. New Phytol 203:32–43.

Syn G., D. Anderson, J.M. Blackwell, and S.E. Jamieson. 2017. *Toxoplasma gondii* infection is associated with mitochondrial dysfunction in-vitro. Front Cell Infect Microbiol 7:512.

Thambithurai D., I. Lanthier, E. Contant, S.S. Killen, and S.A. Binning. 2022. Fish vulnerability to capture by trapping is modulated by individual parasite density. Proc R Soc B 289: 20221956.

Thibault M., P. Blier, and H. Guderley. 1997. Seasonal variation of muscle metabolic organization in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 16:139–155.

Timi J.T. and R. Poulin. 2020. Why ignoring parasites in fish ecology is a mistake. Int J Parasitol 50:755–761.

Tseng Y.-C. and P.-P. Hwang. 2008. Some insights into energy metabolism for osmoregulation in fish. Comp Biochem Physiol 148:419–429.

Turner W.C., W.D. Versfeld, J.W. Kilian, and W.M. Getz. 2012. Synergistic effects of seasonal rainfall, parasites and demography on fluctuations in springbok body condition. J Anim Ecol 81:58–69.

Wendelaar Bonga S.E. 1997. The stress response in fish. Physiol Rev 77:591–625.

Wiegand G. and S.J. Remington. 1986. Citrate synthase: structure, control, and mechanism. Annu Rev Biophys Biophys Chem 15:97–117.

Wuenschel M.J., W.D. McElroy, K. Oliveira, and R.S. McBride. 2019. Measuring fish condition: an evaluation of new and old metrics for three species with contrasting life histories. Can J Fish Aquat Sci 76:886–903.