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Brood predation pressure during parental care does not influence parental enzyme activities related to swimming activity in a teleost fish

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ABSTRACT

Predation is considered one of the main costs to reproduction but is rarely examined from a physiological perspective. In particular, little is known about the influence of brood predation pressure on the physiology of parents engaged in care. Brood defense, even when there is no direct threat to the parent, can be costly as it requires constant vigilance and chasing predators to protect the developing brood and maintain parental investment (i.e., fitness). Our goal was to examine the influence of natural variation in nest predation pressure on the physiology of the teleost smallmouth bass *Micropterus dolomieu*, an animal that provides sole-paternal care for developing offspring. More specifically, we used indicators of anaerobic (lactate dehydrogenase [LDH]) and aerobic capacity (cytochrome c oxidase [CCO] and citrate synthase [CS]) in axial white muscle and pectoral red muscle to test for differences in antipredator performance of nest guarding males across six lakes with natural variation in nest predation pressure. Pectoral red muscle enzyme activities and protein concentrations were highly conserved among populations, while axial white muscle showed differences in LDH activities, CCO activities and protein concentrations. However, there was no evidence for higher metabolic capacities in fish from lakes with increased brood predation pressure. Clearly, factors other than predation pressure have a greater influence on white muscle metabolic capacities. Additional research is needed to clarify the extent to which biotic and abiotic factors influence the enzyme activity and organismal performance in wild animals, particularly at the level of the individual and population.

1. Introduction

Predation is considered one of the greatest costs associated with reproduction (Magnhagen, 1991). Most individuals engaged in reproductive activities become more prone to predation due to physical (e.g., ornamentation, pregnancy, nuptial coloration) or behavioural changes (e.g., mate searching, signaling and calling). As a result most research has explored how predators use the cues of reproducing individuals in order to exploit them (reviewed by Zuk and Kolluru, 1998) or how animals engaged in reproduction reduce the risk of individual predation (reviewed by Lima and Dill, 1990). Alternatively, there are many animals that face low risk of predation during reproduction but whose main goal during this time is to ensure the survival of vulnerable offspring. This specialized behaviour has evolved in most animal taxa (e.g., arachnids, insects, reptiles, fish, mammals, birds) and in environments where offspring face difficult environmental conditions such as limited food availability, extreme temperatures, low oxygen, or high levels of predation (Clutton-Brock, 1991). Nest predator abundance and predation pressure can greatly vary across the reproductive range of a species (Steinhart et al., 2005; Fontaine et al., 2007; Gravel and Cooke, 2009) and the inability of a parent to defend its brood has severe fitness consequences. As such, selective pressures should act on parental performance under these conditions.

The physiology associated with predation pressure has typically been examined from a stress response perspective. Natural variation in predation pressure influences the level of physiological stress response of prey species (Monclus et al., 2009) and much work is being conducted to identify the physiological mechanisms involved in the growth/predation risk trade-off (Slos and Stoks, 2008). Alternatively, the physiological response to variation in predation pressure may also relate to an individual's antipredator performance, such as escape speed or other antipredator defenses. Several physiological tools have been identified as indicators of individual performance and have been used to link organisms to their ecological environment (e.g., Sullivan and Somero, 1983; Kaufman et al., 2006; Selch and Chipps, 2007). One of particular interests is the link between metabolic capacities, the locomotor performance of fish, and the factors that affect this relationship (reviewed by Guderley, 2004).

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Muscle glycolytic (i.e., lactate dehydrogenase [LDH]) and aerobic (i.e., cytochrome c oxidase [CCO], citrate synthase [CS]) enzyme activities have been shown to be correlated with burst swimming speed and endurance swimming capacity in a variety of fish (e.g., Garenc et al., 1999; Martinez et al., 2003). Such enzymes are also ecologically sensitive as they may vary with habitat type (e.g., depth of occurrence Sullivan and Somero, 1980), prey community (Kaufman et al., 2006; Selch and Chipps, 2007) and predation pressure (Odell et al., 2003). These physiological indicators can thus be used as tools to examine questions that relate to performance and environmental variation such as predation pressure.

Here we examine how nest predation pressure influences the physiology of parental care in a species that provides sole-paternal care, the teleost freshwater fish, smallmouth bass (*Micropterus dolomieu*). Parental care activities include fanning eggs to prevent silt deposition, maintaining vigilance while patrolling the nest area (using sustained swimming actions), and protecting offspring from nest predators by chasing away predators (with burst swimming events). In a system with relatively high nest predation pressure, Cooke et al. (2002) determined that parental smallmouth bass swam the equivalent of more than 40 km per day without leaving the immediate vicinity of the nest. Moreover, nearly 20% of the time bass were swimming at speeds in excess of 80% of critical swimming speeds indicative of anaerobic bursting to chase predators. Conversely, in a lake with very few nest predators, Hinch and Collins (1991) observed very few incidences of bursting activity though the fish were similarly vigilant in patrolling the nest area. The parental care period of smallmouth bass typically lasts four weeks (Ridgway, 1988; Cooke et al., 2006) and is known to be physiologically and energetically demanding (Cooke et al., 2002; Cooke, 2004; Hanson and Cooke, 2009). We hypothesize that natural variation in nest predation pressure has the ability to influence parental physiology. More specifically, we predicted that males from lakes with high predation pressure will exhibit greater anaerobic and aerobic muscle enzyme activities in their axial musculature than males from lakes with low predation pressure, due to the need for increased anaerobic burst swimming events associated with engaging predators and increased aerobic patrolling associated with nest vigilance and guarding. Conversely, we expect little difference in enzyme activities in the oxidative pectoral muscles of parental smallmouth bass as pectoral muscles are actively involved in egg fanning but most likely play an inconsequential role in burst swimming events or patrolling, which are typically used to deter nest predators. Studies that integrate animal behaviour and physiology are urgently needed to better understand the role of environmental variation on the performance (Altmann and Altmann, 2003; Gilmour et al. 2005) and ultimately the fitness of individuals (Ricklefs and Wikelski, 2002).

2. Material and methods

2.1. Study sites and sampling design

Fish were sampled from six lakes within a single ecoregion in southeastern Ontario, Canada: Big Rideau Lake, Charleston Lake, Indian Lake, Newboro Lake, Opinicon Lake and Sand Lake. Study lakes were chosen due to the inherent variation in nest predation pressure as documented and described in Gravel and Cooke (2009) with a series of metrics such as number of predators in proximity to nests when male is present (perceived predation pressure) and when male is absent (actual predation pressure), time to egg consumption in the absence of males and proportion of nests predated. By using non-parametric ranking tests, lakes were ordered from lowest to highest in nest predation pressure: Big Rideau Lake < Newboro Lake ≤ Charleston Lake < Indian Lake < Sand Lake < Opinicon Lake. Lake surface area, mean depth and predation pressure metrics are summarized in Table 1 (taken from Gravel and Cooke 2009). These predation pressure metrics were measured on the same individuals which were sampled for muscle enzyme activities. Predation pressure metrics were again measured in 2008 and 2009 and lake ranking has been very similar, lakes with the lowest and highest predation pressure rank identically over the years with some variation in the medium predation pressure lakes (Gravel, M.-A. unpublished data).

Within this ecoregion, differences in lake depth and turbidity cause lakes to warm differentially, allow for temporal variation in peak spawning dates (Kubacki et al., 2002) and enable data collection within one spawning year. At the onset of spring, the six lakes were visited daily by snorkelers. Portions of the littoral zone were swum (approx. 1 to 3 km) and when present, parental males on fresh eggs were identified ($n \leq 30$) and nests were labeled with a numbered tile. All data collection occurred during May and June of 2007. Fish were sampled on fresh eggs and were collected by rod and reel (using heavy angling gear — all angling durations <20 s) within 3 days of egg deposition for physiological analysis of adult males ($n = 10$ nesting adult males per lake).

Parental male fish were removed from their nest and placed in a foam-lined trough filled with fresh lake water for hook removal. Fish were then euthanized by cerebral percussion within 2 min of being on board the boat. Pectoral red muscle and axial white muscle samples were taken with a disposable scalpel, wrapped in foil, and immediately placed in liquid nitrogen until later transfer to a −80 °C freezer. Pectoral red muscle was taken anterior and ventral to the pectoral fin, when it laid flat against the fish, while the axial white muscle sample was taken mid-way down the body, 1 cm above the lateral line.

2.2. Enzyme activities

White and red muscle samples were randomly chosen, thawed on ice, weighed and diluted 10 - fold with homogenizing buffer containing 20 mM HEPES, 1 mM EDTA, and 0.1% Triton X-100. Tissues were homogenized in plastic test tubes constantly immersed in ice with a Janke and Kunkel Ultra Turrax T25 homogenizer (Janke and Kunkel, Staufen, Germany) with 14 cm × 0.75 cm probe at maximal speed (24 000 rpm). All assays were performed in duplicate at 20 °C on a UV/Vis spectrophotometer (Varian Cary 100; Varian Inc., Palo Alto, CA, USA) with an assay volume of 1 mL. Substrate and cofactor concentrations were determined from assay optimization. Lactate dehydrogenase (LDH—EC 1.1.1.27), citrate synthase (CS—EC 4.1.3.7) and cytochrome c oxidase (CCO—EC 1.9.3.1) were measured as in Couture et al. (1998) with the following modifications. For LDH

Table 1
Lake characteristics and predation pressure metrics (predation pressure metrics adapted from Gravel and Cooke, 2009 and Marleau, 2007).

Lake	Surface area (hectares)	Mean depth (m)	Perceived predation pressure (max # predators during 15 min observation — male present) (mean ± SE) $n = 10$ per lake	Actual predation pressure (max # predators during 15 min observation — male absent) (mean ± SE) $n = 10$ per lake	Time to first nest predator arrival (mean ± SE) $n = 10$ per lake	Proportion of nested predated $n = 10$ per lake
Big Rideau	6482	12.3	0.2 ± 0.1	1.7 ± 1.3	11.4 ± 1.9	0.30
Newboro	1850	3	0.6 ± 0.2	2.3 ± 1.2	12.2 ± 1.8	0.56
Charleston	2518	17.4	0.8 ± 0.5	6.3 ± 3.0	10.2 ± 1.8	0.50
Indian	266	10	3.0 ± 1.6	5.8 ± 2.3	8.9 ± 1.8	0.60
Sand	828	4.5	7.2 ± 3.0	14 ± 5.5	6.7 ± 2.1	0.67
Opinicon	7	3	4.1 ± 0.8	16.2 ± 5.5	3.0 ± 1.4	0.90

activity in white axial muscle and red pectoral muscle, dilutions of 1:1000 were made from the homogenized tissues. For CS activity in white axial muscles, assay conditions were changed to 0.1 acetylCoA. For CS activity in red pectoral muscles assay conditions were changed to 0.1 acetylCoA and 0.15 oxalacetate. For CCO activity in red pectoral muscle, dilutions of 1:1000 were made from the homogenized tissues. The reactions were linear over the 5 min period used for the calculation of enzyme activity, and the results are expressed in international units (IU; μmol of substrate converted to product per min) per g tissue mass. Protein concentrations were analyzed as in Lowry et al. (1951) and were determined against a bovine serum albumin (BSA) standard curve. Enzyme activities were also calculated as IU per mg of tissue protein, allowing us, by comparison to enzyme activities expressed on a wet weight basis, to examine whether differences in enzyme activities among groups were due to differences in tissue protein concentration or to up- or down-regulation of the enzymes examined.

2.3. Statistical analysis

All statistical analyses were performed using JMP 7.0 (SAS institute, Cary, NC, USA). Data were tested for normality and homogeneity of variance and non-normal data were log (axial muscle LDH and CS) or square-root (pectoral muscle CS and LDH) transformed to achieve normality. Where appropriate, transformed data were used for statistics but non-transformed data are always presented in figures. The relationship between enzyme activities and muscle protein concentration, as well as the relationship between enzyme activities, were tested using model I regressions. We used one-way ANOVAs to test for differences in mean enzyme activities and protein concentration of axial and pectoral muscle among lake populations. Tests were followed with planned multiple comparisons (Tukey–Kramer, *post hoc*) when significant differences were present. The non-parametric Kruskal–Wallis test was used in one instance when homogeneous variance was not established (fish weight) and was followed by non-parametric multiple comparisons (Zar, 1999). Values presented are means ± standard error (SE) and the significance of all tests was evaluated at $\alpha = 0.05$.

3. Results

3.1. Axial muscle

Parental smallmouth bass sampled for measurements of enzyme activities and protein concentrations did not differ in total length ($F_{5,52} = 0.87$, $p = 0.51$; mean ± SE: 408 ± 5.8 mm) or in total weight ($F_{5,52} = 0.98$, $p = 0.44$; mean ± SE: 996.8 ± 45.8 g). Axial enzyme activities were not significantly correlated to protein concentration (CCO: $R = 0.03$, $p = 0.81$; CS: $R = 0.02$, $p = 0.90$; LDH: $R = 0.22$, $p = 0.09$, Fig. 1A). Indicators of anaerobic capacity as indicated by LDH enzyme activities expressed per g wet mass, differed among populations ($F_{5,52} = 4.65$, $p = 0.001$), but contrary to our predictions, higher values of LDH activity were not associated with the highest predation pressure (Fig. 2A). Fish from lakes with low to intermediate predation pressure (Big Rideau Lake and Charleston Lake) showed the highest LDH activities, while individuals from lakes with higher predation pressure (Opinicon Lake, Sand Lake and Indian Lake) showed intermediate LDH values (Fig. 2A). Of the indicators of aerobic capacity, CCO activity differed among populations ($F_{5,39} = 3.45$, $p = 0.01$, Fig. 2B), while CS did not ($F_{5,53} = 1.52$, $p = 0.2$, Fig. 2C). CCO failed to exhibit the pattern we had predicted with no clear trend between predation pressure and CCO activity. Axial muscle protein concentration differed among populations (Kruskal–Wallis: $\chi^2 = 13.21$, $p = 0.02$, Fig. 2D). Furthermore, differences among populations for LDH activities were reduced when activities were expressed per mg protein ($F_{5,52} = 2.12$, $p = 0.08$), while patterns remained the same for CS ($F_{5,53} = 1.6$, $p = 0.19$) and CCO ($F_{5,39} = 4.5$, $p = 0.003$). Axial LDH activities were not correlated to CCO

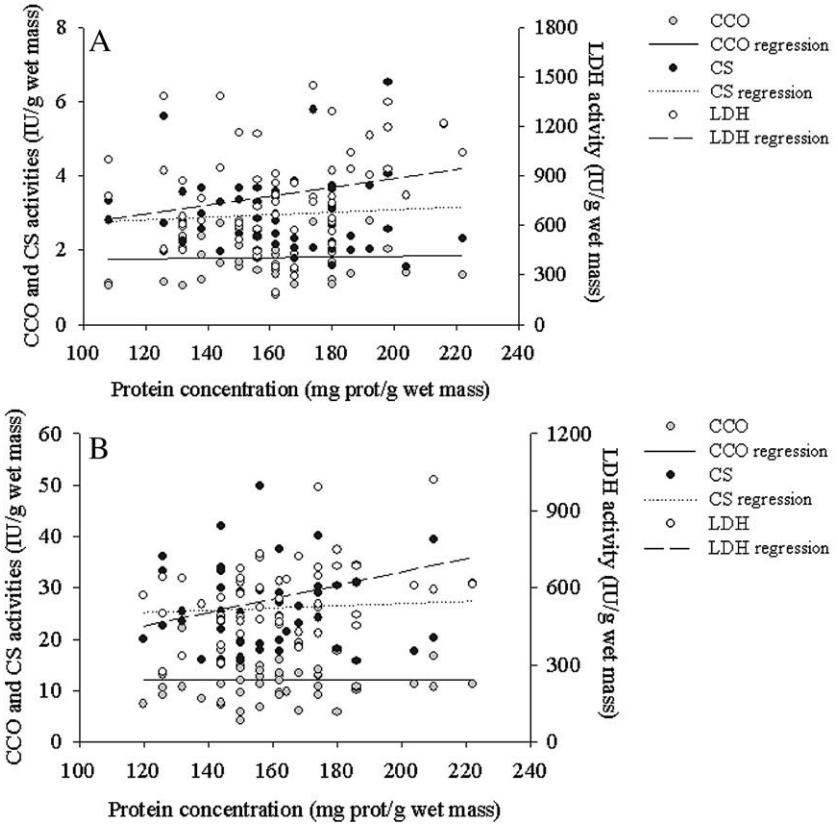

Fig. 1. Relationships between axial muscle protein concentration and CCO, CS and LDH axial muscle activities (A), and between pectoral muscle protein concentration and CCO, CS and LDH pectoral muscle activities (B).

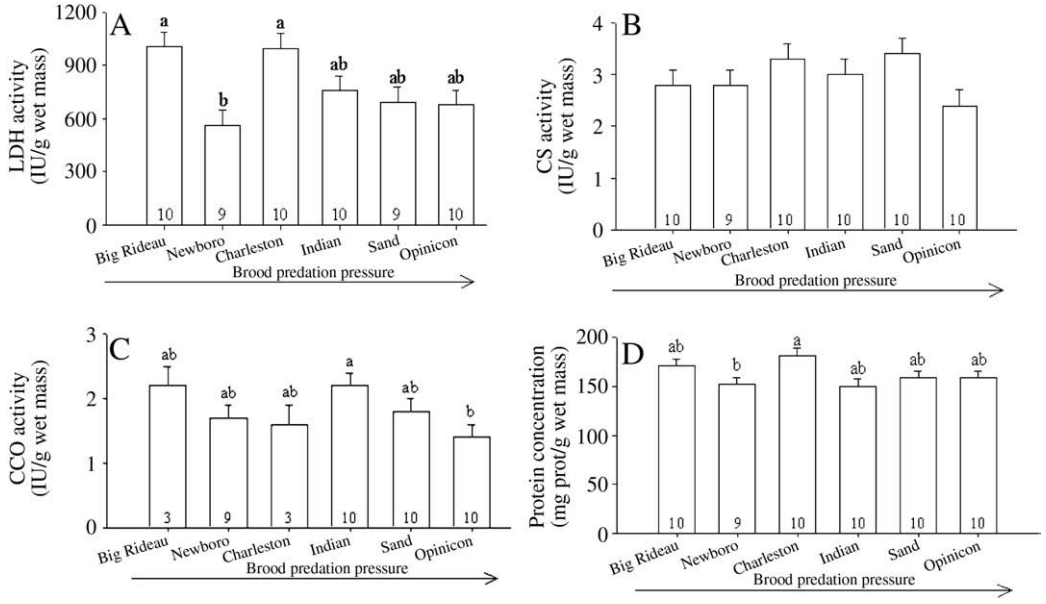

Fig. 2. LDH (A), CS (B), CCO (C) enzyme activities and protein concentration (D) of axial white muscle of parental smallmouth bass across lakes with natural variation in nest predation pressure. Lakes are presented from lowest to highest predation pressure. Dissimilar letters denote significant differences between lakes within a given physiological parameter (Tukey *post hoc* test, $p<0.05$).

or CS activities (LDH vs CCO: $R=17$, $p=0.29$; LDH vs CS: $R=0.08$, $p=0.57$) but CCO and CS activities were positively correlated ($R=0.54$, $p=0.0001$, Fig. 3A).

3.2. Pectoral muscle

Pectoral LDH activities were positively correlated with pectoral muscle protein concentration ($R=0.40$, $P=0.004$), while CCO and CS were not (CCO: $R=0.06$, $p=0.67$; CS: $R=0.001$, $p=0.99$, Fig. 1B). Consistent with our prediction, indicators of anaerobic and aerobic capacities of pectoral muscle did not differ among populations when expressed per g wet mass (LDH: $F_{5,45}=1.56$, $p=0.19$; CS: $F_{5,45}=1.06$, $p=0.39$; CCO: $F_{5,45}=0.85$, $p=0.52$, Fig. 4A–C) or mg protein (LDH: $F_{5,45}=0.09$, $p=0.09$; CS: $F_{5,45}=1.12$, $p=0.36$; CCO: $F_{5,45}=0.63$, $p=0.07$). Parental males from all lakes showed similar protein concentration in their pectoral muscles ($F_{5,45}=0.88$, $p=0.50$, Fig. 4D). Pectoral LDH activities were not correlated to CCO or CS activities (LDH vs CCO: $R=0.17$, $p=0.29$; LDH vs CS: $R=0.08$, $p=0.57$) but CCO and CS activities were positively correlated ($R=0.39$, $p=0.005$, Fig. 3B).

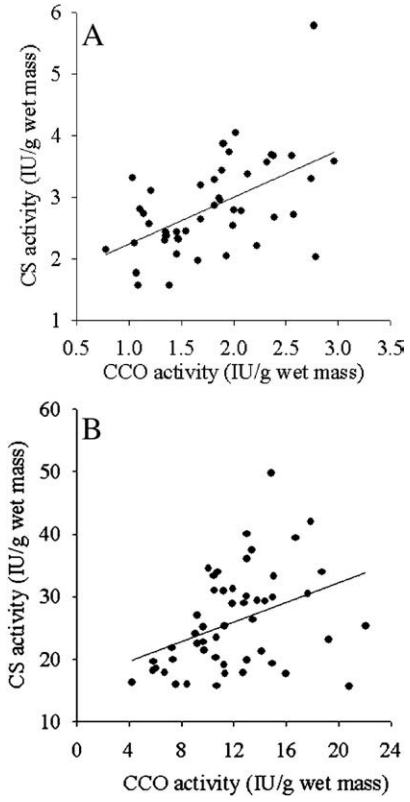

Fig. 3. Relationship between axial muscle (A) and pectoral muscle (B) CCO and CS activities.

4. Discussion

Antipredator behaviours are well studied and relatively well understood (reviewed by Lima and Dill, 1990). However, much less is known about the physiological consequences of predation pressure. Some attention has been placed on the physiological stress response of predation pressure (Scheuerlein et al., 2001) and recent work has explored the trade-offs between growth and predation risk (McPeek et al., 2001; Stoks et al., 2005). In this study, we set out to test if nest predation pressure could influence the physiological performance of a parental care providing species. In agreement with our hypotheses, we found variation in enzyme activities and protein concentration in the axial musculature of the parental smallmouth bass, but no variation of these parameters in the pectoral muscle. However, contrary to our expectations, variations in enzyme activities did not follow the gradient in nest predation pressure. Lakes with the lowest predation pressure often did not group together physiologically (e.g., Big Rideau Lake and Newboro Lake; Fig. 2A) and there was evidence for fish from lakes with low predation pressure (e.g., Big Rideau Lake and Charleston Lake, Fig. 2A) having the highest indicators of anaerobic performance capacity. Such findings are contrary to our prediction which leads to several alternative explanations.

Although muscle enzyme activities provide information on a recent timescale (days–weeks; Nathanailides, 1996) and our results represent the physiological condition of smallmouth bass providing parental care, it is possible that the enzyme activities of fish from these lakes differ for other ecological reasons. Over 95% of nest predators identified in Gravel and Cooke (2009) were *Lepomis spp*,

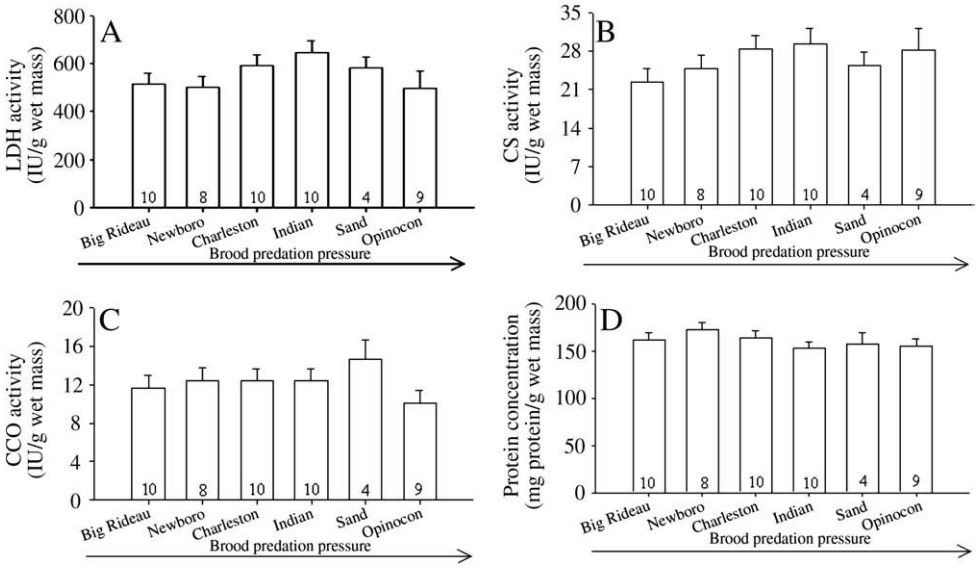

Fig. 4. LDH (A), CS (B), CCO (C) enzyme activities and protein concentration of pectoral red muscle of parental smallmouth bass across lakes with natural variation in nest predation pressure. Lakes are presented from lowest to highest predation pressure. Dissimilar letters denote significant differences between lakes within a given physiological parameter (Tukey *post hoc* test, $p<0.05$).

which outside of the parental care period becomes one of the most common prey species of adult smallmouth bass in lentic centrarchid-dominated habitats (Keast, 1978; Warren, 2009). One possibility is that lower nest predator densities in individual lakes translate to overall lower sunfish densities throughout the active season. We presume such a relationship exists; however, there are no fisheries assessment data on which to evaluate this assumption. Work on other predatory fish show that enzyme activities are influenced by the size, abundance and type of prey (Sherwood et al., 2002; Kaufman et al., 2006). Ontogenetic diet shifts in yellow perch (*Perca flavescens*) result in lower LDH activities and decreased energetic costs for fish that switch from planktivory to piscivory (Sherwood et al., 2002). Similarly, the increase in size and energetic quality of prey reduces the LDH activities of predatory walleye (*Sander vitreus*) (Kaufman et al., 2006). Lakes with low predation pressure such as Big Rideau Lake and Charleston Lake may require foraging smallmouth bass to spend more time chasing fewer prey. Indeed, both of these lakes contain lake trout (*Salvelinus namaycush*) and have large areas that would be considered poor habitat for lepomids (i.e., deep points and rock shoals with minimal vegetation) but would be used by smallmouth bass to forage on crayfish or partially pelagic species such as yellow perch. Physiologically, the enzymatic indicators used in this study may be indicative of annual trends in food abundance rather than predation pressure during parental care. A simple way to explore this question would be to seasonally sample enzyme activities to test if lakes rank similarly across seasons. Work that has explored the influence of metal contaminants on enzyme activities of yellow perch has confirmed that regional differences are conserved throughout seasons (Couture et al., 2008).

The hypothesis of a link between low nest predation pressure and low prey availability does not clarify the discrepancies between some of the lakes with low predation pressure (Fig. 1A). The most obvious difference between Charleston Lake, Big Rideau Lake and Newboro Lake is size. Charleston Lake and Big Rideau are large, deep lakes (2 500 ha; mean depth of 17.4 m and 6 500 ha; mean depth of 10.2 m respectively), while Newboro Lake is much smaller and shallower (1850 ha and mean depth of 3 m). Although the link between water body size and fish physiological indicators has received little attention, characteristics such as growth rate, condition factor, swimming intensity and consumption rate are known to influence physiological indicators, particularly LDH activities (e.g., Sullivan and Somero, 1983; Goolish, 1991; Pelletier et al., 1993). It has long been clear that habitat type and general feeding ecology influence enzyme activities interspecifically (Sullivan and Somero, 1980, 1983), but intraspecific differences in enzyme activities which relate to habitat characteristic have only lately received any attention (Odell et al., 2003; Couture et al., 2008). Our data provide further evidence for intraspecific differences in enzyme activities across a range of habitats and populations, even though all lakes that were sampled were within a narrow geographical range.

Variation in the activity of CCO and not CS among the populations studied may be due to the role of CCO in controlling the oxidation rate of lactate into glycogen in the white muscle after exercise (Goolish, 1991). Smallmouth bass engaged in parental care are twice as active as non-nesting con-specifics and significantly increase levels of burst swimming (Cooke et al., 2002, 2006). Since burst swimming events are related to nest predator chases and behavioural observations indicate that chases were more abundant in the lakes with higher predation pressure (Gravel and Cooke, 2009) we would expect CCO activities to be elevated in lakes with high predation pressure. As with the pattern of LDH activity, this prediction did not hold.

The positive relationship between LDH activities and muscle protein concentration and the lack of differences between LDH enzyme activities among fish from different lakes when enzyme activities are expressed in mg protein support other work (Houlihan et al., 1988; Mendez and Wieser, 1993) which suggests that the cytosolic character of LDH enables it to become a source of protein during periods of fasting. The lack of relationship between the mitochondrial enzymes and muscle protein concentration further support this hypothesis. In our study, the higher levels of axial LDH activities in certain lakes, and the consequent higher anaerobic capacities, were probably achieved as a result of higher protein concentration and not specifically through the upregulation of anaerobic pathways. In contrast, the changes in axial CCO activity are unrelated to muscle protein concentration and are more likely a response to differences in metabolic demands among populations. The strong relationship between both mitochondrial enzymes supports that differences in these enzymes among populations most likely reflect changes in overall aerobic capacities, even if we are unable to identify the cause of this variation. Future studies focused on other lake-specific characteristics such as density, trophic structure or

habitat availability may help to elucidate the relationship between organismal performance and enzyme capacities.

Although there is apparent variation in axial enzyme activities among lakes, pectoral enzyme activities were strongly conserved across the studied populations. The lack of variation in pectoral enzyme activities supports the idea that the variation observed in axial enzyme activities reflects differences in physiological requirements among the populations studied. If differences in protein concentrations and enzyme activities simply reflected differences in protein synthesis among the six populations we would expect similar differences in the axial and pectoral muscles across lakes. However, we cannot exclude the possibility that axial muscle may inherently be more plastic since it is a location for protein storage when growth occurs, while oxidative pectoral muscle may simply be more conservative and less influenced by recent feeding.

Interestingly, this is one of the first studies to evaluate intraspecific variation in enzymatic activities in animals acclimatized to field conditions (but see Couture and Guderley, 1990; Kaufman et al., 2006; Couture et al., 2008). Additional research is needed to clarify the extent to which biotic and abiotic factors influence the enzyme activity and organismal performance in wild animals across a range of ecosystems, contributing to the metabolic theory of ecology (Brown et al., 2004) and clarifying the potential role of these biochemical indicators as predictors of animal performance (Gibb and Dickson, 2002). Although we do not fully understand the causes of differences in metabolic capacities, the evidence is strong that variations in metabolic capacities do reflect differences in biotic and abiotic components of the environment. Research which examines physiological variation among population across large spatial scales (e.g., 'macrophysiology'; Osovitz and Hofmann, 2007) will help us better understand the influences of external factors on fish metabolic capacities.

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