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ORIGINAL PAPER

Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus* spp.) relative to stage of offspring development

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Abstract Parental care requires a complex integration of physiology and behaviour, yet little is known about the physiological and energetic consequences or correlates of these behaviours. Using two species of male black bass (smallmouth bass, *Micropterus dolomieu*; largemouth bass, *M. salmoides*) as a model, the focus of this study was to determine the biochemical and hematological indicators of change in nutritional status and potential for chronic stress. This was accomplished by randomly sampling individuals at four stages across parental care. Additionally, a subset of individuals was repeatedly sampled at three brood development stages to track changes in biochemical factors within the individual. Though there were changes in physiological factors across parental care in randomly sampled fish of both species (declines in plasma glucose in largemouth bass; decreases in hematocrit and plasma chloride in smallmouth bass), repeated sampling of individuals was determined to be a more appropriate sampling technique due to natural variability in biochemical factors among individual fish. Repeated sampling of smallmouth bass did not adversely influence physiological metrics or brood abandonment. However, there were higher incidences of nest abandonment in repeatedly sampled largemouth bass. Amongst the repeatedly sampled smallmouth bass, nutritional indicators such as plasma triglyceride levels decreased indicating individual fasting across the majority of parental care. Increases in plasma calcium and magnesium towards the end of care indicated that feeding most likely resumed when the brood was close to independence after ~3 weeks of care. Lastly, several indicators of chronic stress, such as plasma glucose and chloride levels, increased throughout the parental care period. These sublethal stressors are indicative of decreasing body condition associated with prolonged activity and fasting which may have marked impacts on the ability of an individual to continue parental care for the current brood and impact subsequent individual fitness. Further research into the mechanistic relationships between behaviour, physiology, and energetics during the parental care period will provide a better understanding of the decisions by individuals facing multiple trade-offs that ultimately lead to differences in individual fitness.

Keywords Physiology · Energetics · Parental care · Individual variation · Smallmouth bass · Largemouth bass

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Introduction

Several syntheses have explored the links between fitness and morphology, behaviour, and life history (Endler 1986; Lessells 1991). However, there is a paucity of research investigating the relationship between individual physiological variation, behaviour, and individual fitness, even though these links have been theorized (Endler 1986; Feder 1987; Ricklefs and Wikelski 2002). Generally, links between physiological variation and fitness have been inferred from data rather than implicitly tested (Spicer and Gaston 1999). Amongst behaviours, parental care requires a complex integration of physiology and behaviour (mediated by the endocrine system) to secure individual fitness, yet little is known about the physiological consequences of these behaviours. Parental care represents a trade-off

between multiple interests of the adult providing the care. Adult individuals sacrifice their own health and body condition (Horak et al. 1999; Steinhart et al. 2005), at the risk of mortality (Sabat 1994), as well as other opportunities to mate (both current and future) to ensure increased survival of offspring and subsequent fitness (Williams 1966a; Gross and Sargent 1985; Sargent et al. 1987). As the brood develops towards independence and the probability of individual survivorship increases, the care-giving adult should adjust the amount of care given in favour of minimizing current costs to conserve future reproductive opportunities (Williams 1966a; Gross and Sargent 1985; Gross 2005).

Parental care, especially uniparental male care, is a widespread reproductive behaviour amongst teleost fishes ranging from simple forms such as concealment of eggs to complex forms such as rearing the brood within the body cavity of an adult or live bearing of young (Gross and Sargent 1985). While the energetic costs of parental care have been studied for a number of species (e.g., Sargent and Gross 1986; Coleman and Fischer 1991; Mackereth et al. 1999), little information is currently available about changes to the physiological status of the adult across the parental care period. The majority of previous work on the physiology of parental care in fishes has focused on the endocrine correlates of paternal care (e.g., Knapp et al. 1999; Páll et al. 2002, 2005; Magee et al. 2006; Rodgers et al. 2006). One study has documented the differences in muscle enzyme activity between parental versus bachelor male fish (Guderley and Guevara 1998), but to our knowledge no studies have documented variation in nutritional physiology and biochemistry of individual fish across the parental care period. Furthermore, no studies have repeatedly sampled the same fish throughout the parental care period to document changes at the level of the individual, an approach that has the potential to elucidate inter-individual variation.

Both largemouth (*Micropterus salmoides*) and smallmouth bass (*M. dolomieu*), collectively termed "black bass," exhibit extended parental male care. Black bass are an ideal model for the study of the physiology of parental care in the wild because individual fish can be repeatedly captured via angling (to enable tissue sampling), are large enough to enable the collection of tissue samples (relative to many of the smaller-bodied fishes that have been the focus of behaviour-oriented parental care studies; e.g., cichlids, sticklebacks), have been well-studied with respect to parental care behaviour and energetics providing sufficient information to interpret physiological findings, and because their reproductive success can be easily visually quantified. For both species, when water temperatures reach approximately 14°C in spring, male bass move into the littoral zone where nest construction (the digging out of saucer shaped depressions in the substrate), courtship, spawning, and egg deposition and fertilization occur (Kramer and Smith 1962; Ridgway 1988). After spawning, the female bass leaves the area of the nest while the male bass initiates parental care in the form of active nest defense from potential brood predators as well as fanning the brood to provide proper oxygenation and prevent sedimentation (Hinch and Collins 1991). The male bass will continue to participate in parental care activities until the brood becomes independent, which can often require 1 month (Cooke et al. 2006).

The parental care period of black bass has been noted to be one of the most energetically demanding time periods of an individual's life (Hinch and Collins 1991; Cooke et al. 2002, 2006). While guarding the nest, individuals greatly curtail foraging activities due to the fact that they are unable to leave the brood unattended (Hinch and Collins 1991). At the same time, nest guarding male fish are also some of the most active fish in the population as localized movements on and around the nest equate to movements over tens of kilometers per day (Hinch and Collins 1991; Cooke et al. 2002; Hanson et al. 2007a). Male fish engaged in parental care must rely on endogenous energy reserves to fuel activity during this time (Mackereth et al. 1999). Nest guarding male bass continually move about the nest, executing tight turns to remain above the nest as well as sculling all fins at the same time to remain stationary above the nest while providing oxygenation and preventing silt deposition on the brood (Hinch and Collins 1991; Cooke et al. 2002). As such, it has been theorized that the combination of reliance on endogenous energy supply with increased energy consumption from nest guarding activities results in a continual decline in the energetic and nutritional status of nest guarding males across the parental care period (Mackereth et al. 1999). Drastic declines in endogenous energy reserves can lead to brood abandonment as the current brood is abandoned to secure future reproductive success (Trivers 1972; Sargent and Gross 1986). Additionally, it has been theorized that individual survival through the following winter may be compromised if internal energy reserves are over-utilized (Mackereth et al. 1999).

Using nest-guarding male black bass as a model, the objective of the present study was to determine the nature and magnitude of the energetic and nutritional decline and associated stress physiology across the parental care period through the use of non-lethal sampling. We predicted that blood based indicators of nutritional and energetic status would change as the brood developed and the adult male remained on the nest unable to forage normally and fueling activity through endogenous energy reserves. Additionally, we predicted that patterns in hematology and plasma biochemistry indicative of chronic stress would be evident as parental care progressed. We also tested the utility of repeated blood sampling of individuals across the parental care period. Specifically, we predicted that repeated sampling

of individuals would more accurately show the decline of nutritional indicators across parental care than would comparing the means of separate, randomly sampled groups, while not causing detrimental effects to individuals.

Methods

Field techniques

This study was carried out from May 1st to June 1st, 2006 on Lake Opinicon, eastern Ontario, Canada (44°30′N, 76°20′W). Daily snorkel surveys of the littoral zone were conducted to locate largemouth and smallmouth bass that were actively guarding nests with newly deposited eggs. Upon locating an active bass nest [defined as male guarding newly deposited (<1 day old) eggs], the snorkeler placed a numbered PVC tile near the nest and recorded nest location, nest depth, and number of eggs within the nest (visual, categorical assessment ranging from low of 1 to high of 5; Suski and Philipp 2004). At the time of nest discovery, individuals were randomly assigned to sampling groups. Control fish were not handled beyond that as described above to provide a baseline estimate of nest abandonment within the lake for each species. Subsets of individuals were sampled at each of the four brood developmental stages [eggs (sampled within 1 day of spawning), egg sac fry (newly hatched embryos, approximately 1.5 weeks after spawning), swim up fry (larvae begin to swim >0.5 m above the nest, approximately 2 weeks after spawning), and free swimming fry (larvae swim <1 m above and around the nest, prior to independence, approximately 3 weeks after spawning]. Fish were captured using heavy-action recreational fishing equipment that could be used to angle fish from the boat or underwater (by the diver). In total, 41 largemouth bass (total length mean ± SD; 381 ± 40 mm) and 50 smallmouth bass (total length mean ± SD; 366 ± 38 mm) were blood sampled for this study. All fish were landed within 20 s of hooking to minimize non-parental care related anaerobic exercise. During the entire period that angled fish were held on the boat, they were always in water. Upon capture, fish were quickly blood sampled by the caudal puncture method using a 1.5″, 21 gauge vacutainer syringe (Houston 1990) while being held within a foam lined trough containing fresh lake water. Up to 1.5 ml of blood (representing approximately 3.7% of total blood volume) was collected in a 3-ml, flat-bottomed vacutainer containing lithium heparin to prevent blood coagulation. Total length was recorded as well as the presence or absence of any injury. Individuals were then released within 5 m of the nest in less than 2 min. During the sampling procedure, a snorkeler remained at the nest site and defended the brood until the male returned (typically in under 5 min). Blood samples were centrifuged immediately at 10,000× gravity for 5 min (Clay Adams Compact II Centrifuge). Hematocrit was assessed in the field by measuring the volume of red blood cells by volume of total liquid on centrifuged blood collection tubes using micrometer calipers. Plasma samples were stored in liquid nitrogen for subsequent analysis. Individuals in the last treatment group, repeatedly sampled fish, were sampled at each stage of brood development (with the exception of the swim up fry stage). At the final stage of brood development, due to the fact that fish at this stage roam across large areas and capture by angling becomes ineffective, fish were captured by a snorkeler using a spear gun. Following sampling, fish were euthanized by cerebral percussion. After non-lethal sampling, a snorkeler revisited each nest every 2 days to record presence or absence of the male as well as the progression of the brood through developmental stages.

Lab analyses

Samples were analysed for concentrations of various biochemical constituents indicative of individual nutritional status [alkaline phosphatase (ALP; enzyme number 3.1.3.1), aspartate transaminase (AST; enzyme number 2.6.1.1), creatine kinase (CK; enzyme number 2.7.3.2), lactate dehydrogenase (LDH; enzyme number 1.1.1.27), total protein, phosphorous, triglycerides, cholesterol, and glucose] as well as ions (Mg^+, Ca^{++}, Cl^-, Na^+, K^+) (Wagner and Congleton 2004; Congleton and Wagner 2006). In previous work conducted on Pacific salmonids (*Oncorhynchus* spp.) these biochemical constituents have been shown to reflect the short- and long-term nutritional status of individual fish subjected to fasting or feeding (Wagner and Congleton 2004; Congleton and Wagner 2006). In particular, we measured variables that have been shown to respond to fasting and feeding activity (ALP, CK, total protein, phosphorous, triglycerides, cholesterol, Mg^+, and Ca^{++}; Lall 2002; Wagner and Congleton 2004; Congleton and Wagner 2006) as well as indicators of tissue damage (AST, LDH; Morrissey et al. 2005), and chronic stress (glucose, Cl^-, Na^+, and K^+; Wendelaar Bonga 1997; Barton 2002). All biochemical analyses were conducted on a Roche Hitachi 917 analyzer (Basal, Switzerland) and based upon the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standard reference model (Dimension AR-1MT, Dade Behring Inc., Newark, DE, USA). To ensure proper quality control, all assays (performed by laboratory personnel at Vita-Tech, Markham, ON, Canada) followed procedural guidelines for standardization and quality assurance established by the Veterinary Laboratory Association Quality Assurance Program, New York State Department of Health, College of American Pathologists, and the Canadian Food Inspection Agency External Proficiency Panel.

Statistical analysis

All analyses were performed in the statistical package JMP IN v 4.0 and the level of significance for all tests (α) was assessed at 0.01 to minimize Type I error associated with multiple statistical tests (Zar 1999). All values presented represent mean ± S.E. unless otherwise noted. Normality and heterogeneity of variance of initial physiological data was assessed to determine whether variables needed to be transformed before analysis. Non-normal variables were log-10 transformed prior to subsequent analysis. To determine differences in physiological variables between brood stages, one-way ANOVA's followed by Tukey's HSD post hoc tests were employed (Zar 1999). In instances where homogeneity of variance was violated, Welch's ANOVA was utilized (Zar 1999). To determine the utility of repeated sampling, the mean values of each physiological variable from the repeated sampling events were compared to the means of randomly sampled fish from the same brood developmental period through paired *t*-tests (Zar 1999). Additionally, nest abandonment rates between repeated sampling groups and natural whole lake abandonment were analysed by Chi-square contingency table analysis (Zar 1999). Multiple comparisons across proportions were performed to determine significant differences in abandonment rates according to methods in Zar (1999). Repeatedly sampled fish were analysed separately, but in a similar manner. Repeated measures ANOVA's (or Welch's ANOVA as described above) and Tukey's HSD post hoc tests were used to determine significant differences between sampling periods.

Results

Randomly sampled fish

Overall, very few parameters differed significantly over the course of parental care in both largemouth and smallmouth bass (Tables 1, 2). For largemouth bass across the parental care period, there were only significant alterations in one blood biochemistry variable. Specifically, blood glucose levels in individuals significantly increased after the egg stage of brood development ($P < 0.01$; Fig. 1, Table 3). All other physiological variables did not show any differences across brood development (Table 3).

In smallmouth bass, changes in the levels of hematocrit and chloride levels were noted across parental care. Hematocrit was highest at the commencement of parental care (41.69 ± 2.45%) and declined throughout brood development, reaching its lowest level at the free-swimming fry stage (29.78 ± 2.30%; $P < 0.01$; Fig. 2, Table 3). Chloride levels followed a pattern in which levels declined to the lowest levels during the free swimming fry stage when compared to the egg and egg sac fry stages ($P < 0.01$; Fig. 2, Table 3).

Validation of repeated sampling

Due to high levels of brood abandonment, only comparisons between physiological variables in the second repeated sampling event and control fish at the egg sac fry stage could be performed for largemouth bass. Repeatedly sampled largemouth bass showed decreased levels of phosphorous when compared to controls ($P < 0.01$; Fig. 3, Table 4). No significant differences were detected for other blood biochemistry variables ($P > 0.01$, Table 4).

Similarly, at the same stage of brood development, several differences were noted between values from smallmouth bass that were sampled for the second time and control smallmouth bass at the egg sac fry stage. Specifically, smallmouth bass sampled twice showed had decreased levels of magnesium when compared to control fish at the same brood development stage ($P < 0.01$; Fig. 3, Table 4). No significant differences were detected for other blood biochemistry variables ($P > 0.01$; Table 4). When comparing smallmouth bass sampled for a third time to control fish at the free swimming fry stage, no significant differences in values of physiological variables were noted ($P > 0.01$; Table 4).

Finally, when compared to natural nest abandonment, repeated sampling was found to increase brood abandonment in largemouth bass ($\chi^2 = 9.31$, $df = 2$, $P < 0.01$; Fig. 4). Specifically, brood abandonment amongst repeatedly sampled fish at the third sampling period increased to >80%, more than double the natural abandonment rate (Fig. 4). Though there were statistically significant differences between repeated sampling abandonment rates and natural abandonment rates for smallmouth bass, the final abandonment rate did not increase significantly above control rates for repeatedly sampled fish ($\chi^2 = 25.93$, $df = 2$, $P < 0.01$; Fig. 4).

Repeated sampling

Between the egg and egg sac fry brood development stages, repeatedly sampled largemouth bass did not vary in physiological parameters (Table 5). Due to the increased levels of brood abandonment amongst repeatedly sampled largemouth bass, no statistical analyses could be performed that included fish at the free swimming fry stage. Conversely, smallmouth bass showed differences in multiple physiological parameters. In particular, magnesium levels decreased in the egg sac fry stage as compared to the egg and free swimming fry stages ($P < 0.01$; Fig. 5, Table 5). Chloride and hematocrit decreased across the parental care period ($P < 0.01$; Fig. 5, Table 5). Lastly, plasma calcium levels

Table 1 Physiological variables (mean ± SD with range) measured in largemouth bass treatment groups across this study

Physiological variable	Egg	Egg sac fry single sample	Swim up fry single sample	Free swimming fry single sample	Repeated sampling 2 (egg sac fry stage)
N	8	11	6	7	8
ALP (U/l)	20.88 ± 1.53 (17–30)	18.18 ± 1.24 (9–25)	18.67 ± 0.49 (17–20)	24.14 ± 3.32 (5–30)	16.38 ± 1.87 (5–22)
AST (U/l)	63.13 ± 16.32 (23–165)	71.55 ± 9.96 (19–120)	81.67 ± 42.96 (21–295)	99.71 ± 37.70 (22–250)	39.38 ± 6.75 (13–78)
Calcium (mmol/l)	2.85 ± 0.07 (2.65–3.22)	2.84 ± 0.03 (2.73–3.00)	2.93 ± 0.05 (2.75–3.06)	2.94 ± 0.10 (2.59–3.23)	2.87 ± 0.07 (2.57–3.10)
Chloride (mmol/l)	109 ± 3.31 (96–116)	112.1 ± 2.08 (98–118)	103.5 ± 3.43 (93–112)	104.8 ± 3.25 (98–116)	106 ± 1.86 (97–112)
Cholesterol (mmol/l)	13.08 ± 0.90 (10–16.2)	15.49 ± 1.08 (11.2–21.5)	13.03 ± 0.55 (11.2–14.8)	14.16 ± 0.62 (12.6–16.8)	12.34 ± 0.97 (8.40–17.1)
CK (U/l)	5939 ± 1749.38 (355–14,807)	7525.55 ± 1017.15 (126–12,365)	4241.5 ± 2400.26 (608–15,870)	5504.71 ± 2643.58 (1,054–20,940)	2569.25 ± 881.99 (409–7,337)
Glucose (mmol/l)	2.03 ± 0.09 (1.80–2.6)	2.21 ± 0.12 (1.80–3.0)	2.37 ± 0.20 (1.90–3.2)	2.63 ± 0.10 (2.30–3.0)	2.46 ± 0.16 (1.60–3.0)
Hematocrit (proportion)	0.35 ± 0.03 (0.27–0.5)	0.25 ± 0.01 (0.19–0.31)	0.27 ± 0.03 (0.24–0.46)	0.32 ± 0.03 (0.18–0.37)	0.28 ± 0.02 (0.21–0.34)
LDH (U/l)	460.75 ± 95.82 (113–847)	576.27 ± 105.32 (74–1,429)	939.83 ± 665.63 (94–4,240)	1106.14 ± 563.83 (122–3,990)	229.86 ± 46.76 (57–398)
Magnesium (mmol/l)	1.17 ± 0.02 (1.08–1.27)	1.21 ± 0.02 (1.13–1.33)	1.22 ± 0.03 (1.09–1.35)	1.18 ± 0.05 (1.00–1.37)	1.16 ± 0.02 (1.08–1.27)
Phosphorous (mmol/l)	2.15 ± 0.08 (1.9–2.5)	2.28 ± 0.09 (2.1–2.9)	2.12 ± 0.16 (1.7–2.8)	1.97 ± 0.23 (1.5–3.1)	1.95 ± 0.06 (1.7–2.2)
Potassium (mmol/l)	3.43 ± 0.017 (2.9–4.0)	3.8 ± 0.13 (3.3–4.6)	4.02 ± 0.38 (3.3–5.8)	3.96 ± 0.19 (3.5–4.5)	3.48 ± 0.12 (3.2–4.2)
Sodium (mmol/l)	158.83 ± 1.25 (154–163)	159.60 ± 1.23 (153–166)	160.67 ± 1.31 (156–164)	158.60 ± 2.73 (154–169)	158.50 ± 1.46 (152–164)
Total protein (g/l)	37.88 ± 0.93 (33–42)	39.00 ± 0.75 (37–45)	37.83 ± 0.87 (35–40)	37.71 ± 1.38 (32–44)	38.38 ± 1.95 (31–50)
Triglycerides (mmol/l)	1.19 ± 0.49 (0.43–4.55)	0.83 ± 0.08 (0.49–1.27)	0.63 ± 0.09 (0.45–1.04)	0.58 ± 0.10 (0.27–0.90)	0.59 ± 0.07 (0.38–0.90)

Table 2 Physiological variables (mean ± SD with range) measured in smallmouth bass treatment groups across this study

Physiological Variable	Egg	Egg sac fry single sample	Swim up fry single sample	Free swimming fry single sample	Repeated sampling 2 (egg sac fry stage)	Repeated sampling 3 (free swimming fry stage)
N	10	8	4	8	10	10
ALP (U/l)	38.3 ± 10.29 (13–120)	20.0 ± 3.09 (8–30)	11.25 ± 4.01 (5–23)	33.38 ± 9.27 (8–90)	21.5 ± 6.33 (9–76)	27.1 ± 5.70 (8–61)
AST (U/l)	231.7 ± 66.63 (44–620)	219.13 ± 53.16 (48–470)	69.25 ± 21.20 (24–112)	149.25 ± 36.50 (45–357)	96.9 ± 21.60 (38–274)	157.2 ± 45.37 (40–469)
Calcium (mmol/l)	2.65 ± 0.07 (2.44–2.91)	2.54 ± 0.01 (2.50–2.60)	2.55 ± 0.04 (2.45–2.64)	2.76 ± 0.08 (2.54–2.95)	2.60 ± 0.03 (2.45–2.74)	2.85 ± 0.05 (2.67–3.09)
Chloride (mmol/l)	120.17 ± 2.79 (113–132)	119.83 ± 1.49 (115–126)	115.0 ± 1.68 (113–120)	108.0 ± 2.55 (102–116)	113.0 ± 2.15 (104–123)	103.89 ± 3.58 (90–121)
Cholesterol (mmol/l)	11.2 ± 0.70 (8.4–14.9)	13.51 ± 0.84 (10.5–16.9)	12.73 ± 1.27 (9.5–15.1)	13.10 ± 0.84 (10.1–16.2)	11.14 ± 0.63 (8.60–14.2)	10.91 ± 0.63 (8.50–15.5)
CK (U/l)	7809.5 ± 2745 (1,298–24,920)	8986 ± 3051.82 (1,178–24,091)	2055.5 ± 704.19 (370–3,465)	3895.38 ± 1500.40 (650–12,845)	5398 ± 2496.50 (1,043–27,560)	4361.4 ± 1920.33 (4–17,210)
Glucose (mmol/l)	2.35 ± 0.11 (1.9–2.8)	2.53 ± 0.26 (1.70–3.9)	2.83 ± 0.19 (2.3–3.2)	3.11 ± 0.19 (2.30–3.9)	2.57 ± 0.17 (2.2–3.6)	2.97 ± 0.09 (2.4–3.3)
Hematocrit (proportion)	0.42 ± 0.02 (0.32–0.57)	0.35 ± 0.02 (0.29–0.42)	0.35 ± 0.03 (0.30–0.41)	0.30 ± 0.03 (0.19–0.38)	0.31 ± 0.02 (0.22–0.39)	0.33 ± 0.02 (0.22–0.50)
LDH (U/l)	1776.11 ± 648.96 (169–5,780)	1432 ± 428.98 (210–2,870)	456.5 ± 153.49 (142–809)	833 ± 268.55 (218–2,280)	668.6 ± 302.28 (169–3,350)	761.6 ± 291.67 (1–2,653)
Magnesium (mmol/l)	1.10 ± 0.02 (1.04–1.19)	1.12 ± 0.02 (1.04–1.16)	1.03 ± 0.03 (0.98–1.11)	1.08 ± 0.04 (0.99–1.23)	0.95 ± 0.03 (0.70–1.04)	1.06 ± 0.03 (0.96–1.23)
Phosphorous (mmol/l)	2.57 ± 0.10 (2.3–2.9)	2.31 ± 0.09 (2.0–2.7)	2.18 ± 0.06 (2.0–2.3)	2.18 ± 0.07 (2.0–2.4)	2.24 ± 0.09 (1.8–2.6)	2.29 ± 0.09 (2.0–2.8)
Potassium (mmol/l)	3.2 ± 0.15 (2.8–3.8)	3.53 ± 0.18 (3.2–4.4)	3.38 ± 0.17 (2.9–3.6)	3.6 ± 0.19 (3.0–4.0)	3.33 ± 0.14 (2.6–4.1)	4.43 ± 0.56 (3.2–7.8)
Sodium (mmol/l)	156.5 ± 1.95 (153–166)	155.83 ± 1.01 (153–159)	157.5 ± 1.32 (154–160)	159.8 ± 1.02 (156–162)	152.2 ± 0.82 (149–158)	156.5 ± 1.05 (150–160)
Total protein (g/l)	41.88 ± 1.46 (35–49)	43.14 ± 0.67 (41–46)	40.75 ± 2.66 (33–45)	42.71 ± 1.41 (37–47)	40.9 ± 1.00 (37–47)	40.1 ± 1.30 (35–49)
Triglycerides (mmol/l)	2.44 ± 0.23 (1.40–3.41)	2.69 ± 0.44 (1.12–4.69)	2.76 ± 0.24 (2.24–3.40)	2.11 ± 0.45 (1.06–4.21)	2.22 ± 0.26 (1.38–4.17)	1.61 ± 0.18 (1.12–3.05)

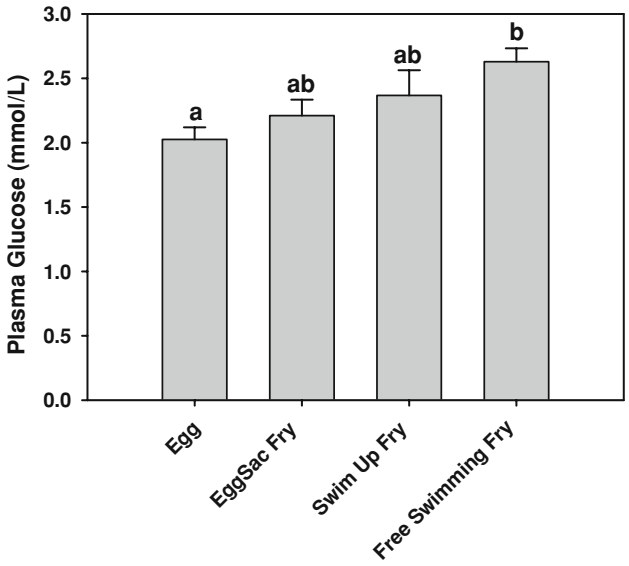

Fig. 1 Changes in plasma glucose levels in randomly sampled nest guarding male largemouth bass across four stages of brood development (egg, egg sac fry, swim up fry, and free swimming fry) during the parental care period. Letter assignments of "*a*" and "*b*" denote significant ($P < 0.01$) differences among brood development stages for largemouth bass. *Error bars* show mean ± 1 SE

increased between the egg sac fry and free swimming fry stages ($P < 0.01$; Fig. 5, Table 5).

Discussion

Evidence of changing nutritional status across the parental care period at the population level (i.e., randomly sampled fish that were not repeatedly sampled) was difficult to obtain in the current study despite the fact that we predicted such alterations given the high levels of parental care activity (i.e., brood defense and nest aeration; Hinch and Collins 1991; Cooke et al. 2002, 2006) and reduced foraging activities (Hinch and Collins 1991; Mackereth et al. 1999). Previous studies have documented large individual variation in biochemical nutritional indicators (Congleton and Wagner 2006). Similarly, in this study, extensive individual variation was noted in the majority of parameters measured (Tables 1, 2). For example, individual values for the enzyme creatine kinase (in U/l) ranged from a low of 608 for to a high of 15,870 within one sampling period (i.e., at the swim up fry stage; Tables 1, 2), though measurements of the enzymatic variables in the current study (especially LDH and CK) may be influenced by sampling strategy (i.e., blood collection via the caudal vasculature can cause elevations in these parameters; Morrissey et al. 2005). AST is a more reliable metric given this blood sampling approach (Morrissey et al. 2005) and it showed similar patterns. Regardless, with natural variation of this magnitude within the measured biochemical parameters, only large effects could be resolved via statistical testing. Many nutritional changes across the parental care period may not be sufficiently large to be noticed with this degree of background variation. Such variation is common in physiological studies and may be indicative of individual differences in behaviour and fitness and reflective of differences in genotype, environment, or individual health and condition (Bennett 1987). For example, in the current study, local environmental conditions (water temperature, wave activity, oxygen levels) and nest predator burdens undoubtedly

Table 3 Comparison of nutritional indicators of nest guarding male largemouth and smallmouth bass (*Micropterus* spp.) randomly sampled across four stages of brood development during the parental care period (eggs, egg sac fry, swim up fry, and free swimming fry) in Lake Opinicon, Ontario

Physiological variable	Largemouth bass			Smallmouth bass		
	df	*F*-ratio	*P*-value	*df*	*F*-ratio	*P*-value
ALP (U/l)	3, 28	0.81[a]	0.51	3, 26	3.25	0.04
AST (U/l)	3, 28	0.22	0.88	3, 26	1.53	0.23
Calcium (mmol/l)	3, 25	0.73	0.55	3, 17	2.51[a]	0.14
Chloride (mmol/l)	3, 23	1.88[a]	0.19	3, 17	6.30	***<0.01***
Cholesterol (mmol/l)	3, 27	1.78[a]	0.18	3, 24	1.87	0.16
CK (U/l)	3, 28	0.69[a]	0.58	3, 25	1.66	0.20
Glucose (mmol/l)	3, 27	5.80[a]	***<0.01***	3, 22	3.31	0.04
Hematocrit (proportion)	3, 28	3.88	0.02	3, 26	5.20	***<0.01***
LDH (U/l)	3, 28	0.20	0.90	3, 24	1.82[a]	0.20
Magnesium (mmol/l)	3, 25	0.56	0.65	3, 18	1.67	0.21
Phosphorous (mmol/l)	3, 26	1.48	0.24	3, 18	3.88[a]	0.05
Potassium (mmol/l)	3, 23	1.44	0.26	3, 17	1.21	0.34
Sodium (mmol/l)	3, 23	0.30	0.83	3, 17	1.47	0.26
Total protein (g/l)	3, 27	0.47	0.71	3, 22	0.41	0.76
Triglycerides (mmol/l)	3, 27	1.64[a]	0.23	3, 24	0.95	0.43

Italicized and boldfaced statistical output indicates significant differences at $\alpha = 0.01$. If variances were homogeneous for these data, analyses were conducted with one-way ANOVA; otherwise, Welch ANOVA was used

[a] Denotes use of Welch ANOVA

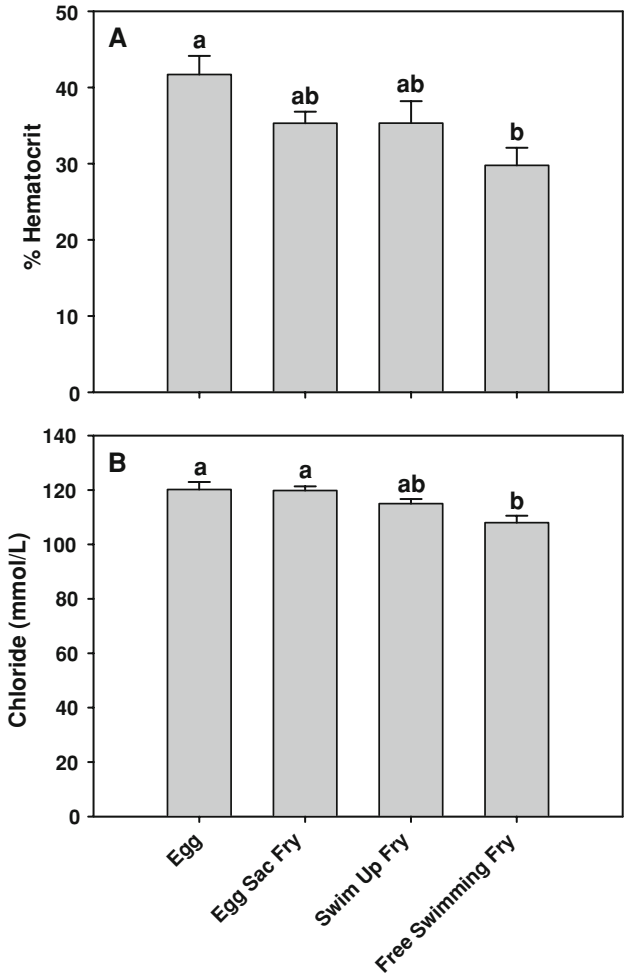

Fig. 2 Changes in **a** hematocrit and **b** plasma chloride levels in randomly sampled nest guarding male smallmouth bass across four stages of brood development (egg, egg sac fry, swim up fry, and free swimming fry) during the parental care period. Letter assignments of "*a*" and "*b*" denote significant ($P < 0.01$) differences among brood development stages for smallmouth bass. *Error bars* show mean ± 1 SE

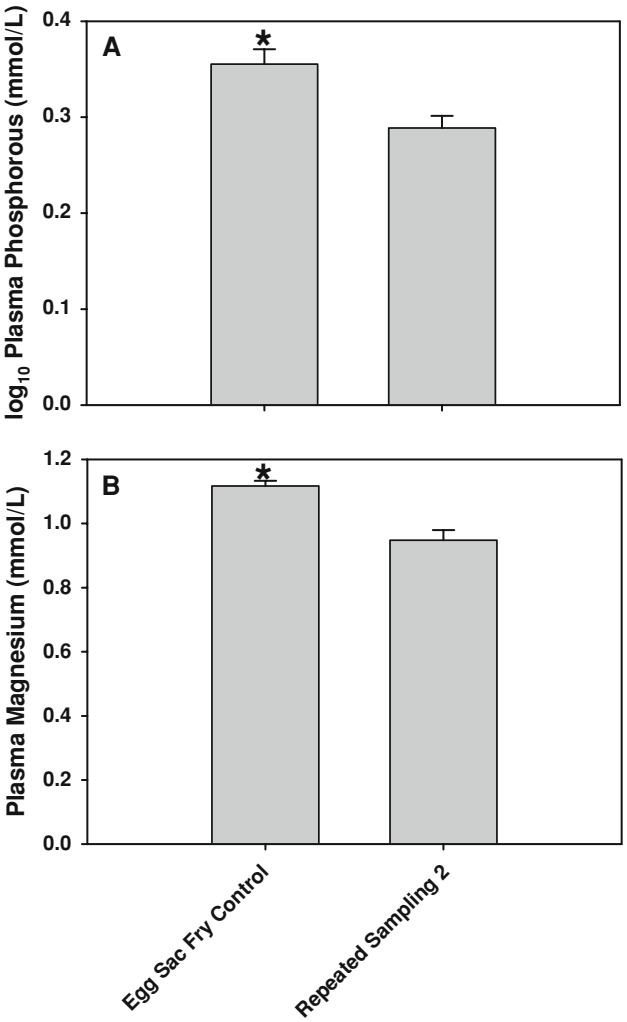

Fig. 3 Comparison of **a** plasma phosphorous levels of nest guarding male largemouth bass and **b** plasma magnesium levels nest guarding male smallmouth bass between the second sampling of repeatedly sampled individuals with control values for fish randomly selected fish both at the egg sac fry brood development stage. Assignment of an *asterisk* denotes significant ($P < 0.01$) differences between repeatedly and randomly sampled fish. *Error bars* show mean ± 1 SE

varied from nest to nest, which may have contributed to variation in organismal behaviour and physiological status.

Comparison of sampling techniques

In the current study, two separate sampling methods (randomly sampling individuals once at a given brood stage or repeatedly sampling individuals at each brood stage) were employed. Repeated sampling had a negative effect on parental care behaviour in largemouth bass. Largemouth bass subjected to repeated sampling had nest abandonment rates that were approximately 2.5 times higher than the natural abandonment rate for largemouth bass in the lake (Fig. 4). Smallmouth bass, however, did not abandon nests at any higher rates than natural nest abandonment (Fig. 4). This increased abandonment by largemouth bass relative to smallmouth bass can be attributed to a difference in parental care investment due to egg size and value (Sargent et al. 1987) and is consistent with parental investment and life-history theory (Cooke et al. 2006). These findings are also consistent with data from catch-and-release studies that reveal that largemouth bass tend to have higher post-angling abandonment rates than smallmouth bass (Hanson et al. 2007b). Also, this could reflect interspecific variation in response to stress, though largemouth bass are generally regarded as being less sensitive to hypoxia and stress than smallmouth bass (Furimsky et al. 2003). Due to the higher incidence of nest abandonment of largemouth bass relative to smallmouth bass, repeated sampling of largemouth bass at the free swimming fry stage was impossible.

To test the utility of repeatedly sampling fish without having the sampling alter physiological and nutritional

Table 4 Contrast between the second sampling of repeatedly sampled nest guarding male largemouth and smallmouth bass (*Micropterus* spp.) at the egg sac fry and free swimming fry brood development stages with control values for fish randomly sampled fish in Lake Opinicon, Ontario

Physiological variable	Largemouth bass egg sac fry stage			Smallmouth bass egg sac fry stage			Smallmouth bass free swimming fry stage		
	df	*t*-value	*P*-value	*df*	*t*-value	*P*-value	*df*	*t*-value	*P*-value
ALP (U/l)	17	0.86[a]	0.43	16	−0.19	0.85	16	−0.49	0.63
AST (U/l)	17	−2.20	0.04	16	−2.21	0.04	16	−0.22	0.83
Calcium (mmol/l)	16	0.39	0.70	14	1.71[a]	0.11	12	0.86[a]	0.42
Chloride (mmol/l)	16	2.15[a]	0.05	14	−2.26	0.04	11	−0.83	0.43
Cholesterol (mmol/l)	16	2.17[a]	0.05	15	−2.29	0.04	15	−2.21	0.04
CK (U/l)	17	2.23[a]	0.04	15	−1.06	0.31	16	−1.12	0.28
Glucose (mmol/l)	16	1.27[a]	0.23	15	0.14	0.89	15	−0.77	0.45
Hematocrit (proportion)	17	1.28	0.22	16	−1.82	0.09	16	0.86	0.40
LDH (U/l)	17	−2.57	0.02	15	1.58[a]	0.14	16	0.69[a]	0.50
Magnesium (mmol/l)	16	−1.71	0.11	15	−4.19	***<0.01***	12	−0.40	0.70
Phosphorous (mmol/l)	16	−3.22	***<0.01***	15	0.59[a]	0.57	12	0.99[a]	0.35
Potassium (mmol/l)	16	−1.89	0.08	14	0.98	0.35	11	−1.14	0.28
Sodium (mmol/l)	16	−0.58	0.57	14	−2.77	0.02	11	−2.11	0.06
Total protein (g/l)	16	−0.48	0.64	15	−1.68	0.11	15	−1.34	0.20
Triglycerides (mmol/l)	16	2.15[a]	0.05	15	−0.83	0.42	15	−0.92	0.37

Italicized and boldfaced statistical output indicates significant differences at $\alpha = 0.01$. If variances were homogeneous for these data, analyses were conducted with one-way ANOVA; otherwise, Welch ANOVA was used

[a] Denotes use of Welch ANOVA

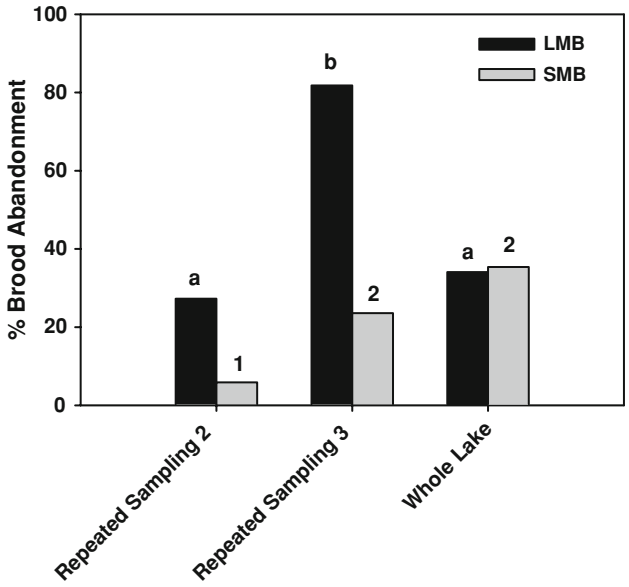

Fig. 4 Abandonment rates of repeatedly sampled largemouth and smallmouth bass compared to non-sampled bass (representing natural, whole-lake abandonment levels). Letter assignments of "*a*", and "*b*" denote significant ($P < 0.01$) differences among groups for largemouth bass, and number assignments of "*1*" and "*2*" denote significant differences among groups for smallmouth bass

condition, we compared the values found for each repeated sampling period to control values determined by singly sampling fish at the analogous brood development stage. The only detectable biochemical differences between repeatedly sampled fish and singly sampled fish occurred at the second sampling period which coincides with the egg sac fry brood stage. Specifically, repeatedly sampled largemouth bass had lower levels of plasma phosphorous than singly sampled fish (Table 4; Fig. 3), and repeatedly sampled smallmouth bass had lower levels of plasma magnesium than singly sampled fish (Table 4; Fig. 3), though the reasons for these differences are unclear. Additionally, there were no differences in any hematology or biochemical parameters between repeatedly sampled smallmouth bass at the third blood sampling period and singly sampled fish at the free swimming fry brood stage (Table 4). The lack of differences between repeated and singly sampled fish indicates that repeated sampling does not have a marked effect on the biochemical parameters measured in this study. In our study, between 3 and 7 days elapsed between repeat sampling periods. Another commonly cited explanation for changes in physiological metrics across stages of offspring development is that environmental conditions were variable. However, the only environmental factor that changed modestly across the parental care period was water temperature (increasing ~3°C between the first and last sampling periods). It was not possible to control for this thermal variation, but these temperatures (both the range and absolute values) are all well within the tolerances of both species and would be considered moderate. As such, we will discuss results from both randomly and repeatedly sampled fish together in the context of changes in physiology across parental care.

Indications of fasting and resumption of feeding

We noted several changes in biochemical parameters that indicate that individuals fasted for the beginning portion of parental care and resumed feeding by the time the brood

Table 5 Comparison of nutritional indicators of repeatedly sampled nest guarding male largemouth bass (*Micropterus salmoides*) across two stages of brood development (eggs and egg sac fry) and smallmouth bass (*Micropterus dolomieu*) across three stages of brood development during the parental care period (eggs, egg sac fry, and free swimming fry) in Lake Opinicon, Ontario

	Largemouth bass			Smallmouth bass		
Physiological variable	*df*	*F*-ratio	*P*-value	*df*	*F*-ratio	*P*-value
ALP (U/l)	1, 14	1.83	0.20	2, 27	1.75	0.20
AST (U/l)	1, 14	1.62	0.23	2, 27	1.28	0.30
Calcium (mmol/l)	1, 13	0.08	0.92	2, 22	8.17	***<0.01***
Chloride (mmol/l)	1, 12	0.41	0.67	2, 21	7.21	***<0.01***
Cholesterol (mmol/l)	1, 14	0.25	0.78	2, 27	0.04	0.95
CK (U/l)	1, 14	1.06	0.37	2, 27	3.07	0.06
Glucose (mmol/l)	1, 14	2.90	0.09	2, 25	5.54	0.01
Hematocrit (proportion)	1, 14	2.45	0.12	2, 27	6.79	***<0.01***
LDH (U/l)	1, 14	3.36	0.06	2, 26	1.93	0.17
Magnesium (mmol/l)	1, 13	1.35	0.29	2, 22	6.61	***<0.01***
Phosphorous (mmol/l)	1, 14	2.18	0.15	2, 22	3.15	0.06
Potassium (mmol/l)	1, 12	0.04	0.96	2, 21	4.66	0.02
Sodium (mmol/l)	1, 12	0.10	0.90	2, 21	4.82	0.02
Total protein (g/l)	1, 14	0.04	0.96	2, 25	0.48	0.62
Triglycerides (mmol/l)	1, 14	1.79	0.20	2, 27	4.93	0.02

Italicized and boldfaced statistical output indicates significant differences at $\alpha = 0.01$

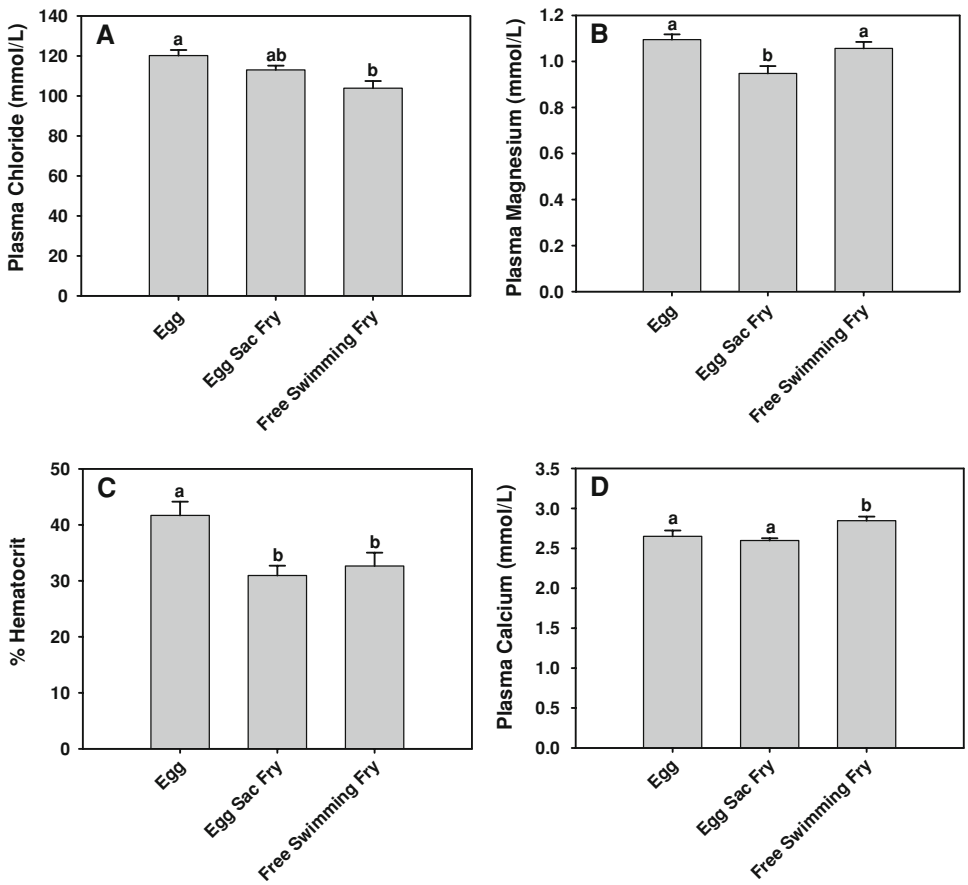

Fig. 5 Comparison of plasma **a** chloride, **b** magnesium, **c** hematocrit, and **d** calcium levels between three repeated sampling periods of nest guarding male smallmouth bass at the egg, egg sac fry, and free swimming fry brood development stages. Letter assignments of "*a*" and "*b*" denote significant ($P < 0.01$) differences among brood development stages for smallmouth bass. *Error bars* show mean ± 1 SE

developed into free swimming fry. Plasma triglyceride levels decreased throughout the parental care period, though this result was not statistically significant at $\alpha = 0.01$ (Table 5). Currently, research indicates that parental care is powered through endogenous energy reserves, primarily muscle energy stores in the form of lipids (Mackereth et al. 1999). Recent research has indicated that circulating levels of lipids in the blood stream are indicative of nutritional status and internal energy stores of the individual as well as recent feeding activity (Wagner and Congleton 2004;

Congleton and Wagner 2006; Polakof et al. 2007). Therefore, the decline of plasma triglyceride across the parental care period is an indicator of extended fasting and is consistent with videographic observations for smallmouth bass during nesting (Hinch and Collins 1991).

Additionally, hematocrit levels decreased from the commencement of parental care to the egg sac fry stage and then remained stable through to the end of sampling in both randomly and repeatedly sampled fish (Figs. 2, 5). Due to the fact that whole blood is being removed from the animal, decreases in hematocrit may be caused by sampling technique. However, the pattern of hematocrit decline within repeatedly sampled fish is consistent with a similar decline amongst singly sampled fish. Since this pattern is conserved through both sampling strategies, we believe that the fluctuations in hematocrit are due to a physiological response to parental care rather than our sampling practices. Consistent with the idea forage intake is markedly decreased during parental care, decreases in hematocrit may be indicative of the use of internal energy stores (rather than exogenous forage) to power parental care activities at the cost of maintaining tissues such as replacing senescent erythrocytes (Rios et al. 2005).

Plasma magnesium levels also fluctuated in a manner indicative of fasting in the current study. Interestingly, the pattern of change in plasma magnesium may also be indicative that fasting only occurs during the first 2 weeks of parental care and normal foraging resumes during the free swimming fry stage (approximately the third week of parental care). Plasma magnesium decreased by 0.16 mmol/l (~16% change from the baseline value at the egg stage) at the egg sac fry stage of brood development and then, by the free swimming fry stage, increased back to the levels at the commencement or parental care (Fig. 5). Plasma magnesium is also a required mineral for enzymatic processes in teleost fishes and is primarily recruited from dietary sources (Lall 2002). In fasted salmonids, circulating magnesium levels decreased in response to fasting (Congleton and Wagner 2006) similar to what was seen in the present study. However, the magnitude of change was greater for salmonids. Incidentally, much research has focused on the role of water temperature in influencing circulating magnesium levels, specifically in the fact that low temperatures tend to decrease levels of plasma magnesium within fish (Burton 1986; Congleton and Wagner 2006). As bass spawning coincides with increasing water temperatures in the spring (Kramer and Smith 1962; Ridgway 1988), decreases in plasma magnesium are more likely to be attributable to the effects of fasting rather than ambient temperature. Additionally, the increase of plasma magnesium at the free swimming fry stage to levels similar to those found at the commencement of parental care are indicative of increased feeding during this time.

Besides fluctuations in plasma magnesium levels, other biochemical metrics indicate that bass may resume feeding towards the end of parental care. By the free swimming fry stage in brood development, the fry have moved into a loosely associated group that fans out over a larger area (Friesen and Ridgway 2000), forcing the male to swim over larger distances to guard the brood and thereby increasing the area over which a male may encounter and consume prey items (Cooke et al. 2002). Circulating calcium levels increased by approximately 0.2 mmol/l (~7%) in parental males by the free swimming fry stage of brood development (Fig. 5). In general, most teleost fish satisfy calcium requirements through the absorption of mineralized calcium from dietary sources (Lall 2002), so increases in circulating levels may be due to digestion of forage. Additionally, increases in circulating calcium levels may be bolstered by the response of the organism to long term fasting in which internal reserves of calcium are mobilized to maintain homeostasis (Yamada 1956; Ikeda et al. 1974; Persson et al. 1997). Calcium is required for various metabolic functions within the body such as nerve transmission, cell membrane function and integrity, and enzyme activity (Lall 2002) as well as the formation of hard structures such as scales and the skeletal system (which may account for up to 95% of body calcium; Berg 1968; Fleming 1974; Persson et al. 1997). Further supporting the idea that feeding resumes by the end of parental care, total protein levels remained relatively consistent across parental care (Table 5). In a study of fasting salmonids, Congleton and Wagner (2006) noted that total circulating protein levels decreased dramatically after the first 3 weeks of fasting. These decreases in circulating plasma protein are thought to be due to the digestion of endogenous proteins for metabolism when outside sources of protein usually derived from forage are unavailable (Sauer and Haider 1979; Navarro and Gutiérrez 1995; Rehulka 1993; Wagner and Congleton 2004). In the present study, no decreases in the levels of plasma protein were noted at the free swimming fry stage (roughly 3 weeks from the onset of parental care), possibly indicative of the resumption of feeding (and intake of exogenous protein) by this time period to preserve homeostasis in the individual.

Indications of chronic stress

Indicators of chronic stress in parental individuals varied considerably as parental care progressed. In randomly sampled largemouth bass, only plasma glucose varied significantly across stages of brood development, representing a 30% increase (0.6 mmol/l) above the baseline values at the egg stage (Fig. 1). Increased plasma glucose is among a suite of commonly measured indicators of chronic stress

and acute stress in fishes (Wedemeyer et al. 1990; Mommsen et al. 1999; Barton 2002) as glucose levels increase as energy reserves are mobilized in response to an acute stressor (Wendelaar Bonga 1997; Barton 2002). In previous studies of fish nutrition, increases in plasma glucose have been frequently attributed to stress due to handling (Wagner and Congleton 2004; Congleton and Wagner 2006) and plasma glucose levels typically peak about 1 h after exposure to an acute stressor (Milligan 1996; Mommsen et al. 1999; Barton 2002). In the current study, handling effects would not be detected given that fish were sampled within seconds of capture, which is reflected by the fact that glucose levels recorded in this study are within the typical range of lab controls for black bass in previous studies (Suski et al. 2003).

In addition, randomly sampled smallmouth bass showed declines in levels of plasma chloride of ~12mmol/l (representing a 10% decrease from the egg stage by the end of parental care) (Fig. 2). Decreases in plasma ion concentrations, such as chloride, often reflect hydromineral imbalances that can result in osmoregulatory dysfunction (Mazeaud and Mazeaud 1981; Barton and Iwama 1991; McDonald and Milligan 1997; Wendelaar Bonga 1997; Wagner and Congleton 2004). Similar to the randomly sampled fish mentioned above, plasma chloride decreased by ~17mmol/l (representing a 14% decrease from the egg stage) across the entire parental care period within the group of repeatedly sampled smallmouth bass (Fig. 5). There is a possibility that the repeated handling of these individual fish across the approximately 3 week-long sampling period could account for the stress response noted in the data. We believe this is not the case due to the fact that a similar pattern in decline in plasma chloride was noted in the singly sampled fish, providing support that these changes are a response to the chronic stress associated with parental care. Furthermore, stress associated with recreational fishing practices (i.e., our capture technique), including ionic imbalances, are rectified within hours and certainly within days for black bass (Gustaveson et al. 1991; Suski et al. 2004, 2006). Together, the fluctuations in plasma glucose and ion concentrations suggest that parental care behaviours represent a chronic stress to the individual for the duration of parental care.

Conclusions

In summary, our results have shown that hematology and biochemical factors associated with endogenous energy stores and parental condition vary across parental care. Interestingly, a rise in indicators of feeding at the free swimming fry stage denotes the resumption of feeding as the brood gains independence. Additionally, factors associated with the response to chronic stress increase across parental care. Overall, changes in nutritional status across the parental care period can have marked impacts on individual fitness (Pottinger 1999). Currently, it is believed that parental care giving male bass largely power brood defense and maintenance behaviours through the use of endogenous energy stores (Mackereth et al. 1999). Many of the biochemical parameters measured in this study reflect either metabolism of these endogenous energy reserves in response to fasting or mobilization of nutrients from ingested food (Congleton and Wagner 2006). Individuals characterized by nutritional indices that indicate poor relative condition prior to spawning, or increased use of energy reserves during parental care relative to conspecifics, may run the risk of expending energy reserves prior to the independence of the brood. Also, the combined sublethal effects of energy depletion coupled with chronic stress could prove to be lethal to the individual. In such a case, the male should abandon the current brood at a cost of any current fitness to ensure his own survival and future reproductive opportunities in keeping with the William's Principle (Williams 1966b; Sargent and Gross 1986). Continuing research into the relationships between the interplay of parental care behaviour and its underlying physiological and energetic consequences will help to elucidate the links between physiology, behaviour, and fitness. This work will afford researchers a better understanding of the trade-offs encountered by the individual that dictate parental decisions and, ultimately, differences in individual fitness.

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References

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517–525

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3–26

Bennett AF (1987) Interindividual variability: an underutilized resource. In: Feder ME, Bennett AF, Burggren WW, Huey RB (eds) New directions in ecological physiology. Cambridge University Press, Cambridge, pp 147–166

Berg A (1968) Studies on the metabolism of calcium and strontium in freshwater fish I—relative contribution of direct and intestinal absorption. Mem Ist Ital Idrobiol 23:161–196

Burton RF (1986) Ionic regulation in fish: the influence of acclimation temperature on plasma composition and apparent set points. Comp Biochem Physiol A Mol Integr Physiol 85:23–28

Coleman RM, Fischer RU (1991) Brood size, male fanning effort and the energetics of a non-shareable parental investment in bluegill sunfish, *Lepomis macrochirus*. Ethology 87:177–188

Congleton JL, Wagner T (2006) Blood-chemistry indicators of nutritional status in juvenile salmonids. J Fish Biol 69:473–490

Cooke SJ, Philipp DP, Weatherhead PJ (2002) Parental care patterns and energetics of smallmouth bass, *Micropterus dolomieu*, and largemouth bass, *M. salmoides*, monitored with activity transmitters. Can J Zool Rev Can Zool 80:756–770

Cooke SJ, Philipp DP, Wahl DH, Weatherhead PJ (2006) Parental care energetics of six syntopic centrarchid fishes. Oecologia 148:235–249

Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton

Feder ME (1987) The analysis of physiological diversity: the prospects for pattern documentation and general questions in ecological physiology. In: Feder ME, Bennett AF, Burggren WW, Huey RB (eds) New directions in ecological physiology. Cambridge University Press, Cambridge, pp 38–75

Fleming WR (1974) Electrolyte metabolism of teleosts—including calcified tissues. In: Florkin M, Scheer BT (eds) Chemical zoology, vol 8. Academic, New York, pp 471–508

Friesen TG, Ridgway MS (2000) Brood size does not influence the behaviour or density of larval and juvenile smallmouth bass under parental care. Behaviour 137:61–74

Furimsky M, Cooke SJ, Suski CD, Wang X, Tufts BL (2003) Respiratory and circulatory effects of hypoxia in largemouth bass and smallmouth bass: an application to "live-release" angling tournaments. Trans Am Fish Soc 132:1065–1075

Gross MR (2005) The evolution of parental care. Q Rev Biol 80:37–45

Gross MR, Sargent RC (1985) The evolution of male and female parental care in fishes. Am Zool 25:807–822

Guderley H, Guevara RC (1998) Physiological correlates of paternal care in male threespine stickleback. Ecoscience 5:438–447

Gustaveson AW, Wydoski RS, Wedemeyer GA (1991) Physiological response of largemouth bass to angling stress. Trans Am Fish Soc 120:629–636

Hanson KC, Cooke SJ, Suski CD, Niezgoda G, Phelan FJS, Tinline R, Philipp DP (2007a) Assessment of largemouth bass (*Micropterus salmoides*) behaviour and activity at multiple spatial and temporal scales utilizing a whole-lake telemetry array. Hydrobiologia 582:243–256

Hanson KC, Cooke SJ, Suski CD, Philipp DP (2007b) Effects of different angling practices on post-release behavior of nest guarding male black bass (*Micropterus* spp.). Fish Manage Ecol 14:141–148

Hinch SG, Collins NC (1991) Importance of diurnal and nocturnal nest defense in the energy budget of male smallmouth bass: insights from direct video observations. Trans Am Fish Soc 120:657–663

Horak P, Jenni-Eiermann S, Ots I (1999) Do great tits (*Parus major*) starve to reproduce? Oecologia 119:293–299

Houston AH (1990) Blood and circulation. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 273–334

Ikeda Y, Ozaki H, Yasuda H (1974) The effects of starvation and reduced diet on the growth of scales in goldfish. Bull Jap Soc Sci Fish 40:859–868

Knapp R, Wingfield JC, Bass AH (1999) Steroid hormones and paternal care in the plainfin midshipman fish (*Porichthys notatus*). Horm Behav 35:81–89

Kramer RH, Smith LL (1962) Formation of year classes in largemouth bass. Trans Am Fish Soc 9:29–41

Lall SP (2002) The minerals. In: Halver JE, Hardy RW (eds) Fish nutrition, 3rd edn edn. Academic, New York, pp 260–309

Lessells CM (1991) The evolution of life histories. In: Krebs JR, Davies NB (eds) Behavioural ecology, 3rd edn edn. Blackwell Scientific Publications, London, pp 32–68

Mackereth RW, Noakes DLG, Ridgway MS (1999) Size-based variation in somatic energy reserves and parental expenditure by male smallmouth bass, *Micropterus dolomieu*. Environ Biol Fishes 56:263–275

Magee SE, Neff BD, Knapp R (2006) Plasma levels of androgens and cortisol in relation to breeding behavior in parental male bluegill sunfish, *Lepomis macrochirus*. Horm Behav 49:598–609

Mazeaud MM, Mazeaud F (1981) Adrenergic responses to stress in fish. In: Pickering AD (ed) Stress and fish. Academic, London, pp 49–75

McDonald D, Milligan L (1997) Ionic, osmotic and acid–base regulation in stress. In: Iwama GK, Pickering AD, Sumpter JP, Schreck CB (eds) Fish stress and health in aquaculture. Cambridge University Press, Cambridge, pp 119–131

Milligan CL (1996) Metabolic recovery from exhaustive exercise in rainbow trout. Comp Biochem Physiol A Mol Integr Physiol 113:51–60

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fisher 9:211–268

Morrissey MB, Suski CD, Esseltine KR, Tufts BL (2005) Incidence and physiological consequences of decompression in smallmouth bass after live-release angling tournaments. Trans Am Fish Soc 134:1038–1047

Navarro I, Gutiérrez J (1995) Fasting and starvation. In: Hochachka PW, Mommsen TP (eds) Biochemistry and molecular biology of fishes, metabolic biochemistry, vol 4. Elsevier Science, New York, pp 393–434

Páll MK, Mayer I, Borg B (2002) Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus*: I. Changes in 11-ketotestoster-one levels during the nesting cycle. Horm Behav 41:377–383

Páll MK, Hellqvist A, Schmitz M, Olsson PE, Mayer I, Borg B (2005) Changes in reproductive physiology and behaviour over the nesting cycle in male three-spined sticklebacks. J Fish Biol 66:1400–1410

Persson P, Johannsson SH, Takagi Y, Björnsson BT (1997) Estradiol-17b and nutritional status affect calcium balance, scale and bone resorption, and bone formation in rainbow trout, *Oncorhynchus mykiss*. J Comp Physiol B 167:468–473

Polakof S, Miguez JM, Soengas JL (2007) Daily changes in parameters of energy metabolism in liver, white muscle, and gills of rainbow trout: dependence on feeding. Comp Biochem Physiol A Mol Integr Physiol 147:363–374

Pottinger TG (1999) The impact of stress on animal reproductive activities. In: Balm PHM (ed) Stress physiology of animals. Sheffield Academic Press, Boca Raton, pp 130–177

Rehulka J (1993) Erythrodermatitis of carp, *Cyprinus carpio* L: an electrophoretic study of blood serum protein fraction levels. Acta Vet Brno 62:187–197

Ricklefs RE, Wikelski M (2002) The physiology/life-history nexus. Trends Ecol Evol 17:462–468

Ridgway MS (1988) Developmental stage of offspring and brood defense in smallmouth bass (*Micropterus dolomieui*). Can J Zool Rev Can Zool 66:1722–1728

Rios FS, Oba ET, Fernandes MN, Kalinin AL, Rantin FT (2005) Erythrocyte senescence and haematological changes induced by starvation in the neotropical fish traíra, *Hoplias malabaricus* (Characiformes, Erythrinidae). Comp Biochem Physiol A Mol Integr Physiol 140:281–287

Rodgers EW, Early RL, Grober MS (2006) Elevated 11-ketotestosterone during paternal behavior in the Bluebanded goby (*Lythrypnus dalli*). Horm Behav 49:610–614

Sabat AN (1994) Cost and benefits of parental effort in a broodguarding fish (*Ambloplites rupestris*, Centrarchidae). Behav Ecol 5:195–201

Sargent RC, Gross MR (1986) Williams' principle: an explanation of parental care in teleost fishes. In: Pitcher TJ (ed) The behavior of teleost fishes. Johns Hopkins University Press, Baltimore, pp 275–293

Sargent RC, Taylor PD, Gross MR (1987) Parental care and the evolution of egg sizes. Am Nat 129:32–46

Sauer DM, Haider G (1979) Enzyme activities in the plasma of rainbow trout, *Salmo gairdneri* Richardson: the effects of nutritional status and salinity. J Fish Biol 14:407–412

Spicer JI, Gaston KJ (1999) Physiological diversity and its ecological implications. Blackwell Science, Oxford

Steinhart GB, Sandrene ME, Weaver S, Stein RA, Marschall EA (2005) Increased parental care cost for nest-guarding fish in a lake with hyperabundant nest predators. Behav Ecol 16:427–434

Suski CD, Philipp DP (2004) Factors affecting the vulnerability to angling of nesting male largemouth and smallmouth bass. Trans Am Fish Soc 133:1100–1106

Suski CD, Killen SS, Morrissey MB, Lund SG, Tufts BL (2003) Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. N Am J Fish Mange 23:760–769

Suski CD, Killen SS, Cooke SJ, Kieffer JD, Philipp DP, Tufts BL (2004) Physiological significance of the weigh-in during live-release angling tournaments for largemouth bass. Trans Am Fish Soc 133:1291–1303

Suski CD, Killen SS, Kieffer JD, Tufts BL (2006) The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. J Fish Biol 68:120–136

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the descent of man. Heinemann Press, London, pp 139–179

Wagner T, Congleton JL (2004) Blood-chemistry correlates of nutritional condition, tissue damage, and stress in migrating juvenile Chinook salmon *Oncorhynchus tshawytscha*. Can J Fish Aquat Sci 61:1066–1074

Wedemeyer GA, Barton BA, McLeay DJ (1990) Stress and acclimation. In: Schreck CB, Moyle PB (eds) Methods for fish biology. American Fisheries Society, Bethesda, pp 451–489

Wendelaar Bonga SE (1997) The stress response in fish. Physiol Rev 77:591–625

Williams GC (1966a) Adaptation and natural selection. Princeton University Press, Princeton

Williams GC (1966b) Natural selection, the costs of reproduction, and a refinement of Lack's principal. Am Nat 100:687–690

Yamada J (1956) On the mechanism of the appearance of the scale structure. VI. Some observations associating with the absorption of scale in the goldfish. Bull Fac Fish Hokkaido Univ 7:202–207

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Englewood Cliffs