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Does nest predation pressure influence the energetic cost of nest guarding in a teleost fish?

M. A. Gravel · S. J. Cooke

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Abstract The energetic costs of providing parental care are widely documented, but rarely do studies consider the role of environmental variation (e.g., predation pressure) in this context. Here, we tested if variation in nest predation pressure influenced the energetic costs of parental care in smallmouth bass (*Micropterus dolomieu*), a teleost fish species that provides lengthy paternal care. First, we documented that nest predation pressure varied among the six lakes studied and the relative predation pressure ranking was consistent across a three year period. We used a combination of traditional proximate body composition (PBC) analyses and electromyogram (EMG) telemetry to quantify activity costs of nesting fish across these populations. The traditional approach revealed declines in energy stores across the parental care period but showed no evidence of an increased energetic cost to parents from populations with higher nest predation pressure. Comparing the distribution of EMG data from the two extremes of predation pressure revealed that males from the site of highest predation spent more time at higher EMG levels relative to the parents from the lake of lowest predation pressure. Although not statistically significant, males from the site of highest predation pressure also spent 21–24 % of their time burst swimming when guarding young offspring compared to 10–11 % for males at the site of lowest predation pressure. These differences in overall activity, a large contributor to the energy use of fish, may translate into longer recovery times and decreased future reproductive opportunities.

Keywords Electromyogram telemetry · Kernel density estimates · *Micropterus dolomieu* · Parental care · Proximate body composition

Introduction

Reproduction is considered a costly activity (Bell 1980) because individuals must allocate a limited amount of resources to growth, maturation and reproduction. Allocating resources to reproduction instead of other functions is often referred to as the 'physiological cost' of reproduction and has played an important role in defining life-history theory (Reznick 1992). Measuring the cost of reproduction within a single environment has been shown in a wide range of taxa (e.g., plants, insects, birds, reptiles and fish; Obeso 1993; Hutchings 1994; Lee et al. 1996; Madsen and Shine 2000; Almbro and Kullberg 2009). These costs

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have been measured in a variety of ways and often examine the trade-offs between reproductive success and growth (e.g., Obeso 1993; Cox and Calsbeek 2010), locomotor performance (e.g., Lee et al. 1996; Almbro and Kullberg 2009), and longevity (e.g., Rose and Charlesworth 1981). For taxa that provide parental care, reproduction can be particularly costly since it also involves energetically demanding behaviours such as feeding offspring, providing heat to offspring, and guarding offspring from predators (Clutton-Brock 1991). Tending broods can cause loss of mass (Townsend 1986; Moreno 1989; Marconato et al. 1993), depletion of energy stores (Fitzgerald et al. 1989; Gillooly and Baylis 1999; Mackereth et al. 1999), and reduced future breeding opportunities (Coleman et al. 1985).

Theoretical models have revealed that environmental influences should affect the costs of providing parental care (Carlisle 1982; Webb et al. 2002; Steinhart et al. 2008), but natural variation in the environment is typically not considered a primary factor. Changes in the costs of parental care associated with different environments have been confirmed by only a handful of taxon-specific empirical studies (Boggs and Ross 1993; Delope et al. 1993; Jones and Reynolds 1999; Green and McCormick 2005; Steinhart et al. 2005; Hale and St Mary 2007). Most often these studies consider the effect of parental food limitation, but some studies have examined the increased costs associated with parasite load (Delope et al. 1993), or in the aquatic environment, low oxygen conditions (Jones and Reynolds 1999) and salinity (Hale and St Mary 2007). Predator abundance and predation pressure can greatly vary across the range of a species (Fontaine et al. 2007; Gravel and Cooke 2009), and the inability of a parent to protect its brood from predators has severe fitness consequences (Philipp et al. 1997; Conway and Martin 2000). Thus, natural variation in nest predation pressure should influence the costs of providing parental care. We tested this hypothesis in a teleost fish that provides extended nest guarding behaviour, the smallmouth bass (*Micropterus dolomieu*).

Smallmouth bass provide male-only parental care that typically lasts four weeks (Ridgway 1988). During this period they fan eggs to help prevent silt deposition, as well as guard eggs and developing offspring from potential nest predators. Due to their parental activities, they have limited opportunities for feeding and must primarily rely on endogenous stores (Hinch and Collins 1991; Mackereth et al. 1999). Furthermore, there is evidence that parental care is energetically costly for this species as nest guarding smallmouth bass are extremely active (Cooke et al. 2002), and lose mass (Gillooly and Baylis 1999) and energy stores (Mackereth et al. 1999) while providing care. Moreover, providing parental care appears to impose a recovery period in which the male must actively feed and replace lost energy stores (Mackereth et al. 1999). In this study, we set out to test whether the energetic costs for parental smallmouth bass vary across populations that naturally differ in nest predation pressure. We predicted that males from lakes with high nest predation pressure would show higher energetic costs relative to males from lakes with lower predation pressure. First, however, we had to test whether predation pressure was consistent over multiple years in a given waterbody and if it varied among lakes. We then measured the energetic costs of providing care using two techniques. Herein, the "traditional approach" refers to lethally sampling individuals to conduct proximate body composition analyses (PBC), and that the "individual-based approach" refers to calibrating observed behaviours to electromyogram (EMG) radio transmitter signals (Cooke et al. 2004), and using EMG measurements as proxy for energy status. EMG transmitters provide information on the locomotor activity levels of fish (Cooke et al. 2004) which is relevant to the costs of reproduction for a parental care and the overall bioenergetics of fish in that locomotor activity is one of the largest and most variable contributors to energy use in fish (Boisclair and Leggett 1989). First, we used the traditional approach to compare the energy stores of individuals at the start and end of parental care. This approach enabled the comparison between the energy stores of different individuals across many populations and examines the effects of predation pressure on the energy stores of parents. Because lethal sampling is used for PBC analyses, different individuals are sampled at the beginning of care and end of care. We predicted that energy stores would be more greatly depleted across the period of parental care for males from lakes of high predation pressure compared to males from lakes with low predation pressure. Second, we used the individual-based approach to quantify the locomotor activity of the same individuals across the parental care period at the two extremes of predation pressure (i.e., the lake with the lowest and highest predator burden). We predicted that males from the site of high predation pressure would show higher EMG values and spend more time engaged in burst swimming events compared to males from the site of low predation pressure. When combined, these techniques have the potential to reveal whether natural variation in the environment influences the cost of providing parental care and more specifically if there are increased energetic costs associated with sites of high nest predation pressure.

Methods

Study site and species

The study took place on six lakes in south-eastern Ontario within the Rideau and Gananoque River watersheds. We showed elsewhere (Gravel and Cooke 2009) that these lakes varied greatly in nest predation pressure in a given year. In order to establish whether variation in predation pressure across lakes remained consistent across years, variation in predation pressure was measured for three consecutive years (2007–2009). Each spring, when water temperatures reached approximately 15°C, snorkelers swam the littoral zones of the lakes to identify nesting smallmouth bass that were guarding fresh fertilized eggs ($n \geq 40$ per lake). Predation pressure was then measured on a random ($n=10$) subset of these fish when males were guarding fresh eggs. Predation pressure was only measured when males guarded fresh fertilized eggs to ensure consistency among the lakes and because this stage is known to be particularly vulnerable to predation because eggs are immobile and energetically valuable for predators (Cooke et al. 2008). Lakes warm differently due to variation in depth and size. Consequently, the sampling dates for the smaller shallower lakes were between May 8 and 16th, while the larger, deeper lakes were sampled between May 20th and 30th. Metrics of predation pressure are outlined in detail in Gravel and Cooke (2009) and consisted of measuring the maximum number of predators in close proximity to the nest (2 m radius) when the male was present (perceived predation pressure) and measuring the maximum number of predators that actively consumed fertilized eggs when the male was absent (actual predation pressure). The perceived predation pressure was assessed for 5 min. Preliminary analyses (Gravel and Cooke 2009) revealed that assessing the perceived predation pressure for 5 or 15 min yielded the same results. To measure actual predation pressure, males were removed from their nest by rod and reel and kept on board the boat in a cooler (40 L) of fresh lake water for 10 min. The proportion of nests depredated and the time to nest predator arrival in the absence of the male were also noted. The maximum values of nest predators were used in statistical analysis because individual predators could not be identified and reporting means would be misleading. Species composition of the nest predator community was similar among lakes and was composed of pumpkinseed sunfish (*Lepomis gibossus*), bluegill sunfish (*L. macrochirus*), and rock bass (*Ambloplites rupestri*), with the majority (i.e., >90 %) of predators observed being Lepomids.

Energy stores

In the spring of 2007 male fish were lethally sampled from the lakes on which the predation pressure metrics were measured. First, males were identified guarding fresh fertilized eggs ($n \geq 40$ per lake). Predation pressure metrics were measured on a random subset of these fish ($n=10$). These same fish were then lethally sampled. Subsequently, the lakes were visited frequently to assess the development of offspring. When males were found to be guarding larvae, a second random sample ($n=10$) were lethally sampled from the originally identified fish. It was necessary to only sample fish identified during the initial sampling period so that we were comparing fish that spawned at the same time and thus experienced the same environmental conditions, as well as were of similar starting size given that it is well known that the size of males initiating reproduction decreases during the spawning period (Mackereth et al. 1999). Fish were removed from their nest with conventional hook-and-line equipment and brought quickly to the boat and euthanized by cerebral percussion. Fish were placed in individual plastic bags and transported in a cooler back to the lab (up to 12 h) and placed in a chest freezer (up to 4 days, -20°C). Fish were thawed and measured for total body weight (g), total length (mm), liver weight (g) and gonad weight (g). Gonadosomatic (GSI) and hepatosomatic (HSI) indices were calculated using following equations: GSI = gonad weight body weight $^{-1}$ * 100 and HSI = liver weight body weight $^{-1}$ * 100. Eviscerated fish were ground in a manual meat grinder. All tissues were placed back in the chest freezer in sealed bags until lipid analysis.

Energy stores were measured in the eviscerated tissue and livers. Techniques were identical to those used by

Redpath et al. (2009) and Gravel et al. (2010a). We measured lipid content using a methanol-chloroform extraction modified from Bligh and Dyer (1959) (Smedes and Askland 1999). Homogenized tissues (2 g) were dried at 80°C overnight (16–20 h) until a constant mass was attained. The dried samples were then reduced to a fine powder with a mortar and pestle and dissolved in a 1:2:0.8 ratio of chloroform, methanol and water and then extracted with a 2:2:1.8 ratio of the same solvents. A second extraction was then performed with 10 % methanol in chloroform solution. The solvent layer of chloroform (containing the lipids) was removed using a pipette and filtered through sodium sulfate and quartz wool. The extracted lipids were left overnight (to allow the chloroform to evaporate), dried for 1 h at 60°C, and weighed to calculate the percent of lipids by dry mass. Replicates were made for all samples and extractions were repeated when differences between samples were larger than 3 %. These values for the dried samples were then expressed in terms of percent of lipid by wet mass.

For the same individuals, other body constituents (water, trace mineral and protein) were determined from aliquots of homogenized eviscerated whole body tissue but were not measured on livers due to their small size. The homogenized tissue (2 g) was dried at 80°C overnight (16–20 h) until a constant mass was attained and re-weighed to assess water content. Water content was determined by measuring the difference between wet mass and dry mass. The dried samples were then combusted for 2 h in a muffle furnace at 500 - 600°C. The resulting trace minerals (ash) were weighed to determine the percentage of ash by wet mass. Protein content was determined using the following relationship: $CP = 100 * (CW + CA + CL)$, where C_P, C_W, C_A and C_L represent percent protein, water, ash and lipid respectively (Crossin and Hinch 2005; Redpath et al. 2009).

Electromyogram (EMG) telemetry

Based on our previous work (Gravel et al. 2010b), we chose lakes at both extremes of predation pressure to examine swimming activity of nesting smallmouth bass. The site with high predation pressure, Lake Opinicon, is a small lake of 790 ha and has a mean depth of 2.4 m. Upper Rideau lake, with low predation pressure, is 6482 ha and has mean depth of 17.4 m (Marleau 2007). Due to their differences in size, the lakes warm differentially. We implanted males guarding fresh fertilized eggs (n=6) on Lake Opinicon on May 6th, 7th and May 8th. Males from Upper Rideau Lake (n=8) were implanted on May 26th, while guarding fresh fertilized eggs. Fish with fresh fertilized eggs were identified by a snorkeler, caught by conventional hook-and-line techniques and brought to the boat for surgery. In the meantime, the snorkeler remained at the nest and defended the eggs from nest predators when necessary. Fish were anesthetised in a large container (50 L) of fresh lake water and clove oil (60 ppm) emulsified in ethanol. Once fish lost equilibrium and showed no response to manual stimulus, they were weighed (g), measured for total length (mm) and transferred to the surgery trough on a moistened sponge, ventral side up. Gills were irrigated with a mixture of fresh lake water and clove oil (30 ppm) emulsified in ethanol. We made a small latero-ventral incision (approx. 20 mm) to insert the EMG transmitter into the body cavity and the gold electrodes were placed in the red axial musculature along the lateral line. The incision was closed with PDS-II absorbable sutures (3/0; Ethicon, Somerville, NJ) and fish were placed in a second container (50 L) of fresh lake water to recover. Fish were released once they had regained equilibrium and reacted to mechanical stimulus (i.e., tail-grabbing). The snorkeler departed from the nest once the male had returned.

Fish were implanted with coded electromyogram (EMG) transmitters (Lotek Wireless Inc. New Market, Ontario, Canada). Two sizes of transmitter were implanted (Lotek, CEMG2-R11-18, 54×11 mm, 4.9 g – in water and Lotek, CEMG-R16-25, 62 mm×16 mm, 13 g - in water) based on the total weight of the fish. Tags always represented <2 % of total fish weight. The transmitters were equipped with an antenna and a pair of electrodes affixed with gold tips (9 carat, 7 mm×1 mm). The electrodes detect electromyogram signals within the red axial muscle and integrate this signal over a 2.5 s period, providing a mean EMG value along with a time stamp. This signal is transmitted to a receiver (SRX 600, Lotek Wireless Inc.) on shore that records the EMG value. The EMG value (min: 0, max: 50) is proportional to muscle activity. Similar types of transmitters have previously been used on black bass (Cooke et al. 2001, 2002). Subtle differences in electrode placement as well as component performance of tags vary slightly between individuals and each tag was individually calibrated *in situ* (Cooke et al. 2004).

Tags were calibrated while males were still guarding eggs. Calibrations were performed 2–4 days post-surgery for fish in Opinicon Lake and 2 days post-surgery for fish in Upper Rideau Lake (see details below). All calibrations were performed underwater by the same snorkeler. The snorkeler set their digital watch to match the recording time on the receiver and observed the fish for five minutes, monitoring all of the swimming movements and writing them on a dive slate. The behaviours were recorded every five seconds. Observed behaviours were categorized as either swimming in place on/near nest or chasing a predator (burst swimming). The fish was then chased by the snorkeler for 30–40 s to elicit burst swimming. A nest predator was then introduced into the nest to elicit an antipredator response. The nest predator consisted of a live bluegill sunfish that was placed in a large glass jar filled with fresh lake water. The behaviours were not randomized since observing the fish on his nest for baseline EMG values may have been affected by first introducing a predator or being chased by a snorkeler. The behaviours observed by the snorkeler were then matched with the EMG values recorded by the receiver and each behaviour was assigned a specific range of EMG values.

Nest guarding behaviours are variable depending on stage of development of the offspring (Ridgway 1988; Cooke et al. 2002, 2008) and typically parents reduce the intensity of care as offspring approach independence (Gross 2005). We categorized offspring development into 3 periods: eggs (mostly fertilized although there was a small proportion of the eggs in nest that are not fertilized), eleutheroembryo (embryos) and pterygiolarvae (larvae) (Wallace 1972; Balon 1975). Nests were snorkelled every 1–3 days to determine the development time of the offspring. Hatching date, pigment development, and the onset of swimming and exogenous feeding were noted. All the fish from the low predation site remained with their offspring until we ceased visiting the nests (24 days). One EMG tag from the low predation site could not be calibrated and sample sizes were reduced to $n=7$. Conversely, all males from the high predation site did not guard their nests for the entire monitoring period (24 days). One male abandoned his nest before eggs hatched (guarded nest for 8 days) and was included in only the egg stage analysis. One tag failed while a male was guarding embryos. This male completed parental care and was included in the analysis up to and including the embryo stage. Another male abandoned while guarding embryos and was included in the analysis up to and including the embryo stage. Finally, one tag failed to calibrated and needed to be removed from all analyses. Our sample size for the high predation site was thus $n=5$ for males guarding eggs, $n=4$ for males guarding embryos, and $n=3$ for males guarding larvae.

Statistical analysis

Statistical analyses were performed in J.M.P 8.0.2 (SAS Institute, Vary, NC, USA), SPSS 17.0 (IBM, Chicago, Illinois, USA) and R 2.11.1 (www.r-project.org). Power was calculated using G*Power 3.1.2 (Heinrich Heine University, Düsseldorf, Germany). Data were tested for normality and homogeneity of variance. When appropriate, non-normal data were log10 transformed to attain normality. We compared predation pressure metrics across lakes and among years using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1995; Dytham 2003) in SPSS because this data could not be transformed to achieve normality. We tested if the time to nest predation pressure differed among lakes using a parametric survival analysis. We used two-way ANOVAs to test if parental males differed in length or weight, using lake and stage of parental care as main effects and then used an ANCOVA model to test if the relationship between total length and weight (both log transformed due to the curvilinear relationship between length and mass, Mackereth et al. 1999) differed between fish guarding eggs and larvae. Because there is a known positive relationship between male size (length) and energy stores (Mackereth et al. 1999; Steinhart et al. 2005), we used general linear models, using total length (TL) as a covariate, to test if the residuals from the relationship between total length and energy indices (all lakes grouped) differed between stages of parental care, across lakes and of greater interest, across the stages of parental care within lakes (interaction term). The level of significance (α) for all tests was assessed at 0.01 to minimize Type I error associated with multiple statistical tests (Zar 1999). If significant differences were found, we used the Tukey-Kramer post-hoc test to identify significantly different groups.

EMG data for each individual fish were calibrated with their behaviours (described above) and then standardized using an activity index to ensure that data could be compared among individuals. The minimum EMG value observed during the calibration period (when snorkeler was monitoring fish and it was stationary) was assigned the value 0 and the maximum EMG value observed during calibrations when bursting was elicited was assigned a value of 1. Any values that fell outside this range were excluded from analysis (less than 4 % of data). EMG data from individual fish were then converted to standardized EMG values ($EMG_{standard}$) using the equation and definitions shown in Table 1. Thus, all EMG values between EMG_{min} and EMG_{max} were replaced with values between 0 and 1. We then used non-parametric kernel density smoothing to examine the distribution of the EMG data for each stage of parental care (egg, embryos and larvae) for both lakes. A conservative normal optimal smoothing method was first used to compute our smoothing parameter, $h = \sigma(4/3n)^{1/5} = 0.017$, where n is the sample size and σ is the standard deviation of the distribution. This approach produced a highly variable estimate due to clustering in the data (i.e., many data points for certain EMG values) (Bowman and Azzalini 1997). Consequently, the smoothing parameter was increased to 0.05 and this was deemed appropriate because it properly described the structure of the data, which avoided oversmoothing (too many peaks) or undersmoothing (too few peaks) (Bowman and Azzalini 1997). We then tested the equality of these distributions at each stage of parental care between lakes using the “sm” package in R (Bowman and Azzalini 2010). The function “sm.density.compare” uses permutations to test for equality between distributions. A bootstrap value of 100 was used in all tests.

The proportion of data points associated with burst swimming events and non-burst swimming events were compared between lakes using a 2×2 contingency and this was repeated for each stage of development (egg, embryos and larvae). The mean proportion of time spent burst swimming between lakes for each stage of parental care was compared using a *t*-test. For this analysis, each behaviour was assigned their values observed during calibration. For example, if EMG values between 20–30 were associated with burst swimming for fish A during calibration, then all values between 20–30 were defined as burst swimming behaviour throughout parental care. Values presented are means±standard error (SE) unless otherwise indicated and the significance of EMG related statistical tests were evaluated at $\alpha=0.05$.

Results

Predation pressure

Perceived and actual predation pressure differed among lakes and was similar across years (Table 2, Fig. 1). Furthermore, the ranking of lake predation pressure was consistent across years (Table 2, Fig. 1). Lakes with lower predation pressure (Upper Rideau, Charleston Lake and Indian lake) had on average less than two individual predators near the nest when males were present and when nests were depredated. Lakes with the highest predation pressure (Sand Lake and Opinicon Lake) had a mean of 3 predators in close proximity to nests when males were present but had 11 predators consume eggs when males were absent. Thus the lake with the highest predation pressure (Opinicon Lake) has over a 100 fold increase in actual nest predation pressure relative to the lake with the lowest actual predation pressure (Upper Rideau Lake). Time to predation event differed significantly among lakes (parametric survival analysis, $\chi^2=48.57$, $p<0.0001$, Fig. 2a) and was not influenced by year ($\chi^2=0.03$, $p<0.98$). Generally, little to no predation occurred for Upper Rideau Lake and Charleston Lake. The proportion of nests depredated among the 6 lakes across the 3 years of data collection

Table 1 Metrics for the conversion of observed EMG values to standardized EMG values

Metric	Definition
$EMG_{standard}$	$(EMG_{obs} - EMG_{min})/(EMG_{max} - EMG_{min})$
EMG_{max}	is the highest recorded EMG value for a given individual
EMG_{min}	is the lowest recorded EMG value for a given individual
EMG_{obs}	is an EMG value between EMG_{min} and EMG_{max}

Table 2 Results from non-parametric two-way ANOVA (Scheirer-Ray-Hare extension of Kruskal-Wallis test) that compares predation pressure metrics from 6 lakes from 2007 to 2009. Significant differences are shown in bold

Response	Source	d.f.	H	P-value
Perceived predation pressure (male present)	Lake	5	16.23	**0.01**
	Year	2	2.94	0.23
	Lake × Year	10	8.27	0.60
Actual predation pressure (male absent)	Lake	5	28.13	**< 0.0001**
	Year	2	2.25	0.32
	Lake × Year	10	7.61	0.67

ranged from 7 % predation (2 of 29 were depredated) to 72 % predation (22 of 29 nests were depredated, Fig. 2b).

Energy stores

Male guarding eggs were on average 7 % longer and 22 % heavier than males guarding larvae (Mean±SE; TL_{egg}: 408.0±6.0 mm, TL_{larvae}: 381.0±6.0 mm, $Weight_{egg}$: 989.0±45.0 g, $Weight_{larvae}$: 806.0±44.0 g), but male size did not differ among lakes (two-way ANOVA, lake: $F_{5,1}$=1.81, p=0.12, stage: $F_{1,1}$=9.60, p=0.002, lake * stage: $F_{5,1}$=1.38, p=0.24). Male weight was also related to the stage of parental care (two-way ANOVA, lake: $F_{5,1}$=1.13, p=0.35, stage: $F_{1,1}$=9.02, p=0.003, lake * stage: $F_{5,1}$=1.36, p=0.24). Because the relationship between total length and weight did not change between fish guarding eggs and fish guarding larvae (ANCOVA, stage: $F_{1,1}$=1.01, p=0.32; TL: $F_{1,1}$=2046.99, p<0.0001; stage * TL: $F_{1,1}$=0.19, p=0.66), the weight loss can be attributed to differences in size between groups and not to loss of mass. Some energy indices decreased across the parental care period (Figs. 3 and 4). GSI showed a positive relationship with male total length, differed between lakes and decreased across the parental care period but did not differ between stages of parental care among lakes (Table 3, Fig. 3a). HSI showed a positive relationship with TL, decreased across parental care and the relationship between HSI and stage of parental care changed between lakes (Table 3, Fig. 3b), with Opinicon and Newboro Lake showing the greatest decrease. All other energy indices were not influenced by male total length (Table 3). Residuals of eviscerated whole body lipid did not change among the stages of parental care period or among lakes (Table 3, Fig. 3c). Residuals of eviscerated whole body protein were influenced by lake and by the stage of parental care (Table 3, Fig. 3d), with a generally increase in protein content across parental care with Indian Lake showing the most positive residuals and Sand Lake showing the most negative residuals. Residuals of liver lipid increased between the stages of parental care and differed among lakes but the influence of stage of parental care did not change among lakes (Table 3, Fig. 3e). Males from Opinicon and Upper Rideau Lake had the most negative residual liver lipids, while males from Charleston Lake had the most positive residuals. Sample sizes are shown in Table 4. Generally, there was no strong relationship between lake-level predation pressure and energy status using traditional energy status metrics.

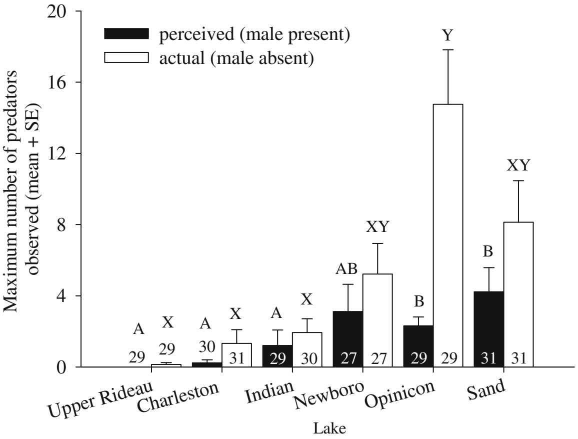

Fig. 1 Perceived (male present) and actual (male absent) predation pressure in 6 lakes from 2007 to 2009. Sample sizes are shown on bars. Dissimilar letters (**a**, **b**) indicates significant differences (p<0.05) across lakes for perceived predation pressure while dissimilar letter (X,Y) indicates significant differences (p<0.05) across lakes for actual predation pressure. (2007 predation pressure metrics are adapted from Gravel and Cooke 2009)

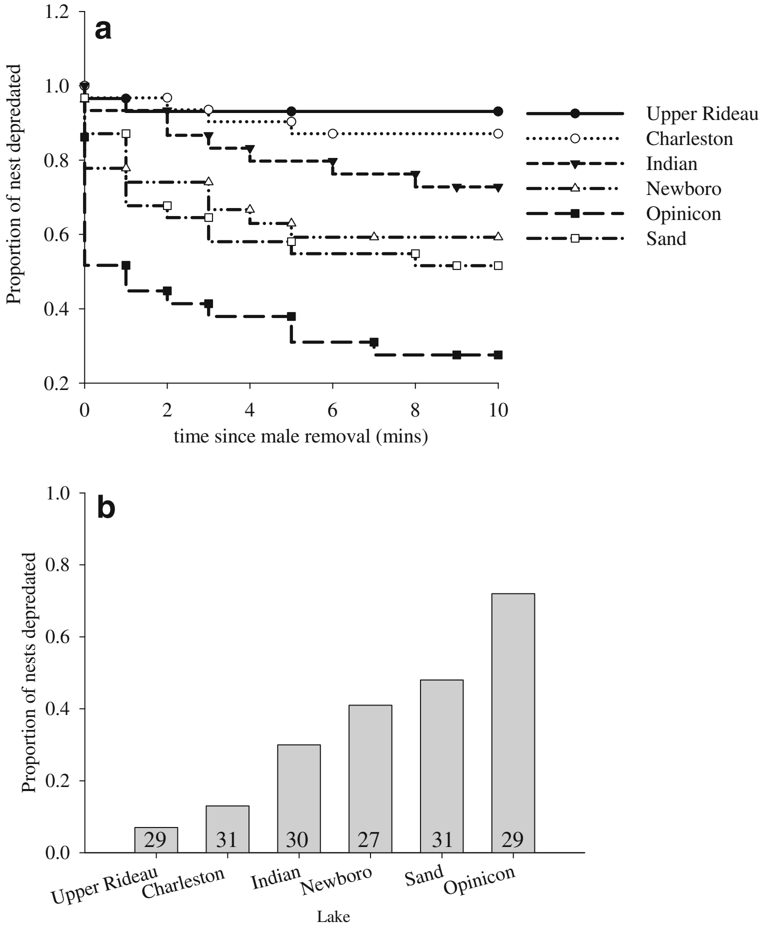

Fig. 2 Time to nest predation (**a**) and proportion of nests depredated (**b**) across 6 lakes from 2007 to 2009. Sample sizes are shown on bars

EMG telemetry

Males implanted with EMG tags in Upper Rideau Lake (mean±SE: 427±9.6 mm) were significantly larger than fish tagged in Opinicon Lake (mean±SE: 358±14.9 mm; *t*-test, $t=3.87$, $p<0.004$). The kernel density estimates of $\text{EMG}_{\text{standard}}$ differed between males from the site of low predation and high predation for all stages of parental care (kernel density comparison, $p<0.0001$, Fig. 4). For the egg stage, we observed that the distribution of $\text{EMG}_{\text{standard}}$ from Upper Rideau Lake (low predation) males showed two peaks in their density estimates; the largest peak was near the lowest $\text{EMG}_{\text{standard}}$ value, while the second smaller peak was near the mid-point of the $\text{EMG}_{\text{standard}}$ values. Conversely, the distribution of $\text{EMG}_{\text{standard}}$ values from Opinicon Lake (high predation) males showed only one peak near the mid-point. The two distributions showed more overlap when males guarded older hatched offspring, with the biggest change being that the Opinicon Lake distribution had more data points registered at lower $\text{EMG}_{\text{standard}}$ (kernel density comparison, $p<0.0001$, Fig. 4b). Finally, the estimates of density continued to significantly differ between the lakes when males guarded larvae (kernel density comparison, $p<0.0001$, Fig. 4c), where Upper Rideau Lake show two peaks compared to only one in Opinicon Lake. Although not statistically significant, parental males from the high predation site spent 24 % of their time engaged in burst swimming when guarding eggs compared to 10 % for parental males at the site of lowest predation pressure (*t*-test, $t=1.39$, $p=0.22$, Fig. 5) and this trend was again observed when guarding embryos (*t*-test, $t=2.01$, $p=0.08$, Fig. 5). There were no differences in the proportion of time engaged in burst swimming activity when males were guarding larvae (*t*-test, $t=0.17$, $p=0.87$; Fig. 5). Due to small sample sizes, the power of these tests was low ($1\text{-}\beta=0.40$, 0.20 and 0.07 for the period spent guarding eggs, embryos and larvae respectively).

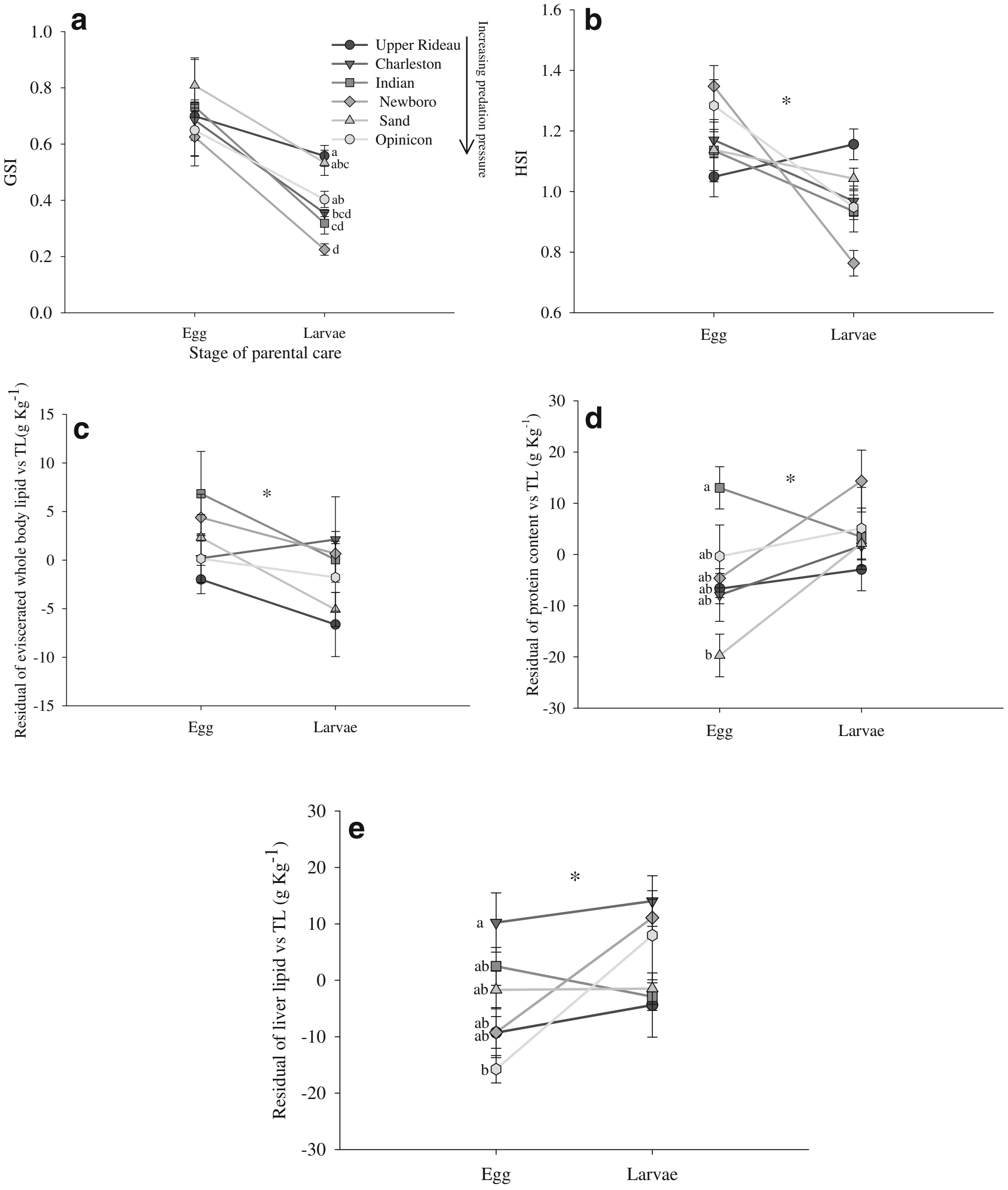

Fig. 3 GSI (**a**), HSI (**b**) and residuals from regression between male smallmouth bass total length and eviscerated whole body lipid (**c**), protein content (**d**) and liver lipid (**e**) as a function of stage of parental care for 6 lakes that differ in nest predation pressure. Different letters (**a**, **b**, **c**, **d**) indicate significant differences between lakes ($p<0.05$) and * indicates significant differences between the stages of parental care ($p<0.05$)

Discussion

We set out to test whether an ecologically relevant environmental factor, nest predation pressure, could influence the energetic costs of providing parental care. We used a species that provides sole-paternal care, the smallmouth bass, as a model. We predicted that males from lakes with higher predation pressure would show greater decreases in energy stores as well as spend more time engaged in burst swimming activities relative to males from lakes with lower predation pressure. Our study revealed that nest predation pressure

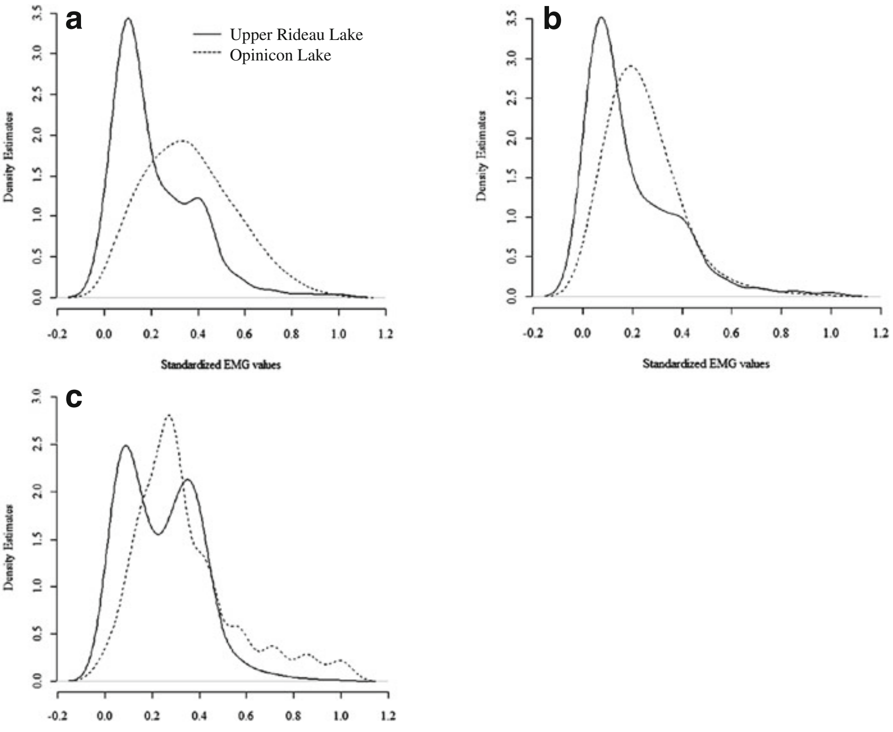

Fig. 4 Kernel density estimates of standardized EMG values for Upper Rideau Lake (low nest predation pressure) and Opinicon Lake (high nest predation pressure) when parental males guarding eggs (**a**), embryos (**b**) and larvae (**c**). Sample sizes are n=7 for Upper Rideau Lake for all stages and n=5 for the egg stage, n=4 for the embryos stage and n=3 for the larvae for Opinicon Lake

varied among lakes and was consistent across years emphasizing the potential for predation pressure to serve as a selective force. We found no support for our hypothesis that predation pressure influenced the cost of parental care when we used proximate body composition to assess energy stores. In contrast, we detected differences in overall locomotor activity between low and high predation sites when we used an approach that examined the activity of free-swimming individuals through time (i.e., higher activity levels in lakes with higher predation pressure).

Consistent with other studies (Gillooly and Baylis 1999; Mackereth et al. 1999), the traditional energetic approach detected a decrease in energy stores of parental males across the period of parental care. However, we failed to detect differences in the energetic costs of parental care with varying predation pressure across lakes. Previous work by Steinhart et al. (2005) compared the energy density and weight loss of parental male smallmouth bass between Lake Erie, a lake with a hyperabundant invasive nest predator, the round goby (*Neogobius melanostomus*), and a lake with lower natural nest predation pressure. Contrary to our results, their work showed a reduction in parental weight and body energy density in parents from the lake with increased predation pressure (i.e., hyperabundant invasive nest predators) relative to the lake with lower and native nest predation pressure. Steinhart et al. (2004) report that nest predation pressure was 4.3 round goby/min (males absent) for the Lake Erie population. Presented in that manner, our data shows that predation pressure for the lake with the highest predation pressure (Opinicon Lake) would be on average 9.7 predators/min (males absent). In addition, Steinhart et al. (2005) reports that nesting males were involved nine times more frequently in predator chases in the lake with round gobies relative to the lake without that invasive species. Our previous work has shown that males from the site of lowest predation pressure did not engage in any predator chases, while males from our high predation site did engage in 10 or more predator chases within a 10 min period (Gravel and Cooke 2009). If weight loss and energy loss is solely attributed to an increase in predator abundance and associated predator chases, we would also expect males from Opinicon Lake to show similar changes in energy status. One interesting distinction between the populations in our study and the Lake Erie population may be the historical levels of predation pressure. Round goby were first discovered in Lake Erie in 1993 and their abundance has greatly increased since their introduction (Corkum et al.

Table 3 Results from General Linear Models, using total length (TL) as a covariate and testing the influence of predation pressure (lake), stage of offspring development and their interaction on the residuals from the regression between male TL and different energy indices. Significant differences are shown in bold

Response	Source	d.f.	F	P-value
GSI	TL	1	13.30	**0.0004**
	Lake	5	8.53	**< 0.0001**
	Stage	1	93.22	**< 0.0001**
	Lake × Stage	5	1.27	0.28
HSI	TL	1	11.94	**0.0008**
	Lake	5	0.31	0.90
	Stage	1	24.53	**< 0.0001**
	Lake × Stage	5	5.87	**< 0.0001**
Residuals of eviscerated whole body lipid (g Kg^{-1})	TL	1	0.22	0.88
	Lake	5	5.63	0.11
	Stage	1	4.85	0.03
	Lake × Stage	5	0.71	0.62
Residuals of liver lipid (g Kg^{-1})	TL	1	0.51	0.48
	Lake	5	5.63	**< 0.0001**
	Stage	1	10.06	**0.002**
	Lake × Stage	5	2.00	0.08
Residuals of eviscerated whole body water content	TL	1	0.12	0.73
	Lake	5	3.51	**0.006**
	Stage	1	0.01	0.91
	Lake × Stage	5	2.55	0.03
Residuals of eviscerated whole body ash content	TL	1	2.81	0.10
	Lake	5	7.35	**< 0.0001**
	Stage	1	6.38	**0.01**
	Lake × Stage	5	1.15	0.34
Residuals of eviscerated whole body protein content	TL	1	2.62	0.11
	Lake	5	3.78	**0.003**
	Stage	1	10.78	**0.001**
	Lake × Stage	5	2.72	0.02

2004). Because smallmouth bass demonstrate some nest site fidelity (Ridgway et al. 1991; Barthel et al. 2008) and predator abundance could be consistent across many years, selection pressure may play a role in adaptation to local predation pressures. Perhaps males from the populations we sampled are better able to deal with increased predation pressure relative to populations with a relatively novel and abundant predator such as the round goby.

Although depletions in energy stores did not reflect variation in predation pressure, there were lake specific differences. Evidently there are other important lake-level effects, other than predation pressure, that influence some of the energy indices measured in this study. One important difference among these lakes is their size (Gravel et al. 2010b). Upper Rideau and Charleston Lake are large and deep lakes (surface area: 6482 hectares, mean depth: 12.3 m and surface area: 2518, mean depth: 17.4 m respectively, Marleau 2007) which provide different habitat, prey availability, and prey composition than smaller shallower lakes (mean surface area±SE and mean depth±SE for Indian, Newboro, Sand and Opinicon Lakes: 737±408 hectares and 5.1±1.7 m, Marleau 2007). However, no clear pattern emerges between energy stores and lake size. The energy stores of parental males from the larger lakes are not consistently distinguishable from males from the smaller lakes. Dunlop et al. (2005) have demonstrated that difference in adult smallmouth bass densities can influence life-history traits (e.g., size at reproduction, reproductive investment) in recently divergent populations. Additional research would need to focus on these

Table 4 Sample sizes for parental males sampled for indicators of energy status (HSI, GSI, eviscerated body lipid, liver lipid and protein content)

Lake	Stage	n
Upper Rideau	egg	9
	larvae	10
Charleston	egg	10
	larvae	10
Indian	egg	10
	larvae	10
Newboro	egg	8
	larvae	10
Sand	egg	10
	larvae	11
Opinicon	egg	11
	larvae	10

life-history parameters (e.g., growth rate, age at maturity), as well as ecological parameters such as prey communities and densities, in order to identify the lake-specific effects that may be driving the differences in energy status.

Previous research shows that the traditional sampling technique has been used with success to identify variation in the energy status of fish providing parental care (e.g., Fitzgerald et al. 1989; Mackereth et al. 1999). However, this sampling technique requires lethal sampling and limits ability to repeatedly measure individuals. In turn, this leads to biases at certain developmental stages due to differential nest abandonment. Males that have prematurely abandoned their nests due to loss of energy stores, nest predation, or other causes are not included in the sampling of later stages of offspring development. The variation that may exist in energy stores may only be obvious in males that need to prematurely abandon their broods. Successful males may simply have energy stores above this "abandonment threshold". At the end of parental care, the values of the energetic indicators may be inflated because the fish that have fallen below the critical level have already abandoned.

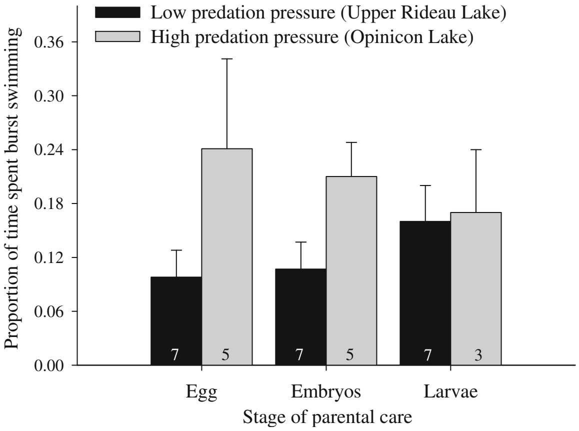

Fig. 5 Mean proportion of time spent burst swimming (+ SE) for parental males from populations that differ in nest predation pressure when guarding eggs, embryos and larvae. Sample sizes are shown on bars

The individual-based approach showed evidence that the energetic cost of providing parental care differed between sites of low and high predation pressure due to differences in abandonment rates between the sites. We observed 100 % success at the low predation site and 67 % success at site of high predation pressure. Monitoring abandonment rates may be a useful metric for examining the costs of providing care. Since nesting smallmouth bass are iteroparous and providing parental care is considered a substantial investment into current reproduction (Gross 2005), abandoning a current brood prematurely may be a good strategy to increase future reproductive opportunities (Coleman et al. 1985). In fact, a study on parental pumpkinseed showed that nesting success was higher in a lake with lower abundances of nest predators compared to nesting success in a lake with higher abundances of nest predators (Popiel et al. 1996). In addition, modeling studies have attempted to predict when parents should abandon their current broods (Carlisle 1982; Webb et al. 2002) and a recent such study by Steinhart et al. (2008) revealed that abandonment rates of parental smallmouth bass may increase when the probability of adult survival decreases and as the cost of parental care increases.

Further evidence of an increased cost of parental care in areas of high predation pressure comes from the activity data that was provided by EMG telemetry. Aquatic offspring are believed to be particularly vulnerable at the egg stage (Ridgway 1988; Hinch and Collins 1991) and therefore differences in EMG density estimates would be expected to be most pronounced. The density estimate for the low predation site is relatively narrow and left-skewed, and thus approaches the lowest EMG values recorded during the parental care period. The distribution of the EMG values from the high predation site is wider and more centralized (Fig. 4). Therefore, during the period of highest vulnerability for offspring, males from the site of high predation pressure are spending more time at higher EMG values but also show greater variance in their EMG values. In other words, fish from the site of high predation pressure have higher muscle activities on average but also show greater variation in their muscle activity, while the fish at the low predation site have lower mean muscle activities and also show less variation in their muscle activities. In addition, the EMG data showed that parental males from the site of high predation pressure spend almost twice the amount of time engaged in burst swimming events when guarding eggs or embryos. Because locomotor activity is one of the largest contributors to energy use in fish (Boisclair and Leggett 1989), chasing predators may translate into higher energetic and hence reproductive costs in lakes with increased predation pressure. For a temperate species such as the smallmouth bass, this could translate into longer post-reproduction recovery times (e.g., need to consume more food for longer) or potential for more frequent “reproductive holidays” (Dunlop et al. 2005; Barthel et al. 2008). Individuals may not acquire enough energy reserves to endure the winter months and spawn in the early spring. In consequence, they may need to delay reproduction to the following year.

Alternatively, parents at sites of high predation pressure may simply need to increase their energy intake during parental care in order to maintain these higher activities. Although smallmouth bass drastically decrease their food intake during parental care and demonstrate voluntary anorexia (Hinch and Collins 1991; Hanson et al. 2009) the level to which parents reduce food intake differs among populations (Gravel et al. 2010a). There is also clear evidence that consuming food during parental care impedes burst swimming ability and reduces aggressiveness towards brood predators (Hanson et al. 2009). Our work shows that males from sites of high predation pressure are more often engaged in aggressive behaviours towards brood predators (Gravel and Cooke 2009) and spend more time burst swimming than males from low predation sites, but somehow show no difference in depletion of energy stores. Although gut content only provides a snapshot of overall food consumption, we were able to compare gut contents from the fish which were sampled for the traditional energetic approach and parents from the different lakes did not differ in gut content wet-weight (Gravel unpubl. data). Recent work has shown that reproduction costs can be masked by individual differences in resource availability (Hamel et al. 2010). These general habitat differences and their associated local adaptations may also play a role in individual condition and energy status of parental smallmouth bass.

The difference in sampled male total length between these two lakes could also contribute to differences in EMG values. For this species, larger males are also older and more experienced than smaller males. Size and age contribute to the reproductive success of males for this species (Wiegmann and Baylis 1995; Wiegmann et al. 1997) and thus may also contribute to their reproductive behaviours. Older large males, with limited future breeding opportunities, will continue to provide parental care to their current brood following a stressor, while younger smaller males, with greater future reproductive opportunities, will abandon their current brood following a stressor (Connie O’Connor unpubl. data). Perhaps the smaller fish sampled at the site of high predation pressure (Opinicon Lake) are less experienced and demonstrate more irregular guarding behaviours than larger, older nesting males sampled at the low predation site (Upper Rideau Lake).

Overall, our study showed that traditional indicators of energy status did not detect an increased cost of parental care in populations with higher predation pressure. An individual-based approached detected physiological differences in the swimming behaviours of parental males between sites of high and low predation pressure. Parents from the site of high predation pressure show trends of spending more time burst swimming and were more often engaged in high levels of activity. Because locomotor activity is an important contributor to energy use in fish, it is possible that these differences in activity translate into longer recovery times and decreased future reproductive opportunities, particularly for temperate species where the growing season is limited. Given that patterns of nest predation among lakes appear to be consistent across years, and that activity levels of nest guarding males vary among high and low predator lakes, our study reveals that predation pressure may represent a physiological cost and thus has the potential to influence life-history. As such, there may be life-history consequences associated with different levels of nest predation pressure. Our study emphasizes the need to incorporate field-based estimates (i.e., repeated measures) of energy use rather than only relying on metrics determined from lethal sampling of different individuals across multiple developmental stages.

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