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Exploring relationships between cardiovascular activity and parental care behavior in nesting smallmouth bass: A field study using heart rate loggers

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ABSTRACT

Research in a variety of vertebrate taxa has found that cardiac function is a major limiting factor in the ability of animals to cope with physiological challenges, and thus is suggested to play an important role in mediating fitness-related behaviors in the wild. Yet, there remains a paucity of empirical assessments of the relationships between physiological performance and biological fitness in wild animals, partially due to challenges in measuring these metrics remotely. Using male smallmouth bass (*Micropterus dolomieu*) as a model, we tested for relationships between cardiac performance (measured using heart rate loggers) and fitness-related behaviors (assessed using videography and snorkeler observations) in the wild during the parental care period. Our results showed that heart rates were not significantly related to any measured parental care behaviors (e.g., nest tending) except for individual aggression level. After accounting for the effect of water temperature on heart rate, we found within-individual heart rate differed between days and also differed between nights. There was, however, evidence of diel variation in heart rate, where heart rate was higher during the day than at night. Although fitness is thought to be dependent on physiological capacity for exercise in wild animals, inter-individual variation in heart rate alone does not appear to relate to parental care behavior in smallmouth bass at the temporal scales examined here (i.e., hours to days). Further studies are required to confirm relationships between physiological performance and parental care behavior to elucidate the apparently complex relationships between physiology, behavior, and fitness in wild animals.

1. Introduction

Many wild vertebrates engage in parental care as a reproductive strategy to maximize offspring survival (Clutton-Brock, 1991; Klug and Bonsall, 2014). During the parental care period, individuals are frequently exposed to intense physical exercise, a lack of energy intake via foraging, and increased exposure to predators (e.g. Roff, 1983; Bustnes and Erikstad, 1991; Magnhagen, 1991). Research on various vertebrate taxa has suggested that energy demanding behaviors, such as those associated with parental care behaviors, rely strongly on aerobic scope and energy acquisition and utilization (Priede, 1977; Reardon and Chapman, 2010; discussed in Brownscombe et al., 2017). Cardiac function likely mediates parental care behavior and fitness given its relationship with oxygen delivery and metabolism (Mirkovic and

Rombough, 1998) and circulation of signaling molecules (i.e. hormones), which are known to affect parental care (e.g. Dey et al., 2010). In fish, oxygen delivery to tissues, required to synthesize ATP (energy), is limited by the performance of the cardiovascular system (reviewed in Farrell et al., 2009; Eliason et al., 2013). Oxygen consumption increases with exercise (Ji, 1999; Farrell, 2009; Eliason et al., 2013), which is regulated through adjustments in cardiac output and arteriovenous oxygen difference (Farrell et al., 2009; discussed in Eliason and Antilla, 2017). Cardiac output is a function of heart rate (f_H) and stroke volume. Yet in some species, including centrarchids, stroke volume has been found to remain relatively constant in various situations. For instance, when exhibiting temperature stress, smallmouth bass f_H and cardiac output increased while resting stroke volume remained unaffected (Schreer et al., 2001). These same trends were also reported in studies

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investigating the effects of seasonal changes (Cooke, 2004) and exhaustive exercise (Schreer et al., 2001; Cooke et al., 2003a) on largemouth bass (*Micropterus salmoides*; Lacépède, 1802; Centrarchidae) cardiovascular performance, leading to the conclusion that largemouth and smallmouth bass (*Micropterus dolomieu*; Lacépède, 1802; Centrarchidae) are predominantly frequency modulators. Hence, f_H provides an effective real-time continuous proxy for metabolic performance in centrarchids and may play a critical role in mediating energy demanding behaviors (Schreer et al., 2001).

Parental care is reasonably common among teleost fishes (Blumer, 1979). It is therefore not surprising that there have been numerous laboratory and field studies focused on understanding the factors that influence variation in individual fitness using teleost fish as models. Smallmouth bass in particular have been the focus of much fieldwork on the physiology, energetics, behavior, and individual reproductive success (reviewed in Cooke and Philipp, 2009). During the four to six-week reproductive stage, male smallmouth bass create a nest, court with females, tend to the nest (e.g. fan eggs, remove debris), and protect their brood from predators and conspecifics (Ridgway, 1988; Cooke et al., 2002a). Leading up to the reproduction period, energy is allocated towards gonad development and nest creation (Bevelhimer and Breck, 2009). After reproduction, energy allocation shifts to supplement heightened activity associated with parental care behaviors (e.g. fanning; Hinch and Collins, 1991; Cooke et al., 2002a), that requires enhanced oxygen delivery throughout the body (Cooke, 2004; Cooke et al., 2004a). Parental care also requires constant vigilance, where if a bass leaves the nest to feed, the eggs would likely be predated within seconds or minutes, depending on the predation pressure in the system (Steinhart et al., 2004; Gravel and Cooke, 2009). Therefore, during parental care, the male parent has limited feeding opportunities (Hinch and Collins, 1991; Steinhart et al., 2004). Consequently, endogenous energy reserves decline during the parental care period (Mackereth et al., 1998; Gillooly and Baylis, 1999) and parental males adjust their behavior (e.g., locomotor activity dedicated to parental care; Ridgway, 1988; Wiegmann and Baylis, 1995; Cooke et al., 2002a) as part of a trade-off between current and future reproduction (Stearns, 1989). If the energetic demand of parental care becomes excessive (exceeds a tolerance threshold), the parent may abandon the offspring, resulting in zero biological fitness for that reproduction period (Chellappa and Huntingford, 1989). It is therefore important for a parental male smallmouth bass to have a sufficient metabolic capacity to supply the body with the oxygen required to meet these energy demands during the prolonged parental care period, which often includes the need for short bouts of high intensity exercise (Brownscombe et al., 2017).

Researchers have rationalized strong relations between parental care investment (such as parental behavior) and intraspecific physiological performance in wild animals (e.g. Ricklefs and Wikelski, 2002; Westneat et al., 2011; Brownscombe et al., 2017), however the number of studies focusing on assessing these relationships is comparatively scarce. Instead, physiological diversity (i.e. variation in physiological performance among individuals) is often ignored (given inherent research challenges; Costa and Sinervo, 2004), treated as noise, or controlled against in scientific literature (Bennett, 1987; Spicer and Gaston, 2009). Neglecting to experimentally validate the hypothesized relationships between physiological performance and parental care behavior has created gaps in the current understanding of the inter-relatedness between animal physiology, reproductive behavior, fitness and, therefore, animal life history (Brownscombe et al., 2017).

The relationships between cardiovascular performance and metabolism, and physiological capacity for exercise and parental care behavior has motivated the hypothesis that fitness and fitness-related behaviors are strongly influenced by cardiovascular performance (Franklin and Davie, 1992; Cooke, 2004; Farrell et al., 2008; Cooke et al., 2010). Additionally, previous research has shown that smallmouth bass (and other centrarchid species) undergo behavioral and physiological adaptations, including modulating f_H to enhance

reproductive performance (Cooke et al., 2010). However, to our knowledge, no study has formally tested the hypothesis in free-swimming fish. Using free-swimming nesting smallmouth bass as a model species, this study explored the relationship between routine f_H and parental care behavior. More specifically, we evaluated whether the proportion of the scope for f_H (calculated as peak f_H - resting f_H) that an individual routinely utilizes is related to the individual's relative energy expenditure rate and therefore, whether it is related to parental care behaviors (i.e., time spent nest tending, nest defending, nest guarding, and aggression score) and reproductive fitness (i.e., brood success, determined by whether the parent abandoned the nest prematurely).

2. Methods

Fish were collected under the Ontario Ministry of Natural Resources licence #1079390, and the study was designed in accordance with the Canadian Council of Animal Care protocol, under the permit number BT-026 administered through the Carleton University Animal Care Committee.

2.1. Day 1

2.1.1. Nest identification

From May 23 to 28, 2016, snorkelling surveys identified 24 nesting male smallmouth bass (mean L_T 422 mm \pm 78 mm; mean \pm SD) on nests in Big Rideau Lake (Ottawa, Ontario, Canada, 44.7706° N, 76.2152° W). Brood size was estimated by scoring the nests on a scale from 1 to 5, where 1 was a nest with few eggs and 5 was a nest with thousands of eggs (Philipp et al., 1997; Kubacki et al., 2002). All snorkelers were trained at the beginning of the season until there was consistency in brood size scoring among snorkelers and within individual snorkelers. The eggs are quite visible such that this simple method has been used reliably in a number of studies (e.g., Siepker et al., 2006; Algera et al., 2017a). Zuckerman et al. (2014) reported that fish with a brood score < 3 and egg development > 4 days (out of approximately 6 days of egg stage and 22 days of parental care; Cooke et al., 2002a) were more likely to abandon. Therefore, to reduce the risk of smallmouth bass abandoning their nest with the f_H logger, only fish with a brood score of 3 or more, and an egg stage of 4 days or less were used in this study. Nests that met the criteria were labelled using weighted numbered tags.

2.1.2. Surgery and instrumentation

Smallmouth bass were collected off their nest by angling coupled with assistance from the snorkeler providing hand signals to ensure the correct bass was caught. Fish were landed within 20 s after hookset to reduce anaerobic exercise and stress associated with angling (Cooke et al., 2003a; Lawrence et al., 2018), and brought into the boat using a rubberized net to avoid injury. Once un-hooked, bass were placed onto a surgery table with water being continuously pumped over the gills, and were electro-sedated using fish handling gloves (Smith-Root, Inc., Washington, USA, <http://www.smith-root.com>; 10 mA). An approximately 5 cm longitudinal incision was made half-way between the pectoral and pelvic fins, posterior to the pericardial membrane. A f_H logger (DST milli HRT, 8 g, 13 mm \times 39.5 mm, Star-Oddi, Iceland; <http://www.star-oddi.com/>) was inserted immediately posterior of the pericardial membrane and sutured to the ventral musculature (PDS II polydioxanone suture; violet monofilament, 3-0). f_H loggers were programmed to monitor ECG at 100 Hz, and to record f_H every 2 min and ECG every 1.5 h to validate f_H readings. The incision was closed using four to five square knot sutures, and the total length of the fish was measured, after which the fish was recovered and then released close to the nest. Throughout the entire process, a snorkeler was protecting the nest from predators and conspecifics until the adult bass returned to the nest and resumed its nesting behaviors. The loggers began collecting data at midnight on the same day of insertion (on average 9 h post-

surgery).

2.2. Day 2–3

2.2.1. Behavior footage and predator exposure

Video footage of routine behaviors was recorded for 1 h on day 2 and 50 min on day 3. Behavioral footage was collected using a GoPro (HERO3+) supported by a 30 cm tall stand, placed 1 m from the edge of each nest (Struthers et al., 2015). Additionally, on day 3, after recording 50 min of routine behavior, a pumpkinseed (*Lepomis gibbosus*; Linnaeus, 1758; $L_T = 130\text{--}150$ mm) in a 2 L clear, glass jar with open water circulation was placed on the edge of the nest, at the opposite side from the GoPro, for the subsequent 10 min. The latter provided behavioral footage in the presence of a nest predator and was used to assess the parental male aggression score as a metric of character (i.e., bold vs. shy) (Hanson et al., 2009). At the end of the hour, both the GoPro and the predator were removed.

2.3. Day 4

2.3.1. Estimating maximum f_H and logger removal

Again, with the assistance of a snorkeler, the instrumented small-mouth bass were re-angled off their nest. If the bass could not be recaptured from the boat, the snorkeler angled the bass given their better perspective of the fish. As soon as the bass was caught, the snorkeler placed a cage over the nest to prevent the eggs from being preyed on. Approximately 2 mL of blood was sampled from the caudal vasculature for hematocrit analysis. This was done within 3 min of initial hooking and capture (Lawrence et al., 2018) using a lithium heparinized vacutainer and a 21 gauge needle (B.D. Vacutainer, NJ, USA). This was immediately followed by a 3 min forced exercise period, which involved placing the bass in a 115 L (90 cm diameter \times 30 cm height) basin filled with fresh lake water, where a researcher continuously attempted to grab the caudal peduncle to cause the fish to burst swim. The bass was then placed into a closed holding container (66 cm \times 34 cm \times 31 cm) for 1 h. Water changes were made continuously over this time frame to avoid exposing the fish to hypoxia during recovery. The purpose of the chase and 1 h wait was to obtain an estimate of the fish's maximum f_H (Cooke et al., 2002b).

One hour after the fish was placed into the holding container, the f_H logger was removed using the same surgical set-up and electro-anesthesia as implantation. The incision was re-opened to retrieve the logger, and sealed in the same manner. The fish were released close to the nest, at which point the cage covering the eggs was removed and the snorkeler protected the nest until the bass returned and resumed their nesting behaviors.

2.4. Day 5 – onward

2.4.1. Monitoring egg development

Throughout the study and until June 6th, 2016, a snorkeler monitored the nest every other day for brood success. A nest was deemed successful if the offspring matured to free-swimming fry.

2.5. Data processing

2.5.1. Fish size and condition

During initial capture, fish length was measured to the nearest mm to represent fish size. To minimize stress, fish mass was not obtained to minimize stress on the fish (i.e., air exposure and drying). As a proxy for fish condition, hematocrit was measured from blood sampled after recapture (Gallaughan and Farrell, 1998). Blood was collected in heparinized capillary tubes (75 mm Drummond Hemato-Clad, ammonium heparin, Toronto, ON, Canada) and centrifuged for 5 min at 8000g (Clay Adams™, NJ, USA). Packed red blood cells were measured to the nearest mm via a ruler.

2.5.2. Behavior data

Using JWatcher (Blumstein et al. 2000; <http://www.jwatcher.ucla.edu/>), video footage from day 2 and day 3 were analysed separately. For both days of nesting behavior footage, only footage from minute 20 to minute 40 in the hour-long behavioral footage was analysed. This allowed the nesting bass to acclimatize to the GoPro, and ensured that the boat and the snorkeler were not present when behavior was being recorded. The time and duration of nesting behaviors were recorded and organized into one of three behavioral categories: 1. Nest tending, which involves actively maintaining the nest. Examples of nest tending behaviors included removing debris from the nest or fanning the eggs. 2. Nest guarding, which involves staying on or near the nest but not conducting any nest tending behaviors. This included patrolling the nest by slowly swimming around the nest, hovering over the nest or within 1 m from the nest. 3. Nest defending, which consists of deterring predators and conspecifics from the nest. This included behaviors such as hovering with dorsal fin erect or burst swimming to chase predators and conspecifics. Additionally, the number of nest predators and conspecifics that appeared in the footage were recorded.

Individual aggression score was assessed by adding the time that the bass' mouth was on the jar (rounded to the nearest second), the number of strikes on the jar (mouth bumps the jar), and the number of yawns (Hanson et al., 2009; Zolderdo et al., 2016). Additionally, the time until the first attack, measured from when the jar was dropped to when the first aggression behavior occurred.

2.5.3. f_H data

A total of 22 nesting smallmouth bass were tagged, where 3 fish abandoned with the logger and 2 fish had failed f_H records. Therefore, 19 fish were used for behavioral analysis and only 17 fish were included in the f_H analyses. f_H data were validated using the Pattern Finder software (v. 1.11.0, Star-Oddi, Iceland) to ensure the logger algorithm was correctly calculating the f_H from the ECG traces. Since we could not validate all f_H records with ECG traces and no studies have recorded heart rate above 130 bpm in centrarchids in the literature, data exceeding 130 bpm were deemed erroneous and were removed from the dataset.

Temperature (measured by the f_H loggers) changed by ~ 7.5 °C during the eight-day study period. A simple linear regression model was employed to investigate relationships between temperature and f_H , where the slope of the relationship provided an estimate for Q_{10} . Since temperature is related to f_H (Reynolds, 1977) and behavior (Cooke et al., 2003c), the effect of temperature on f_H and fish behavior was detrended by determining the residuals from the linear regression between f_H and temperature, and the linear regression between behavior and temperature (Jakob et al., 1996) and using these residuals in further analyses.

f_H data were analysed by determining the routine, resting, max, min, and scope for f_H (max f_H – resting f_H). Occasionally, the 3 min chase did not yield a peak in f_H , likely due to poor swimming performance during the chase. Therefore, max f_H was determined as the highest f_H record throughout the f_H trace. Routine f_H was calculated as the average of the f_H trace excluding when the fish was captured and chased. Resting f_H was calculated as the lowest 10th percentile f_H values, also excluding when the fish was captured and chased. For all individuals, f_H measurements included 2 days and two nights.

2.6. Statistical analyses

First, we needed to determine whether parental care behavior differed between day 2 and day 3. This was assessed using a one-sample t -test, which confirmed there was no difference in time spent on each behavior between day 2 and day 3 (difference between times on each behavior did not significantly differ from 0; p -value = .99 in all cases). This was also confirmed with a Spearman's rank order correlation analysis where the time an individual spent on each behavior had a

Table 1

Final linear mixed effect and beta regression models used to test the relationship between f_H and parental care behaviors.

Model type	Response variable	Covariates
Linear mixed effect	Time	Behavior Egg score Time
	f_H	Round (i.e., day 2 vs day 3) Cycle (i.e., day vs night)
	$R\%f_H$	Nest defending Egg score Time to first attack Time swimming around jar Aggression score
Beta regression	$R\%f_H$	

positive correlation between day 2 and day 3 (guarding: $\rho = 0.70$, p -value $< .001$; tending: $\rho = 0.70$, p -value = .0017, and defending: $\rho = 0.41$, p -value = .054). Therefore, further analyses used the average time spent on each behavior, predator count during the behavioral footage, and aggression score between day 2 and day 3. In all models, fish length and hematocrit were included to account for fish size and fish condition, respectfully.

A linear mixed effect model was fit to compare the time spent on each parental care behavior (time as the response variable and behavior interacting with egg score as the predictor variables), where individual was treated as a random effect to account for dependencies (Table 1). The model was simplified using the drop1 command in R, and significant predictors were assessed using p -values based on the F -distribution. For significant variables, a Bonferroni adjusted Tukey post-hoc analysis was used for pairwise comparisons. Next, routine f_H was used to determine whether there is a relationship between routine f_H and overall time spent on each behaviour (model outlined in Table 1). However, to account for differences in heart rate capacities (i.e., varying resting and maximum f_H) fish heart rate was standardized according to where their routine f_H fell within their scope for f_H in the form of percentage. We labelled this as the percentage of routine f_H within scope for f_H ($R\%f_H$), which was calculated as ((routine-resting) \div scope for f_H) \times 100, and allowed comparison between fish according to the proportion of the scope for f_H that was routinely being used. Given the response variable, $R\%f_H$, is a proportion and continuous, a beta regression (Kieschnick and McCullough, 2003; betareg package, Cribari-Neto and Zeileis, 2010) model with temperature detrended $R\%f_H$ as the response variable and temperature detrended nesting behaviors, aggression, fish length, egg score, and hematocrit as covariates, was used to relate f_H to parental care behavior. To avoid overfitting the model, random forest analysis (randomForest package, Liaw and Wiener, 2002) was used to identify the five covariates with the greatest influence on f_H variability that were to be included in the model according to %IncMSE (i.e., the ranking of covariates according to the percent increase in means square error of the explanatory variable related to the dependent variable), and stepwise model selection was conducted using AICc to account for the small sample size (Table 2).

It was observed that f_H varied slightly between the two days, and the direction and magnitude of change varied differently among each fish. Therefore, to determine whether change in f_H over time related to parental care behavior, a linear mixed effect model was used to relate f_H (using the temperature detrended data recorded every 2 min) to nesting behaviors, aggression, fish length, egg score, and hematocrit. Again, random forest analysis was used to select the top five covariates to avoid overfitting the model. Three linear mixed models were fit, all with individual fish as the random effect, where model 1 was set to have random intercepts, model 2 was set to have random slopes and intercepts, and model 3 was set to have random slopes. All models were fit using the nlme package in R (Pinheiro et al., 2014). A one-way ANOVA was used to select the best model (model 3, random slopes)

Table 2

Output for beta regression model relating percent routine f_H within scope for f_H ($R\%f_H$) to the five most influential covariates according to the random forest analysis (data were temperature detrended). Nest defending = proportion of time parental male spent deterring predators and conspecifics; Egg score = estimated nest size; Time to first attack = time until first aggression behavior during the predator simulation measured from when the jar was dropped; Aggression score = number of times the bass made contact with the jar or yawns during the predator simulation; Time swimming around jar = time that the parental male bass spent facing the jar without attacking during the predator simulation. Statistical analysis was conducted on 17 individuals and evaluated at an $\alpha = 0.05$.

Covariate	Estimate	Std. error	z value	P(> z)
Intercept	-1.0	0.30	-3.51	0.0004
Nest defending	0.006.7	0.013	0.51	0.60
Egg score	0.020	0.13	1.6	0.11
Time to first attack	-1.7e-5	1.6e-5	-1.1	0.29
Time swimming around jar	-1.8e-6	2.9e-6	-0.63	0.52
Aggression score	0.00052	0.0020	0.26	0.80

Table 3

Output for linear mixed effect model simplification using log ratio tests to compare the model against reduced models via the 'drop1' command in RStudio. The model was testing the relationship between f_H (using the temperature detrended data recorded every 2 min) and nesting behaviors and aggression level. The hyphen represents the model with all variables retained. Since all AIC values in this case were similar (differed by 4), the significance of each variable was tested to select covariates to drop to avoid overfitting our model. Time swimming around jar = time that the parental male bass spent facing the jar without attacking during the predator simulation; Aggression score = number of times the bass made contact with the jar or yawned during the predator simulation; Time = duration that heart rate (f_H) was monitored; Nest guarding = proportion of time parental male spent on or near the nest but not conducting any nest tending behaviors; Nest tending = proportion of time parental male spent on nest tending behaviors. Colon represents an interaction between covariates. Statistical analysis was conducted on 17 individuals and evaluated at an $\alpha = 0.05$. Significance is identified in bold.

Covariate dropped	df	Likelihood ratio test	AIC	Pr(> Chi)
-	-	-	161,809	-
Time	1	4.8	161,812	0.028
Aggression score	1	1.5	161,808	0.22
Time swimming around jar	1	1.9	161,809	0.17
Nest tending:Nest guarding	1	0.56	161,808	0.45

according to the lowest BIC value (model 1 = 162,146.2; model 2 = 161,958.6; model 3 = 161,958.4). Stepwise model reduction using the drop1 command suggested the model could be further reduced to only include time as a covariate (Table 3).

Finally, Spearman's rank correlation was used to determine if $R\%f_H$ differed between the 2 days or the two nights during the nesting period. Diel $R\%f_H$ values were calculated for each day and each night using the routine f_H for each day and each night and determining where they fell within the overall scope for f_H . Diel patterns in $R\%f_H$ were assessed using a linear mixed effect model with individual as a repeated measure. Since only 2 fish abandoned their nest and did not have offspring attain free-swimming fry, we could not statistically relate f_H to successful brood development. Instead, the relationship between $R\%f_H$ and parental care performance and brood success was visually compared for potential trends. All statistical analyses were conducted using RStudio (R Core Team, 2017).

3. Results

3.1. Characterizing f_H

Nesting smallmouth bass ($L_T = 441 \text{ mm} \pm 44 \text{ mm}$; mean \pm SD,

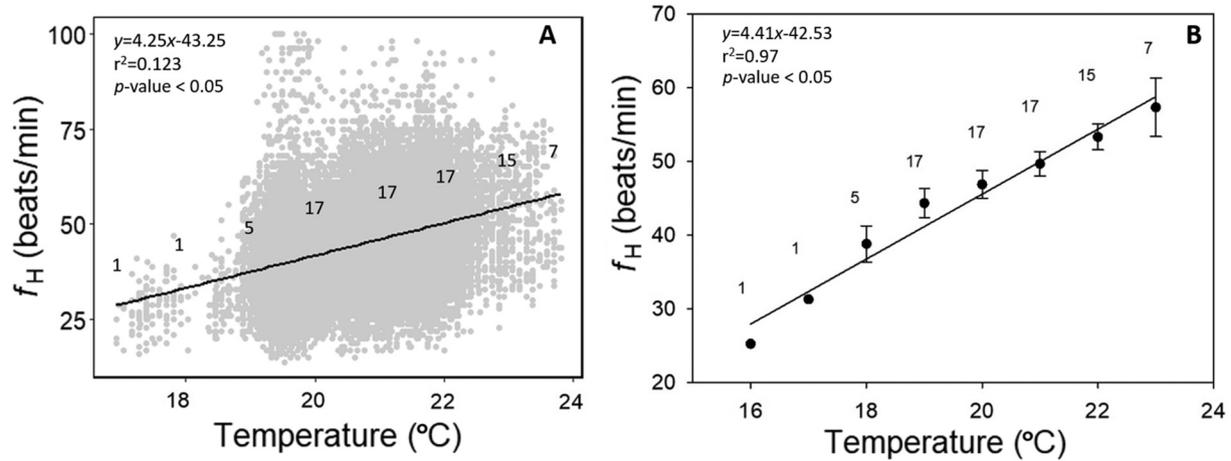


Fig. 1. Regression plot showing (A) raw and (B) mean f_H of 17 nesting male smallmouth bass in response to water temperature increase in 1 °C increments. The regression line shows that f_H increases according to the equation $f_H = 4.25$ (temperature °C) - 43.25, with a coefficient of determination (r^2) of 0.123, p -value < .05, but on average f_H increases according to the equation $f_H = 4.41$ (temperature °C) - 42.53, with an r^2 of 0.97, p -value < .001. Error bars represent the standard error for each mean. Numbers over each point describe the number of individuals exposed to each temperature.

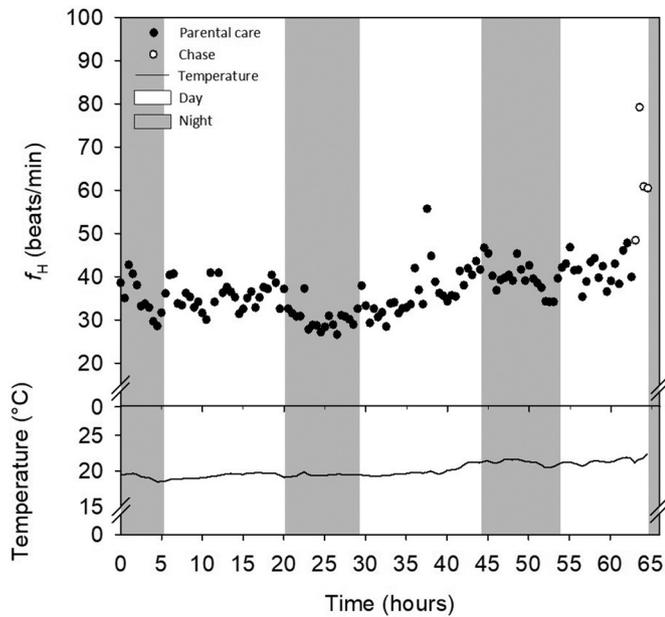


Fig. 2. Scatterplot of an individual fish f_H (beats min^{-1}) averaged for every half hour over a 64 h period. Black dots represent f_H during parental care. White dots represent f_H during the recapture and chase. Solid line shows the water temperature. White bands represent daytime (5:30) and grey bands represent night (20:30).

$n = 17$) had an average routine f_H of 47.4 beats $\text{min}^{-1} \pm 1.6$ beats min^{-1} , with an average resting f_H of 24.8 beats $\text{min}^{-1} \pm 1.7$ beats min^{-1} , and a minimum f_H and maximum f_H of 17.9 beats $\text{min}^{-1} \pm 0.9$ beats min^{-1} and 124.6 beats $\text{min}^{-1} \pm 2.7$ beats min^{-1} , respectively (water temperature ranged from 16 °C–23 °C (Fig. 1; Appendix A)). An example of a smallmouth bass f_H trace during 64 h of parental care is presented in Fig. 2. In all cases, hematocrit levels were above the poor condition threshold of 20% (mean = 31.0% \pm 2.2%; Appendix A; Fränge, 1992; Gallagher and Farrell, 1998).

There was a significant positive relationship between temperature and f_H ($p < .001$), where f_H increased on average by 4.41 beats min^{-1} (8.22% \pm 2.0%) with every degree Celsius increase between 16 °C to 23 °C (Figs. 1 and 2). When corrected against temperature effects, the average scope for f_H changed from 99.9 beats $\text{min}^{-1} \pm 2.2$ beats min^{-1} to 101.1 beats $\text{min}^{-1} \pm 2.8$ beats min^{-1} . Hence, on average,

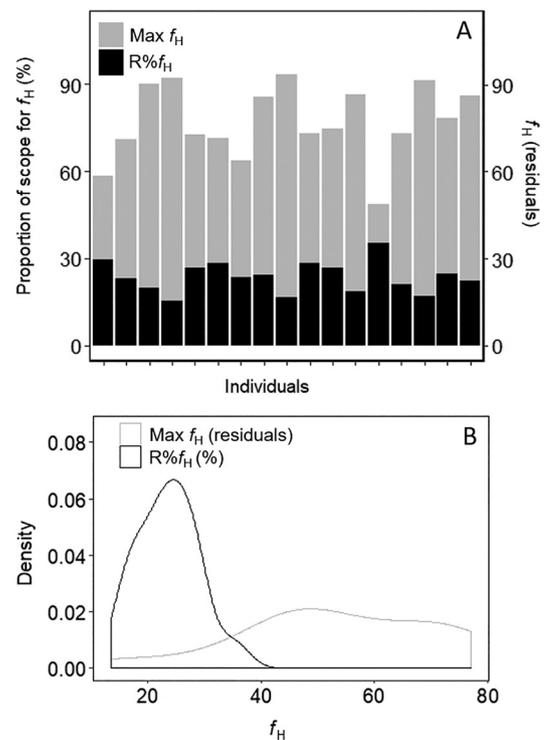


Fig. 3. Double bar plot (A) and frequency distribution plot (B) showing intraspecific variability and range in f_H in nesting male smallmouth bass. Double bar plot (A) shows the recorded individual variability in f_H performance of nesting male smallmouth bass. The recorded routine f_H within scope for f_H (R% f_H) (%) of individual nesting smallmouth bass (the left axis) is presented in grey. The recorded maximum f_H (in residuals to correct for the influence of temperature of f_H) of each individual (right axis) is presented in black. Density distribution plot (B) shows the frequency of individual variation in percent R% f_H (%) (black) and the maximum f_H (residuals) (grey) in nesting smallmouth bass after temperature correction ($n = 17$).

the routine f_H of nesting male smallmouth bass operated at 18.13% \pm 1.06% of the scope for f_H without temperature correction, and at 23.6% \pm 1.2% of the scope for f_H after temperature correction, with a 20% range for both cases (Fig. 3).

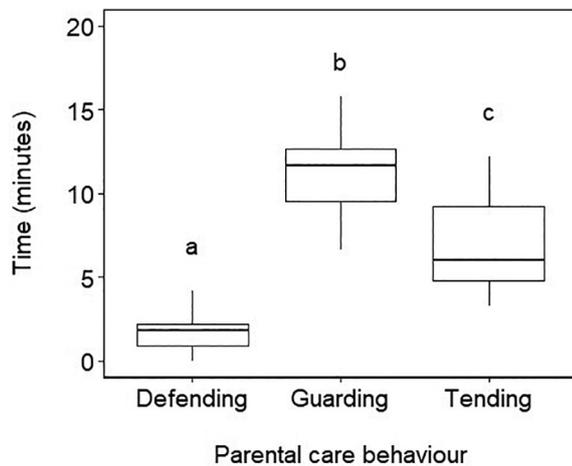


Fig. 4. Percentage of time male smallmouth bass spent on each nesting behavior over the 40 min of monitored behavior during nesting period ($n = 19$). Different letters show significant differences between groups ($p < .001$ for all cases).

3.2. Relationship between f_H and nesting behavior

During the nesting period, male smallmouth bass spent a significantly greater proportion of their time guarding the nest ($56.7\% \pm 3.0$), followed by tending to the nest ($34.6\% \pm 3.2\%$), which was six fold and four fold greater than the time spent defending the nest from predators and conspecifics ($8.7\% \pm 1.2\%$), respectively ($p < .001$ for all cases; Fig. 4). Regardless of the observable individual variation in f_H and parental care behavior (Figs. 3–5), random forest analysis yielded no predictability power between f_H (specifically $R\%f_H$) and parental care behaviors, fish condition, and nest predation pressure (percent variance explained = -41.98%). A beta regression model containing the five most influential covariates (according to the random forest analysis: nest defending, egg score, time to first attack, time spent swimming around the jar, and aggression score) yielded no significant relationship between parental care behaviors and $R\%f_H$ (all p -values $> .05$; Table 2). A model including only aggression score as a covariate (according to the lowest AICc) yielded a significant relationship between $R\%f_H$ and aggression score (p -value = $.0075$; Fig. 6). Since only two f_H loggers could be retrieved from fish that abandoned the nest, the relationship between f_H and the likelihood of nest abandonment could not be statistically compared. However, the two fish that abandoned their brood prematurely were among the fish with a higher routine f_H , resting f_H (top fiftieth percentile), minimum f_H (all within the top fiftieth percentile), and the lower maximum f_H (lowest quarter percentile), suggesting a potential relationship between f_H and brood success (Fig. 5C). Furthermore, smaller nest scores appeared to coincide with fish that had a higher resting f_H and a lower $R\%f_H$ (Fig. 5E). However, when tested, no significant relationship was detected for either case (all p -value $> .05$). Similarly, there was no detectable relationship between change in f_H over time and nesting behaviors (p -values $> .05$; Table 3). The only significant relationship (p -value = $.028$) suggested that f_H may decrease as the nesting period progresses (Fig. 7).

Although f_H was not repeatable among days or nights (p -value $> .05$), nesting smallmouth bass f_H showed diel variation. More specifically, $R\%f_H$ was 10% higher during the day than at night (p -value = $.003$; Fig. 8).

4. Discussion

The present study explored whether cardiac performance mediates fitness and fitness related behaviors. In doing so, it is also the first study

to monitor cardiac function in free-swimming fish during parental care. Despite the intraspecific variation in f_H recorded in this study, and the apparent relationship between metabolic performance and fitness presented in recent literature (Ricklefs and Wikelski, 2002; Westneat et al., 2011; reviewed in Brownscombe et al., 2017), the present study did not detect a relationship between an individual's routine f_H and parental care behavior in smallmouth bass. A significant positive relationship between $R\%f_H$ and parental male aggression level was detected (Fig. 6), however this did not affect parental care behaviors, specifically nest defending, nest tending and nest guarding behavior. Although f_H is related to an individual's aggression level, the lack of relationship between f_H and parental care behaviors suggests that f_H alone does not appear to be directly related to parental care behavior in smallmouth bass.

The recorded f_H mostly conformed with the rates reported in previous literature (Schreer et al., 2001 ($16^\circ\text{C} = 34.5 \text{ beats min}^{-1} \pm 2.7 \text{ beats min}^{-1}$ and $20^\circ\text{C} = 52.8 \text{ beats min}^{-1} \pm 2.5 \text{ beats min}^{-1}$ for routine f_H and scope for f_H ranging between 1.8 and 2.6 fold); Cooke, 2004 ($21^\circ\text{C} = \sim 49 \text{ beats min}^{-1}$ for routine); Cooke et al., 2010 ($21^\circ\text{C} = \sim 50 \text{ beats min}^{-1}$ for routine); Cooke et al., 2004b (in largemouth bass (*Micropterus salmoides*) $17^\circ\text{C} = \sim 48 \text{ beats min}^{-1}$, $21^\circ\text{C} = \sim 50 \text{ beats min}^{-1}$, and $25^\circ\text{C} = \sim 60 \text{ beats min}^{-1}$ for routine f_H ; $17^\circ\text{C} = \sim 80 \text{ beats min}^{-1}$, $21^\circ\text{C} = \sim 90 \text{ beats min}^{-1}$, and $25^\circ\text{C} = \sim 120 \text{ beats min}^{-1}$ for max f_H), measured within the same temperature range ($16\text{--}23^\circ\text{C}$; average and standard deviation of $20.63^\circ\text{C} \pm 1.00^\circ\text{C}$). Differences in f_H records compared to the previously cited literature values are likely due to differences between the f_H loggers used in the present study compared to other devices, where ECG was not recorded to validate all f_H readings. For each f_H record, the loggers provide a quality index ranging from 0 to 3 scoring the ability of the algorithm to estimate f_H values, where 0 = good f_H estimates, 3 = bad quality f_H estimates. However, these have yet to be validated in smallmouth bass and the ability of the loggers to record higher f_H frequencies while set to collect data at 100 Hz is unclear. Therefore, analysis was conducted using all recorded f_H data within range ($< 130 \text{ beats min}^{-1}$) and repeated using cleaned traces (i.e., removed all values with a quality index of 2 or 3; maximum $f_H = 95.4 \text{ beats min}^{-1} \pm 2.1 \text{ beats min}^{-1}$; data not provided). Both methods yielded the same results.

Although f_H was only monitored for up to 2.5 days during the egg developmental stage, f_H fluctuated throughout the period (Fig. 2). Previous research has shown that environmental factors, activity, and hormones can affect fish f_H (discussed in Hoar et al., 1992 and Farrell, 1993; Vornanen, 2017). For instance, cardiac output increases with temperature, where, in teleost fish, the pacemaker rate increases with acute temperature changes such that the Q_{10} for f_H is ~ 2.0 (Farrell, 1992). In the case of nesting smallmouth bass, we showed that f_H increased by $\sim 8\%$ with every 1°C increase (Fig. 1). Temperature has also been shown to strongly influence the timing of smallmouth bass reproduction (Graham and Orth, 1986), however during parental care, lack of patterns between temperature variation and nesting behavior reported in a previous study suggests smallmouth bass adjust their energy expenditures according to their environment (Cooke et al., 2003a). This adjustment in behavior is likely partially responsible for the observed increase in f_H with temperature as well.

Irrespective of temperature, physiological variation persisted among individuals (Fig. 5), including interindividual $R\%f_H$ (Fig. 3). Although it has been demonstrated that morphological variation, specifically male size (Wiegmann and Baylis, 1995; Algera et al., 2017a), is positively related to brood size and fitness, there was no relationship between brood size, male size, and f_H in the current study. Nest abandonment rates appeared to be higher in nesting males with higher resting f_H , routine f_H , minimum f_H and maximum f_H (Fig. 5), however these apparent trends could not be statistically tested and may be artifacts of varying physiological states among individuals or small sample size; further investigations are required to confirm these trends.

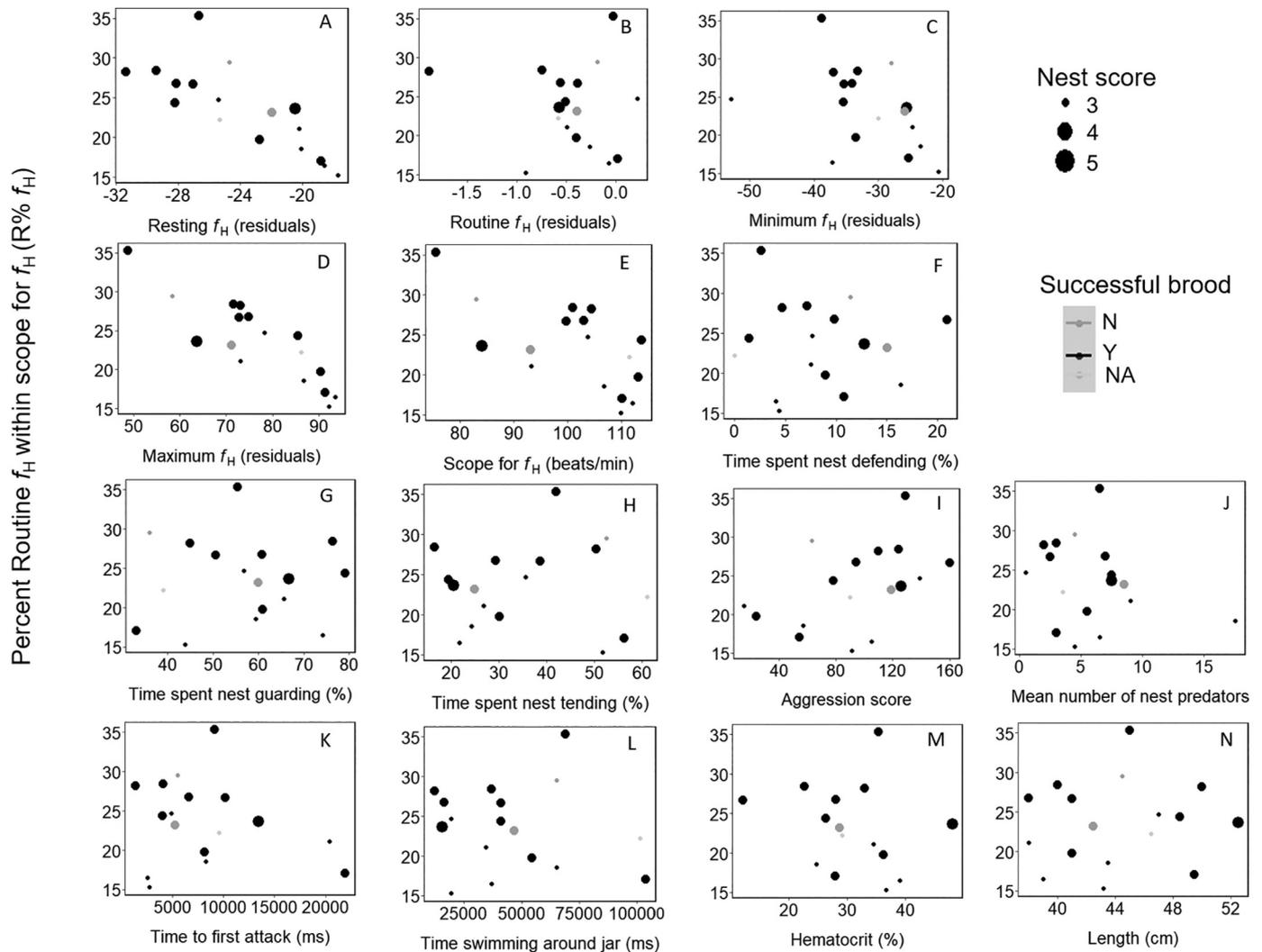


Fig. 5. Scatterplots comparing individual percent routine f_H within scope for f_H ($R\%f_H$), brood success, and nest score to individual (A) resting f_H ; (B) routine f_H ; (C) minimum f_H ; (D) maximum f_H ; (E) scope for f_H ; (F) time spent on nest defending behaviors; (G) time spent on nest guarding behaviors; (H) time spent on nest tending behaviors; (I) aggression score; (J) nest predation pressure; (K) time to first attack; (L) time spent swimming around the jar; (M) hematocrit; and (N) Length. $n = 17$ for all panels except M, where $n = 15$. Dot size represents the nest score.

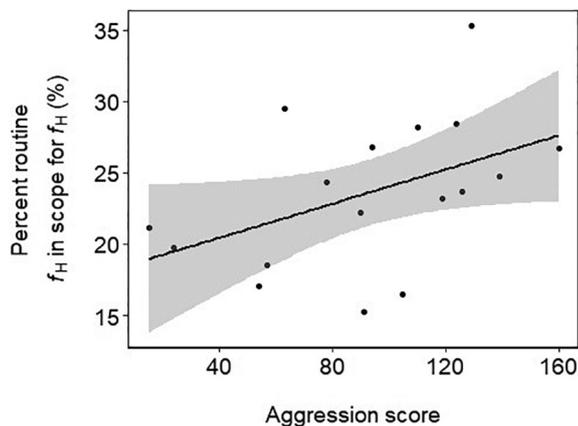


Fig. 6. Regression plot showing the relationship between percent routine f_H within scope for f_H ($R\%f_H$) and parental male smallmouth bass aggression score (p -value = .0075). Temperature was corrected against. Points represent individual fish responses, $n = 17$, and the grey shaded region represents the 95% confidence region.

Additionally, previous literature reported a 60% higher metabolic rate (Hinch and Collins, 1991) and enhanced cardiovascular performance (Cooke et al., 2010) in nesting smallmouth bass compared to non-nesting individuals, while Gillooly and Baylis (1999) demonstrated that energy expenditure during parental care is positively related to reproductive success. These trends are likely due to the enhanced energetic demands of parental care behaviors and heightened activity (Cooke et al., 2002a; Cooke et al., 2004a; Bevelhimer and Breck, 2009). However, the fish in the present study on average used only 24% of their scope for f_H . This could suggest that the parental care activity is low enough that it is not physiologically demanding on the cardiovascular system, and relationships between physiological performance and reproductive behavior may become more apparent in fish with high spawning activity, such as salmon (*Oncorhynchus* sp. and *Salmo* sp.) that experience energetically-demanding competition during spawning (Healey et al., 2003; Esteve, 2005). This is also likely because the present study was restricted to successful parental males that managed to court a female and already had fertilized eggs in their nest. Including males with lower nest scores in future studies may allow for a larger intraspecific variation in $R\%f_H$ to be detected.

Although the positive relationship between $R\%f_H$ and the aggression score in the present study was slight (given that $R\%f_H$ differed by

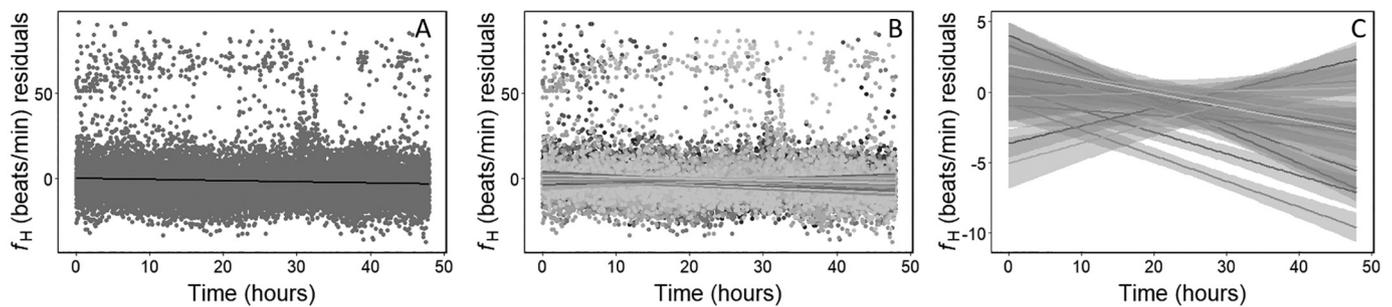


Fig. 7. Linear mixed effect model showing parental male smallmouth bass f_H overtime where (A) individual fish are pooled, (B) raw data and slopes for each individual parental male bass, and (C) trends of each individual parental male bass. Individuals are represented by different shades. f_H was recorded every 2 min over a 48 h period, at 100 Hz.

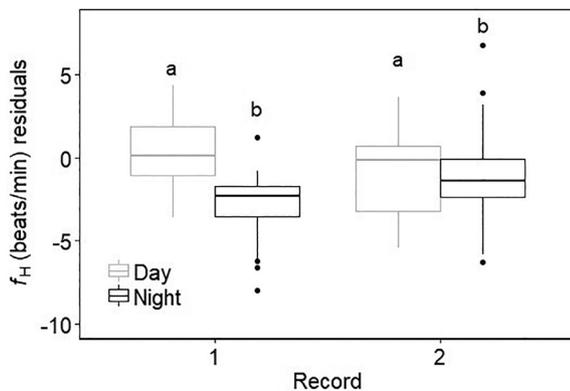


Fig. 8. Boxplot showing the diurnal average, upper quartile, lower quartile, and standard error of nesting smallmouth bass percent routine f_H within scope for f_H ($R\%f_H$) over a 48 h period (two days and two nights). Grey represents $R\%f_H$ during the day, black represent $R\%f_H$ during night. Different letters show significant differences between groups ($p = .0032$).

approximately 8% among individuals with low versus high aggression score), this relationship supports previous findings in literature (e.g. Ballew et al., 2017 who suggested individual boldness is related to fitness). We also revealed that more aggressive individuals routinely use a greater proportion of their scope for f_H (Fig. 6). Future studies should investigate whether a stronger relationship exists between f_H and parental care behavior in smallmouth bass nesting in lakes with higher predation pressure. Irrespective of this trend, the smallmouth bass in the present study mostly exhibited nest tending and nest guarding behaviors, with the least amount of time spent on nest defending behaviors. This was likely because of the low predation pressure in Big Rideau Lake (Gravel and Cooke, 2009), allowing the fish to allocate more time on their nest and less time deterring predators and conspecifics. Since nest tending behaviors, such as fanning and removing debris are highly energetically demanding (Lachance and Fitzgerald, 1992; Cooke et al., 2002a), it is not unexpected that the time was split with nest guarding behaviors, which are less energetically demanding. However, given the lack of relationship between f_H and parental care behavior, the variation in f_H cannot be attributed to interindividual variation in parental care activity. Instead variation in nesting behavior could simply be a product of behavioral syndromes (Sih et al., 2004; Bergmüller, 2010; Conrad et al., 2011), other endocrine factors such as stress hormones (Dey et al., 2010; Zolderdo et al., 2016; Algera et al., 2017a; Brownscombe et al., 2017), or because this study only involved fish that managed to attract a female and reproduce, while excluding fish that failed to spawn. Further studies are required to investigate these relationships using individuals that succeeded in spawning and individuals that abandoned their nest. Lastly, given that cardiac output is a product of f_H and stroke volume (Farrell, 1993; Vornanen, 2017), we can not be certain that scope for f_H is an effective proxy for cardiac

capacity. Plasticity in fish f_H occurs as a result of prolonged environmental changes (discussed in Gamperl and Farrell, 2004), and even minor changes in stroke volume could have significant changes in cardiac output that could not be detected in the present study. Therefore, although changes in an individual's scope for f_H during this study is unlikely given the short duration of this study and that all fish were monitored at the same stage in the parental care period, we cannot rule out the possibility that aerobic scope and exercise influence nesting smallmouth bass behavior without verifying this relationship using all cardiovascular parameters in future studies.

This was the first study to monitor f_H in nest guarding fish in the wild. Individual f_H profiles suggests f_H may decrease as the parental period progresses and follow a consistent diurnal pattern overtime, with no consistency among individuals. Due to the trade-off between the longer f_H monitoring period and the increasing risk of smallmouth bass abandoning the nest with the f_H loggers and therefore the f_H data, f_H was only monitored for up to 2.5 days. Hence, it is likely that the observed inconsistency and decreasing trend in f_H could be a result of the fish still recovering from the surgery, small sample size, or short monitoring duration. Nevertheless, it is known that f_H is modulated by environmental factors and by the supply or demands of oxygen and metabolites (Stecyk et al., 2011; Eliason and Anttila, 2017; Vornanen, 2017). Given that the present study showed fluctuations in nesting smallmouth bass f_H that cannot be explained by temperature, nesting behavior, individual aggression level, size, nor condition (hematocrit), it is likely that variation in f_H is predominantly related to another factor or a combination of factors that were not included in this study, such as photoperiod, hormones, blood plasma constituents (Hoar et al., 1992; Farrell, 1993; Vornanen, 2017), predation risk on the parent male (Cooke et al., 2003b), noise pollution (Graham and Cooke, 2008), or nest location (Lachance and Fitzgerald, 1992). Further research should investigate whether f_H becomes repeatable and stabilizes after 1–2 days of recovery and a longer monitoring period.

The observed diel pattern in the nesting smallmouth bass f_H profiles (Fig. 8) confirms that f_H is less dependent on parental care behavior given that previous research has demonstrated there is no diel variation in nesting smallmouth bass parental care behavior (Hinch and Collins, 1991; Cooke et al., 2002a). This trend could be due to diel pattern in activity (routine and burst swimming), where activity is higher during the day than at night, however these differences in activity are small (Algera et al., 2017b). Since the effect of temperature on f_H was corrected against, the magnitude of the diel variation in f_H (10%) suggests that other extrinsic and intrinsic factors may also affect smallmouth bass metabolic performance. Previous research has shown diel cycles occurring in blood plasma constituents, such as cortisol, which increases with the onset of light and continues to increase with activity (Pickering and Pottinger, 1983; Meier, 1984; Cousineau et al., 2014). Although cortisol drives other endocrine and metabolic rhythms, there is a wide variation in the response of f_H and other cardiovascular parameters (Pickering and Pottinger, 1983). Furthermore, Algera et al.

(2017b) showed that experimentally elevated cortisol levels in nesting smallmouth bass decrease fitness by reducing locomotor activity, burst swimming, and routine swimming, while increasing resting behavior. Other hormones have also been reported to exhibit diel rhythms in teleost fish (e.g. prolactin, melatonin, insulin, gonadal steroids), as well as plasma electrolytes ($[Na^+]$, $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$), plasma metabolites (e.g. glucose – observed in sea bass during a 7 day fasting period; McDonald and Milligan, 1992), some of which act on f_H by modulating the relative levels of adrenergic and cholinergic tones causing f_H to deviate from the intrinsic pacemaker frequency (McDonald and Milligan, 1992; Farrell, 1993; Vornanen, 2017). The combination of diel cycling hormones, plasma electrolytes, and plasma metabolites are likely to drive the observed diel pattern in nesting smallmouth bass f_H . This further supports the observation that f_H is predominantly influenced by endogenous and environmental factors and less influenced by behavior during the parental care period.

The approach used in the present study allowed smallmouth bass f_H and parental care behavior to be monitored in the natural environment. However, the effects of the surgery cannot be ignored. To account for post-surgery recovery, the above analysis was repeated using only day 3 f_H data. This yielded the same results as when f_H data from both day 2 and 3 were used, confirming the reported results (data not shown). Additionally, it is worth noting that the diel pattern becomes less distinct as time progresses (Fig. 8), suggesting the trend may be due to environmental variability or an artifact of the surgery. A longer monitoring period and the development of less invasive methods for monitoring f_H in free-swimming fish are required to better investigate these trends.

5. Conclusion

Although smallmouth bass show individual physiological and behavioral variation during parental care (Cooke et al. 2003b; Gravel et al., 2010), there was no direct relationship detected between f_H and parental care behavior in our study. In fact, f_H appears to be unrelated to behavior during the nesting period, as shown by the diel variation in f_H while behavior remained unchanged. Despite the strong theoretical rationales relating physiological performance and fitness, this study is among many others failing to support this relationship (e.g. Wiegmann and Baylis, 1995; Hatfield and Schluter, 1999; Hanson et al., 2009; discussed in Spicer and Gaston, 2009, and Brownscombe et al., 2017). While evidence that activity is fundamental to parental care performance in smallmouth bass (Gillooly and Baylis, 1999; Cooke et al., 2002a) indeed implies a relationship between metabolic performance and fitness, the mechanisms remain elusive. Further research on a variety of vertebrate taxa is required to understand the complex relationship between intraspecific variability in physiological performance and fitness, which can ultimately provide new platforms for which animal energetics, behavior, life history, and population dynamics can be studied.

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Declaration of interest

None

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2019.04.012>.

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