



Cortisol treatment affects locomotor activity and swimming behaviour of male smallmouth bass engaged in paternal care: A field study using acceleration biologgers



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ABSTRACT

Paternal care, where the male provides sole care for the developing brood, is a common form of reproductive investment among teleost fish and ubiquitous in the *Centrarchidae* family. Throughout the parental care period, nesting males expend energy in a variety of swimming behaviours, including routine and burst swimming, vigilantly monitoring the nest area and protecting the brood from predators. Parental care is an energetically demanding period, which is presumably made even more difficult if fish are exposed to additional challenges such as those arising from human disturbance, resulting in activation of the hypothalamic-pituitary-interrenal axis (i.e., elevation of cortisol). To study this situation, we examined the effects of experimental manipulation of the stress hormone cortisol on locomotor activity and behaviour of nest guarding male smallmouth bass (*Micropterus dolomieu*). We exogenously elevated circulating cortisol levels (via intracoelomic implants) and attached tri-axial accelerometers to wild smallmouth bass for three days. During the recovery period (i.e., ≤ 4 h post-release), cortisol-treated fish exhibited significantly reduced locomotor activity and performed significantly less burst and routine swimming relative to control fish, indicating cortisol uptake was rapid, as were the associated behavioural responses. Post-recovery (i.e., > 4 h post-release), fish with high cortisol exhibited lower locomotor activity and reduced routine swimming relative to controls. Fish were less active and reduced routine and burst swimming at night compared to daylight hours, an effect independent of cortisol treatment. Collectively, our results suggest that cortisol treatment (as a proxy for anthropogenic disturbance and stress) contributed to altered behaviour, and consequently cortisol-treated males decreased parental investment in their brood, which could have potential fitness implications.

1. Introduction

Many taxa provide extended parental care for their developing offspring. By providing parental care, defined here as the post-fertilization behaviours exhibited while caring for offspring, parents increase the chances of offspring survival, thus increasing the potential for reproductive success and fitness of the parents [1]. Parental care occurs in all vertebrate taxa including mammals [2], birds [3], reptiles [4,5], amphibians [6,7], and fish [8,9]. Of all vertebrate taxa, fish exhibit the greatest diversity of parental care modes [1,10], including paternal care (sole male care), maternal care (sole female care), bi-parental care (both parents), and combinations thereof [9,11]. Among teleost fishes, paternal care is the most common mode [8].

Paternal care is ubiquitous in the freshwater fish *Centrarchidae*

family [8,12] and has been particularly well-studied for the black bass (*Micropterus spp.*). The four to six week parental care period is energetically costly for male black bass [12–15]. Locomotor activity levels must be maintained by nesting males throughout parental care [13] to vigorously defend their developing offspring from omnipresent and persistent brood predators [16–18]. Considerable time is devoted to nest-tending activities (i.e., fanning eggs, removing debris and dead eggs from the nest) to ensure that eggs and developing offspring remain well oxygenated and healthy [19]. Time consuming parental care duties [13,19] and alterations in appetite-regulating hormones [20–22] both lead to reduced food intake, meaning that paternal care largely is driven by endogenous energy stores [15]. Black bass parental investment and reproductive success are linked such that nesting males that exhibit increased defensive behaviours are known to have a higher

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probability of reproductive success compared to those exhibiting diminished defensive behaviours [23]. Owing to the challenges of paternal care and the trade-offs in energy allocation to current versus future reproductive success [24,25], premature nest abandonment is not uncommon, particularly in fish with limited energy reserves (e.g., small fish or those in poor condition; [26]) or among those with small broods [23,27].

In addition to the increased energetic demands directly associated with paternal care, parental fish endure other challenges such as exposure to piscivorous predators (e.g., heron, osprey) owing to their nesting locations in the littoral zone [28], and inclement weather that can expose both parent and developing offspring to variation in temperature, elevated water turbidity (from suspended sediment) and the effects of currents/wave action [29]. The challenges faced by black bass during paternal care may be magnified by anthropogenic disturbances and stressors common in freshwater systems, including shoreline development and associated habitat alterations [30], ecological light pollution [31,32], noise and wave disturbances from vessels powered by combustion engines [33], and fisheries interactions (e.g., catch-and-release angling; [23,34]). Some of these additional challenges have the potential to cause physiological stress (i.e., activation of the hypothalamic-pituitary-interrenal axis in fish and elevation of circulating glucocorticoids; [35,36]), thereby potentially altering behaviour and energy-use (reviewed in [37]) and potentially fitness [38] and population-level processes [39].

A stress response involves elevation of circulating glucocorticoids (GCs), with cortisol being the primary GC in teleost fish [35,36,40,41]. An increasingly common approach to study how anthropogenic stressors may alter the energetics and behaviour of wild animals, including fish, is through experimental GC manipulation [42,43]. Parental male black bass treated with cortisol exhibit increased nest abandonment relative to controls (e.g., [44,45]). However, alterations in the behaviour of nesting males prior to abandonment have not been detected (e.g., [44–46]), which may be a consequence of using snorkeling observers to monitor behaviour, because snorkelers typically monitor simple behaviours for several minutes (e.g., response to a brood predator model, monitoring time spent on nest, willingness to engage predators), with observation periods occurring only during daylight conditions. Some researchers that have studied black bass parental care behaviour and energetics (independent of GC manipulation) used video cameras to continuously monitor behaviour over several days [12,19], but cameras have a restricted field of view and recordings are heavily influenced by water clarity and light [47]. More recently, new developments in bio-logging technology have enabled researchers to affix tri-axial accelerometers to fish to monitor the fine-scale behaviours and energy-use of free-swimming fish, such that animals can be studied day and night with greater temporal resolution (e.g., [48,49]).

Given the above, we aimed to determine whether elevation of cortisol (intended to mimic disturbances/stress associated with human activity) influenced the behaviour and energy use of wild fish engaged in paternal care. We hypothesized that experimental elevation of cortisol would affect locomotor activity and swimming behaviours of nesting male smallmouth bass. Cortisol mobilizes energy reserves during a stress response, a beneficial attribute in dealing with a stressful situation [35], but prolonged cortisol elevation can be deleterious to fish condition [35,37]. Because nesting fish with experimentally elevated cortisol exhibit decreased nest success (e.g., [44,45]), and nest-tending and brood predator defense are key locomotor-based behaviours linked to reproductive success [23], we predicted that nesting males with elevated cortisol levels would exhibit reduced locomotor activity and swimming behaviours compared to control fish, with the extent of behavioural alterations being magnified in fish experiencing supra-physiological cortisol levels.

2. Materials and methods

2.1. Experimental animals and protocol

The study was conducted between May 15th and 28th, 2015 in four lakes in the Rideau River system (Opinicon Lake, Sand Lake, Indian Lake, and Big Rideau Lake) located in southeastern Ontario. These lakes have a similar species composition and predator burden [50], and previous research indicated that the physiological cost of parental care is similar in these lakes [51]. Surface water temperature ranged from 12 to 16 °C during the study. Nesting male smallmouth bass (*Micropterus dolomieu*) guarding unhatched embryos ('eggs') 0 to 3 days old were located through snorkel surveys and angled from the nest, with fight time minimized (< 20 s) to reduce angling-associated stress [44,46]. All nests selected for inclusion in the study were located in a rocky substrate in water depths ranging from 0.5 to 1.5 m. Following capture, fish were placed in a foam-lined trough containing fresh lake water and total length (TL) was measured. To limit handling time, mass was estimated from an established length-mass relationship [44] using the Eq. (1) where mass is reported in grams and TL in millimeters.

$$\log_{10} \text{mass} = -7.1004 + 3.884(\log_{10} \text{TL}) \quad (1)$$

Following the general methods of Brownscombe et al. [52], accelerometers (see below for accelerometer details) were firmly attached through the dorsal musculature using 13.6 kg Dacron line and a backing pad; accelerometers were placed on the external surface of the right side of the fish under the soft area of the dorsal fin.

Fish were randomly assigned to one of three treatment groups; control fish that were untreated, fish that received an intraperitoneal injection of cocoa butter (5 mL kg⁻¹; NOW Foods, Bloomingdale, IL) mixed with a low (5 mg mL⁻¹) concentration of cortisol (hydrocortisone 21-hemisuccinate, Santa Cruz Biotechnology, Dallas, TX), and fish that received a high (10 mg mL⁻¹) cortisol concentration, again as an intraperitoneal implant in cocoa butter. The high cortisol concentration in this study (10 mg mL⁻¹) has been found to produce high elevated circulating cortisol levels in nesting black bass for 5–6 days [44,45]. A sham treatment group (cocoa butter alone) was not included in the experimental design owing to inconsistent cortisol responses associated with sham treatment (see [53]). Prior to release, fish (with tag affixed) were rotated along known axes (forward pitching motion and rolled to the right) in a cooler containing fresh lake water, noting the corresponding times, to aid in calibrating accelerometers. Capture, tagging, cortisol administration (if applicable), and accelerometer calibration encompassed < 7 min.

Fish were released within 5 m of the nest. A snorkeler guarded the nest in the male's absence until the male resumed parental behaviours, typically < 5 min. All males were ≥ 390 mm TL and had an egg score of ≥ 3 in an attempt to limit premature nest abandonment. Egg score is a qualitative measure of the density of eggs within the nest, with a (minimum) score of one being low density (< 500 eggs) and a (maximum) score of five being high density (> 4000 eggs; [34,54]). All procedures were approved by the Carleton University animal care committee (B12-06), in accordance with the guidelines of the Canadian Council on Animal Care for the use of animals in research and teaching.

After 3 days, fish were recaptured using rod and reel (angling fight time < 20 s) and placed into a foam-lined trough containing fresh lake water for the withdrawal of a blood sample and accelerometer removal. Time from capture to blood sampling was < 120 s for each fish. Blood samples (approximately 1 mL) were withdrawn from the caudal vasculature using a 22 mm, 21-gauge needle and 4 mL heparinized vacutainer (B.D. Vacutainer, Franklin Lakes, NJ), and were immediately placed into a water-ice slurry for no longer than 90 min. Blood samples were centrifuged at 10,000 g for 5 min and separated plasma was frozen in liquid nitrogen and stored at -80 °C until analysis. Analysis of cortisol concentrations was conducted as previously described [45] using a commercially-available radioimmunoassay (ImmuChem, MP

Biomedicals, Solon, OH). Samples were measured in two different assays, each in duplicate, with intra-assay variation of 7.5% and 1.1% and inter-assay variation of 8.3%.

Two accelerometer models were used (model X16mini, 17 g in air, 51 mm × 25 mm × 13 mm, 25 Hz recording frequency, ± 16 g range, 2048 count/g sensitivity; model X8M-3, 15 g in air, 49 mm × 25 mm × 13 mm, 25 Hz recording frequency, ± 8 g range, 1024 count/g sensitivity; Gulf Coast Data Concepts, Waveland, MS), with both being programmed to continuously record acceleration measurements on three axes (x = heave, y = surge, z = sway). Accelerometer models were randomly distributed among treatments. The average weight of accelerometers including backing plates and tape was ~28 g in air, corresponding to < 2% of fish weight for all fish in this study to minimize tagging burden [55]. Visual observations confirmed that swimming behaviours did not appear impeded by accelerometer attachment, with tagged fish resuming the same general swimming behaviours (i.e., chasing brood predators, monitoring the nest area, fanning eggs) as untagged fish (personal observations of several divers).

2.2. Data and statistical analyses

Accelerometer data analysis was conducted using IGOR Pro 6.0 (WaveMetrics Inc., Lake Oswego, OR) with the Ethographer package [56]. Acceleration data recordings were cropped to account for experimental manipulation of the fish. The initial 7 min of the first hour of data were removed to account for accelerometer attachment and cortisol treatment. The last hour of data was removed to account for fish recapture for accelerometer retrieval. Thus, for each fish a total of 64 h of accelerometer data was available for analysis, with exception of one fish, for which 37 h were available. Raw acceleration data were first separated into static (gravity) and dynamic (fish movement) components by a weighted smoother over each axis with a 2 s moving window; this smoothing interval that has been found to be appropriate for small teleost fishes [52,57]. Overall dynamic body acceleration (ODBA), the variable commonly used to estimate relative activity in teleost fishes [52,57], was calculated as the absolute sum of the dynamic acceleration of the three axes (x , y , z).

Accelerometer data also were analyzed to identify different types of swimming behaviour, namely burst swimming, routine swimming, and resting. The frequency of occurrence of these activities was determined. Burst swimming differs from routine swimming in being characterized as aggressive, of short duration, and energetically demanding [13,58]; burst swimming is exhibited, for example, by nesting fish chasing away a brood predator. Routine swimming included turns and general monitoring of the nest area. Resting behaviour included periods where fish were stationary or engaged in limited locomotor activities such as performing nest checks to remove dead eggs and debris. To identify these behaviours within the accelerometer data, first the swimming behaviours of several fish were visually observed by snorkelers with digital watches synchronized to the accelerometer, who noted the time and duration that a fish exhibited behaviours of interest. These observations served to develop swimming behaviour criteria for a 'trainer' data set via a classification tree [52,59]. Predictor variables in the classification tree model included the mean and standard deviation of dynamic acceleration for each axis (x , y , z), pitch, and roll. Pitch and roll were calculated using Eqs. (2) and (3), respectively.

$$\text{Pitch} = \arcsine(\text{surge static}) \times (180/\pi) \quad (2)$$

$$\text{Roll} = \arcsine(\text{heave static}) \times (180/\pi) \quad (3)$$

The final classification tree model was pruned to include three nodes (one node for each swimming behaviour), and was cross-validated, yielding misclassification error of 5%. To classify routine swimming, burst swimming, and resting behaviours on fish that were not observed, the pruned classification tree model was applied to the

accelerometer algorithm outputs [52]. Predicted swimming behaviours were classified at a probability of > 0.70 from classification tree criteria output. Predicted swimming behaviour was determined on a per second (s^{-1}) time interval such that each second of the 64-h study duration is classified as a burst, routine, or resting behaviour.

Owing to individual variation in clearance rates, cortisol-treated fish were categorized based on their measured circulating cortisol values for data analyses. 'Low' cortisol fish were deemed to be those having measured cortisol values of < 50 up to 1000 ng ml⁻¹, documented in fish during a stress response [60,61]; and 'high' cortisol fish included those with plasma cortisol levels > 1000 ng ml⁻¹, a supra-physiological level [44,45]. Control fish, those that did not receive a cortisol treatment, remained unchanged in their categorization for all analyses. A one-way analysis of variance (ANOVA) was conducted to determine whether differences in circulating cortisol concentrations among treatments were statistically significant. Measured cortisol values were log₁₀ transformed to meet the assumptions of normality and equal variance for parametric tests. As the fish in the present study were angled and handled, separate analyses were conducted on data collected prior to and following recovery, where the recovery period was considered to be the initial 4 h of data collection. This recovery period was chosen on the basis of previous research that indicated that circulating cortisol levels return to pre-capture levels within 2 to 6 h in teleost fishes [62]. Also, Schreer et al. [63] found that recovery from an exhaustive angling event occurred within 215 mins, and fish in the present study were only briefly angled (< 20 s). The effects of cortisol treatment on locomotor activity (ODBA) and swimming behaviours (burst and routine swimming) during the recovery period (i.e., ≤ 4 h post-release) were tested using a linear mixed effects model (LME) with cortisol treatment (no cortisol, low cortisol, high cortisol) as a fixed effect. The effects of cortisol treatment on locomotor activity and swimming behaviours during the post-recovery period (i.e., > 4 h post-release) were tested using a LME with cortisol treatment (no cortisol, low cortisol, high cortisol), diel period (day, night), and their interaction terms as fixed effects. Diel period was included as a fixed effect because smallmouth bass are less active at night [31,64]. Random effects in recovery and post-recovery LME included a random intercept of individual fish (fish ID) nested within water surface temperature. Water temperature measurements ranged from 12 to 14 °C during accelerometer attachment and cortisol treatment, and were categorized as "low" or "expected"; "low" indicated a water temperature lower than that at which smallmouth bass typically spawn (i.e., < 14 °C), while "expected" was a water temperature approaching what is typical of spawning bass (i.e., 14 °C; [65]). From the model including all explanatory variables and interactions, a stepwise backward model selection with single term deletions using likelihood ratio tests was conducted; first the significance and optimal structure of the random component was established with restricted maximum likelihood estimation (REML), and then the optimal structure of the fixed component was established with maximum likelihood estimation (ML), as recommended by Zuur et al. [66]. Linear mixed effects models were fit using "lmer" from the lme4 R-package [67]. Model validation was conducted as per Zuur et al. [66]. Owing to patterns in the residuals, log transformation was applied to ODBA and square root transformation to swimming behaviour response variables, resulting in improved model fit. Fixed effect posterior simulations ($N = 10,000$) were generated using "sim" from the arm R-package [68]. Fixed effect significance was considered to exist if estimates from the simulated posterior distributions contained zero at the 95% credible interval. That is, the 95% credible intervals of fixed effects estimates that did not contain zero were considered significant. Statistical analyses were conducted using RStudio (v. 0.99.896, Boston, MA) and R (v. 3.2.3, R Foundation for Statistical Computing, Vienna, Austria).

Table 1
Body length and circulating cortisol concentrations measured 3 days post-treatment for nesting male smallmouth bass (*Micropterus dolomieu*).

Treatment	Body length (TL, mm)	[Cortisol] (ng mL ⁻¹)
Control	426 ± 12 ^a	15 ± 2 ^a
Low cortisol	456 ± 5 ^{ab}	537 ± 153 ^b
High cortisol	459 ± 4 ^b	2691 ± 469 ^c

Values are means ± SE for N = 6 control, N = 5 low cortisol, and N = 5 high cortisol fish. Significant differences among groups (α = 0.05) are denoted with different letters. P-values are found in text.

3. Results

Analyses included 6 control, 5 ‘low cortisol’, and 5 ‘high cortisol’ fish. Body length was significantly different among groups (one-way ANOVA: $F = 4.40$, $df = 2$, p -value = 0.035). A pair-wise comparison revealed a difference in body length between control and high cortisol groups (Tukey’s HSD: p -value = 0.049; Table 1). The significant difference in body size (total length) was driven by a single fish in the control group; if this fish was removed from the analysis, there was no difference among treatments. Owing to the small sample sizes we opted to retain this fish in the data set. Measured circulating cortisol concentration was lowest in the control group (Table 1), ranging from 10 to 19 ng mL⁻¹. The low and high cortisol groups included fish with measured circulating cortisol values ranging from 128 to 897 ng mL⁻¹, and 1527 to 4277 ng mL⁻¹, respectively. Cortisol values were significantly different among groups (one-way ANOVA: $F = 127.7$,

$df = 2$, p -value < 0.0001; Tukey’s HSD: p -value < 0.001, all cases; Table 1).

3.1. Locomotor activity (ODBA)

Fish in all groups began the experiment with a similar level of locomotor activity (ODBA, Fig. 1A). There was considerable variation in activity level among individuals in all treatments groups for the first ~30 min of the study period (Fig. 1A). However, after 30 min, individual variation was more consistent, and both low and high cortisol fish began to exhibit lower activity levels than control fish. When examined on an hourly basis, control fish maintained the highest level of locomotor activity, with both cortisol groups exhibiting lower mean locomotor activity than control fish for the 64-h study duration (Fig. 1B). During the recovery period (≤ 4 h post-release), a linear mixed effects model (LME) revealed significant differences in locomotor activity among low and high cortisol-treated fish relative to controls (Table 2). During the post-recovery period (> 4 h post-release), a LME revealed significant differences in locomotor activity between high cortisol-treated fish relative to controls (Table 2). Fish exhibited significantly higher locomotor activity level during daylight than at night, an effect independent of cortisol treatment (Table 2; Fig. 3A). Multiple comparisons revealed that high cortisol fish exhibited lower daytime locomotor activity relative to controls (Tukey’s HSD: p -value < 0.001; Fig. 3). Significance of fixed effects remained unchanged with a LME in which the significant treatment and diel interaction terms were removed.

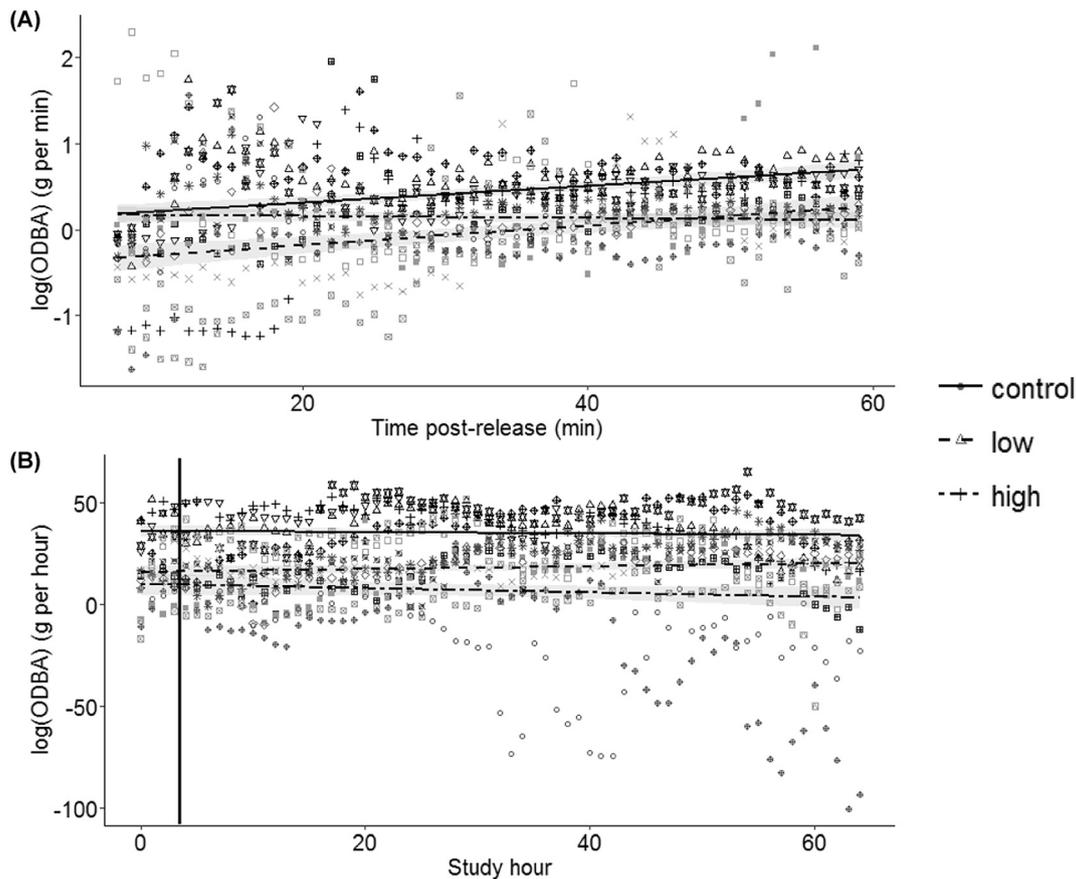


Fig. 1. Post-release locomotor activity (as overall dynamic body acceleration, ODBA) of nesting smallmouth bass for the first hour of the study period (A) and for the duration (64 h) of the study period (B). Solid line and black symbols depict data for control fish (N = 6); low cortisol fish (N = 5) are depicted with a dashed-dot line and light grey symbols; high cortisol fish (N = 5) are depicted with a dashed line and dark grey symbols. A linear trend line with 95% confidence intervals is shown for the observed data. Data in (B) were analyzed using a linear mixed effects model and simulated posterior distributions (see Table 2 for values; credible intervals do not correspond to confidence intervals shown in figure). The solid vertical line in (B) denotes where the post-recovery period (> 4 h post-release) begins.

Table 2

Linear mixed effects models testing the effects of cortisol treatment (Treatment) and diel period (Diel) on the locomotor activity and swimming behaviours of nesting male smallmouth bass. Mean and 95% credible interval of fixed effects were calculated from posterior simulations ($N = 10,000$). Recovery and post-recovery models encompass the first 4 h and the remaining 60 h of the study duration, respectively. Significant terms are denoted in boldface.

Response	Factor	Mean	Upper CI	Lower CI
Recovery				
Locomotor activity (ODBA)	Intercept	32.05	41.98	22.42
	Treatment (low)	-19.43	-5.04	-32.61
	Treatment (high)	-20.97	-6.49	-34.54
Routine	Intercept	4.73	5.56	3.93
	Treatment (low)	-1.99	-0.90	-3.07
	Treatment (high)	-1.96	-0.86	-3.12
Burst	Intercept	1.15	1.50	0.78
	Treatment (low)	-0.44	0.01	-0.87
	Treatment (high)	-0.65	-0.21	-1.08
Post-recovery				
Locomotor activity (ODBA)	Intercept	39.14	50.77	26.83
	Treatment (low)	-16.61	1.85	-34.69
	Treatment (high)	-30.94	-13.74	-48.62
	Diel (night)	-7.88	-4.97	-10.80
	Treatment (low) × Diel (night)	-1.56	2.73	-5.73
	Treatment (high) × Diel (night)	3.63	7.74	-0.43
Routine	Intercept	5.29	6.43	4.08
	Treatment (low)	-1.33	0.06	-2.69
	Treatment (high)	-1.37	-0.01	-2.73
	Diel (night)	-0.69	-0.49	-0.92
	Treatment (low) × Diel (night)	0.16	0.49	-0.15
	Treatment (high) × Diel (night)	0.88	1.19	0.58
Burst	Intercept	1.29	1.71	0.89
	Treatment (low)	-0.18	0.12	-0.47
	Treatment (high)	-0.25	0.04	-0.58
	Diel (night)	-0.54	-0.44	-0.63
	Treatment (low) × Diel (night)	0.20	0.34	0.06
	Treatment (high) × Diel (night)	0.33	0.47	0.19

For all analyses control $N = 6$, high cortisol $N = 5$, and low cortisol $N = 5$. ODBA, overall dynamic body acceleration; low = low cortisol; high = high cortisol. Multiple comparisons for significant interaction terms other than those indicated are found in text.

3.2. Routine swimming

The time spent routine swimming was variable among individuals in all treatments groups, particularly in the controls (Fig. 2A). Routine swimming declined in control fish as the study period progressed, but despite this trend, control fish consistently performed more routine swimming per hour than cortisol-treated fish through the majority of the study period (Fig. 2A). Both cortisol groups exhibited a positive trend in the time spent routine swimming through the study period, with values steadily increasing to levels similar to those of control fish around hour 60 (Fig. 2A). During the recovery period, a LME revealed significant differences in routine swimming among low and high cortisol-treated fish relative to controls (Table 2). During the post-recovery period, a LME revealed a significant difference in routine swimming among high cortisol-treated fish relative to controls (Table 2). Overall, fish performed more routine swimming during the day than at night, an effect that was independent of cortisol treatment (Table 2; Fig. 3B). Multiple comparisons revealed no significant differences in routine swimming among cortisol-treated fish and controls during daylight or night.

3.3. Burst swimming

Control fish consistently spent more time burst swimming per hour

throughout the study period (Fig. 2B). By contrast, the low and high cortisol groups exhibited increased use of burst swimming throughout the study period, with both groups initially performing less burst swimming than control fish, and rates then steadily increasing to values similar to those of control fish after ~30 h (Fig. 2B). During the recovery period, a LME revealed significant differences in burst swimming among low and high cortisol-treated fish relative to controls (Table 2). During the post-recovery period, a LME revealed no significant in burst swimming among low and high cortisol-treated fish relative to controls (Table 2). Fish performed more burst swimming during the day than at night independent of cortisol treatment (Table 2; Fig. 3C). Multiple comparisons revealed no significant differences in burst swimming among cortisol-treated fish and controls during daylight or night.

3.4. Resting behaviour

Trends in resting behaviour for each group (not visually depicted) were the mirror image of those for routine and burst swimming shown in Fig. 2A and B. The time spent resting gradually increased in control fish as the study period progressed, with control fish consistently spending less time per hour resting than cortisol-treated fish through the majority of the study period. Through the study period, both cortisol groups exhibited a negative trend in the time spent resting, with values steadily decreasing to levels similar to those of control fish around hour 60. Resting behaviour was not statistically analyzed.

4. Discussion

In this study we tested whether experimental cortisol elevation (intended to mimic the stress-induced GC response typical of anthropogenic activity; [43]) elicited changes in the locomotor activity and swimming behaviour of wild, nesting male smallmouth bass to gain further insight into the effects of cortisol, a key stress hormone in fish, on parental care. As predicted, during the recovery period cortisol-treated smallmouth bass exhibited significantly lower locomotor activity (i.e., ODBA) and routine and burst swimming than control fish, and the effects were magnified in fish with high (supra-physiological) cortisol levels. During the post-recovery period, fish with high cortisol levels exhibited significantly lower locomotor activity and reduced routine swimming relative to controls. Low cortisol fish tended to exhibit reduced burst swimming relative to controls during the post-recovery period, but this trend was not statistically significant. Diel period had an overall effect on smallmouth bass locomotor activity and swimming behaviour such that fish spent less time burst swimming at night compared to the day. The corresponding locomotor activity and swimming behaviour responses during the recovery period suggest that physiologically-relevant circulating cortisol levels, which are those reflective of a stress response [i.e., low cortisol group in the present study [54,57] and supra-physiological levels [i.e., high cortisol group in the present study [44,45], produce a similar behavioural response in nesting smallmouth bass. Although there was a statistical difference in mean body length between the control and high cortisol group, the difference in body length amounted to a one-year age difference in smallmouth bass [69], which has been shown not to be biologically significant in terms of parental care behaviour in mature smallmouth bass [70].

Nest-tending smallmouth bass with high cortisol levels exhibited lower locomotor activity (ODBA) and routine swimming throughout the post-recovery study period compared to control fish, and low cortisol-treated fish tended to perform less burst swimming than control fish in the post-recovery period. Studies that experimentally manipulated cortisol in other teleosts [e.g., rainbow trout *Oncorhynchus mykiss*, [71]; common carp *Cyprinus carpio* [72]] found no effect of chronically elevated cortisol concentrations on aerobic swimming performance as measured by U_{crit} . These findings suggest that swimming function per se

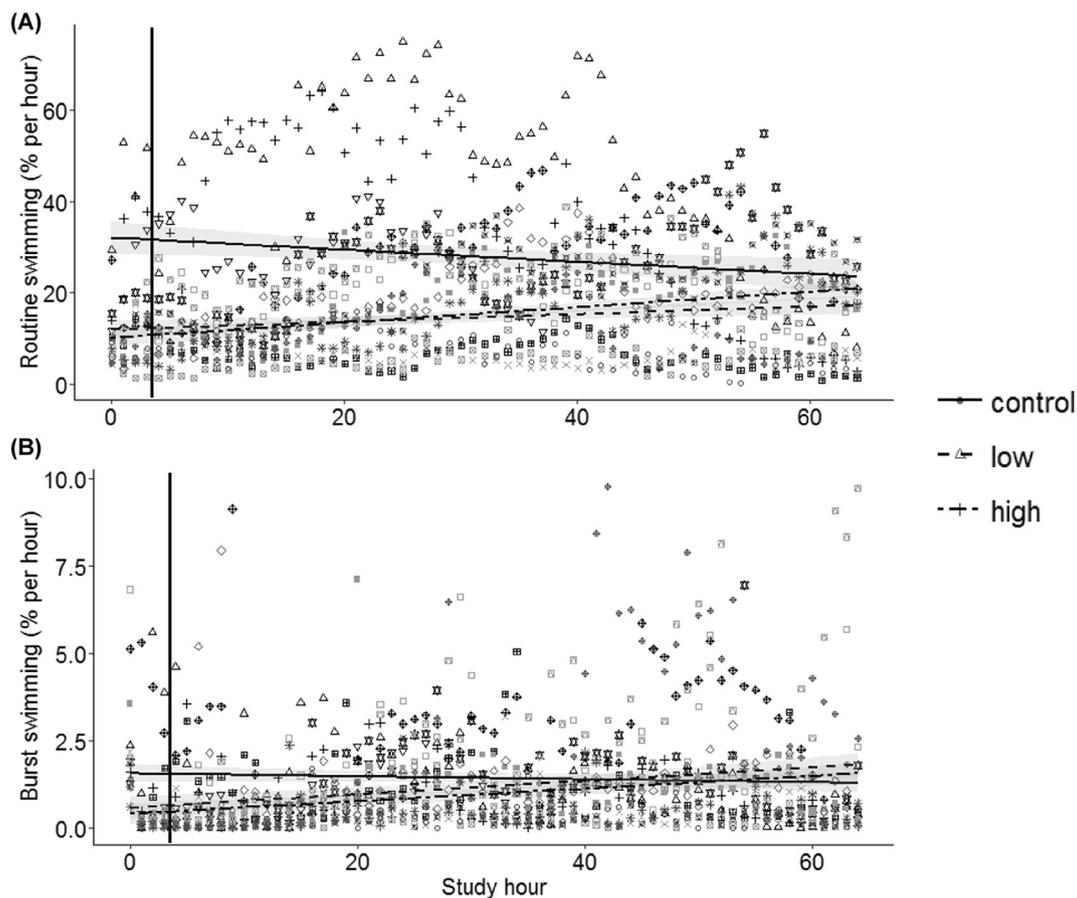


Fig. 2. Proportion of each hour nesting male smallmouth bass performed routine (A) and burst swimming (B) during the study period (64 h). Solid line and black symbols depict data for control fish ($N = 6$); low cortisol fish ($N = 5$) are depicted with a dashed-dot line and light grey symbols; high cortisol fish ($N = 5$) are depicted with a dashed line and dark grey symbols. A linear trend line with 95% confidence intervals is shown for the observed data. Data were analyzed using a linear mixed effects model and simulated posterior distributions (see Table 2 for values; credible intervals do not correspond to confidence intervals shown in figure). The solid vertical line denotes where the post-recovery period (> 4 h post-release) begins.

(e.g., U_{crit}) was not impaired by cortisol treatment in nesting smallmouth bass, but rather that elevated cortisol levels may be affecting behavioural choices (i.e., motivation and investment), with cortisol-treated fish choosing to engage in routine and burst swimming less often than control fish. Cortisol influences metabolic processes (reviewed by [40]). For example, cortisol treatment elevates metabolic rate [73,86,72], and elevated cortisol levels can contribute to energy mobilization even when teleosts are inactive. Liew et al. [72] found that common carp with exogenously elevated cortisol levels mobilized liver and muscle proteins while in a resting state. Other teleosts have exhibited a decreased hepatosomatic index (rainbow trout [74]; bluegill sunfish *Lepomis macrochirus* [87]) and condition factor [74] owing to chronically elevated cortisol levels. Thus, it is possible that the chronically elevated cortisol levels may be contributing to decreases in physiological condition and mobilization of energy reserves during periods of inactivity in the smallmouth bass in the present study. Our results suggest that in response, the cortisol-treated fish may be reducing activity level, favouring self-maintenance over the continued energetic demands of swimming activities. Overall dynamic body acceleration (ODBA), used to estimate activity level in this study, correlates highly with metabolic rates [75–77]. As such, the lower ODBA exhibited by the cortisol-treated fish implies that the cortisol-treated fish exhibit a lower energy expenditure. Interestingly, manipulation of cortisol titres in wild creek chub (*Semotilus atromaculatus*) was not associated with changes in behaviour in mesocosm or field environments [78], suggesting that the effects of cortisol treatment on behavioural choices may only become apparent under conditions of increased energy demand or environmental challenge. In agreement with this

possibility, O'Connor et al. [79] failed to find differences in behaviour between cortisol-treated and control largemouth bass, except in response to an environmental challenge (i.e., winter hypoxia).

Parental care is energetically demanding for nesting black bass [13], contributing to lost body mass [14] and depleted endogenous energy reserves [12,15]. Chronic elevation of cortisol in nesting black bass adds to this physiological burden because cortisol increases metabolic rate, mobilizes energy reserves and lowers growth rate [71,86,72]. For example, increased incidence of Saprolegnial fungal infections was observed in cortisol-treated nesting largemouth bass [45]. Zolderdo et al. [46] found that cortisol-treated smallmouth bass showed evidence of altered immune function through changes in white blood cell counts (i.e., lymphocytes, monocytes, neutrophils), and that chronically elevated cortisol may contribute to oxidative stress in nesting smallmouth bass. Under these conditions, cortisol-treated nesting black bass may have few options but to lower activity levels, particularly if nest abandonment is to be avoided. Although the time frame of the present study did not allow the incidence of nest abandonment to be examined, previous studies have reported that cortisol treatment is associated with increased nest abandonment in parental male black bass [44,45]. Thus, our results may indicate that parental males are attempting to maintain parental investment in their current reproductive effort, but limiting energetic expenditure to ensure opportunity for future reproductive potential [25,80]. Alternatively or additionally, locomotor activity may have a non-linear relationship with offspring survival; for example, a moderate or large reduction in activity level may result in a small reduction in brood size.

Control fish, serving as an indicator of the typical swimming

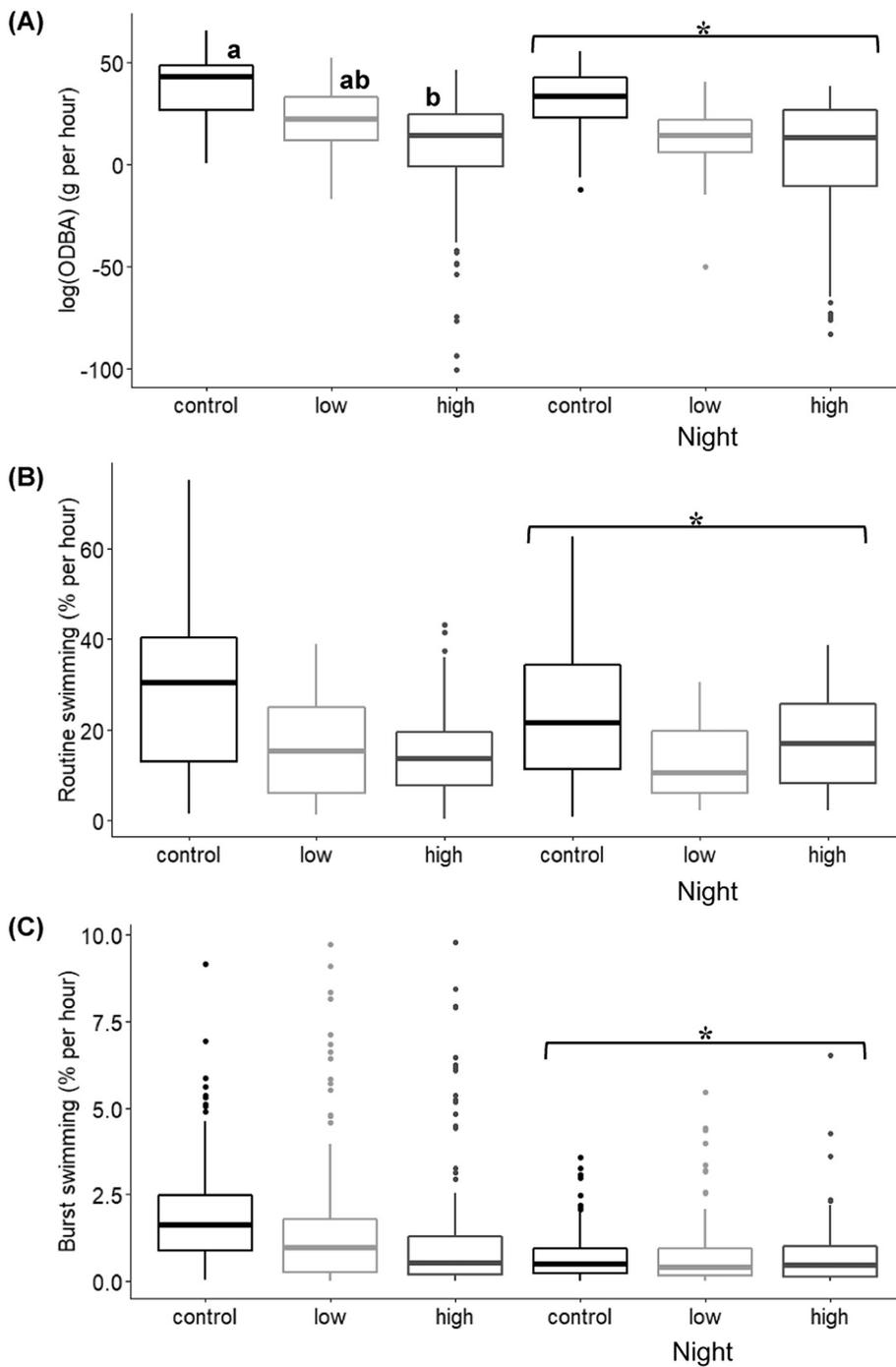


Fig. 3. Nesting male smallmouth bass locomotor activity (as overall dynamic body acceleration, ODBA; A), and proportion of each hour performing routine (B) and burst swimming (C) during the post-recovery routine (> 4 h post-release) according to diel period. Values are presented for control (black, $N = 6$), low cortisol (light grey, $N = 5$), and high cortisol (dark grey, $N = 5$) fish. Within a diel period, groups that share a letter are not significantly different. Significant differences between day and night (independent of treatment) are indicated with an asterisk. Data were analyzed using a linear mixed effects model and simulated posterior distributions (see Table 2 for values). Multiple comparisons were conducted using a Tukey's HSD method.

behaviours characteristic of parental care for nesting black bass, performed more routine swimming and tended to perform more burst swimming than low cortisol fish. Significant two-way interactions between cortisol treatment and diel period indicate that high-cortisol fish performed less routine swimming during the day relative to controls. Parental investment in nest defense behaviours in parental black bass and other centrarchids is higher when guarding a large brood [27,65,81], because larger broods have a higher reproductive value. Using electromyogram telemetry transmitters, Cooke et al. [13] estimated that nesting black bass swim upwards of 40 km day⁻¹ as they monitor their nest area. Studies by Gravel and Cooke [50] and Steinhart et al. [17] found that when predation pressure increases, male black bass guarding eggs spent more time performing burst swims, an essential behaviour in protecting the nest from brood predators. Hanson

et al. [20–22] found no correlation between cortisol levels and nesting male smallmouth bass aggression, however in that case fish were exposed to an acute stressor. Consequently, the observed reduction in nest defense and monitoring behaviours (i.e., reduced routine and burst swimming) exhibited by exogenously cortisol-treated fish in the present study may result in impaired parental investment in brood defense and/or maintenance, which in turn may ultimately have fitness related impacts through reduced nest success with the current brood. Given this impact of elevated cortisol levels on locomotor activity and, presumably, parental care, it is perhaps not surprising that parental male bass exhibit attenuation of the cortisol response to a stressor during the early stages of offspring development, when the energetic demands of parental care are at their highest [61,82].

Temporal factors affected activity level and swimming behaviours.

The difference in locomotor activity level and swimming behaviours between control and cortisol-treated fish was evident in the recovery period, suggesting that cortisol uptake was rapid. The eventual convergence in routine and burst swimming behaviours of cortisol-treated and control fish late in the study period corresponds with the results of previous studies, which found no difference in control and cortisol-treated black bass parental care behaviours when fish were assessed four days post-cortisol treatment [44–46]. Our study indicates that altered behavioural responses attributable to experimental cortisol manipulation commence rapidly, and are only evident when observed at a fine scale. Burst swimming differed according to diel period such that smallmouth bass exhibited reduced burst swimming at night. The reductions in activity level (ODBA) and swimming behaviour at night is not surprising, as nesting smallmouth bass have fewer brood predators to engage at that time. Although nocturnal brood predators (e.g., bullhead catfish *Ameiurus spp.*) are present in the study lake system [83], the predator burden imposed by these nocturnal species would be negligible in comparison to that imposed by other centrarchids (e.g., *Lepomis spp.*) and conspecifics, the dominant brood predators in lakes within this study system [83,50], which are primarily active during daylight hours [64].

Variation in water temperature, which ranged from 12 to 16 °C during the study period, was an unavoidable limitation in the present study. Temperature impacts most metabolic processes [84], and thus may have influenced fish activity in this study. Surface temperature data were recorded for each fish in our study at the time of capture (and associated tagging and treatment), but temperature was not continuously monitored throughout the study period. It is possible that fish sampled on warmer days (i.e., higher surface water temperature) had increased activity relative to those sampled on cooler days. It is also possible that cortisol absorption and pharmacokinetics may have varied across temperatures. Furthermore, black bass typically begin spawning when the water temperature approaches 15 °C [65], thus fish that begin spawning at cooler temperatures may exhibit different activity patterns than those spawning in warmer temperatures. Owing to a lack of thermal data, we cannot adequately rule out that temperature was not a significant co-factor in the decreased locomotor activity and swimming behaviours observed in cortisol-treated black bass. Nonetheless, fish were allocated to different treatments randomly, nests were in similar habitats and in reasonable close proximity (within a given waterbody), tagging occurred over a narrow window of time related to when we found “new” eggs, and the water temperature at time of initial treatment only ranged across 2 °C (i.e., 12 to 14 °C) collectively suggesting that vastly different thermal exposure among individual fish or treatments were unlikely.

The present study is the first to examine locomotor activity and parental care behaviours in response to exogenously elevated cortisol at a fine scale over an extended time period, adding important new information to the growing body of literature documenting the effects of elevated cortisol levels on teleost reproduction. Many teleost species that provide parental care construct their nest in the littoral zone near the shoreline (e.g., [12,85]). Our results are pertinent to recreational fisheries management given that anglers could reduce potential negative effects on nesting male black bass by reducing capture stress (e.g., reducing fight times, minimizing handling and air exposure, releasing fish as rapidly as possible) although avoiding bass capture entirely during this period would be prudent [34]. Owing to anthropogenic shoreline development, fish in nearshore areas are expected to have the highest exposure to stressors such as shoreline development, increased light pollution, and boat operation and noise. In the present study, changes in parental care behaviours in response to exogenously elevated cortisol were observed. However, fish in the present study were not explicitly exposed to the aforementioned shoreline development stressors. Our study focused only on a single reproductive event, whereas fish may trade-off the value of their current brood with the potential for future reproductive success [9,80]. Furthermore, though

the present study examined parental care activities at a fine scale over 64 h early in the parental care period, when demands on the male are particularly high, the parental care period encompasses four to six weeks in total. For future work, it will be important to track nest success in conjunction with activity level and swimming behaviour. Studies conducted at different temporal scales (single breeding season, multi-year) to directly quantify and examine the relationship(s) among circulating cortisol concentrations, parental care behaviours, and reproductive success would be beneficial in understanding how stress influences parental care decisions and ultimately, lifetime fitness.

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References

- [1] T.H. Clutton-Brock, *The Evolution of Parental Care*, Princeton University Press, 1991.
- [2] D.J. Gubernick, P.H. Klopfer, *Parental care in mammals*, Plenum Press, New York, NY, 1981.
- [3] B.S. Tullberg, M. Ah-King, H. Temrin, Phylogenetic reconstruction of parental-care systems in the ancestors of birds, *Philos. Trans. R. Soc. B* 357 (2002) 251–257.
- [4] S.G. Platt, J.B. Thorbjarnarson, Nesting ecology of the American crocodile in the coastal zone of Belize, *Copeia* 2000 (2000) 869–873.
- [5] R. Shine, T.R.L. Madsen, M.J. Elphick, P.S. Harlow, The influence of nest temperature and maternal brooding on hatchling phenotypes in water pythons, *Ecology* 78 (1997) 1713–1721.
- [6] K. Summers, Mating strategies in two species of dart-poison frogs: a comparison study, *Anim. Behav.* 43 (1992) 907–919.
- [7] J. Tumulty, V. Morales, K. Summers, The bi-parental care hypothesis for the evolution of monogamy: experimental evidence in an amphibian, *Behav. Ecol.* 25 (2014) 262–270.
- [8] L.S. Blumer, A bibliography and categorization of bony fishes exhibiting parental care, *Zool. J. Linnean Soc.* 76 (1982) 1–22.
- [9] M. Gross, R. Sargent, The evolution of male and female parental care in fishes, *Am. Zool.* 25 (1985) 807–822.
- [10] J.N. Webb, A.I. Houston, J.M. McNamara, T. Székely, Multiple patterns of parental care, *Anim. Behav.* 58 (1999) 983–993.
- [11] N. Goodwin, S. Balshine-Earn, J.D. Reynolds, Evolutionary transitions in parental care in cichlid fish, *Proc. R. Soc. Lond. B* 265 (1998) 2265–2272.
- [12] S.J. Cooke, D.P. Philipp, D.H. Wahl, P.J. Weatherhead, Energetics of parental care in six syntopic centrarchid fishes, *Oecologia* 148 (2006) 235–249.
- [13] S.J. Cooke, D.P. Philipp, D.H. Wahl, P.J. Weatherhead, Parental care patterns and energetics of smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*Micropterus salmoides*) monitored with activity transmitters, *Can. J. Zool.* 80 (2002) 756–770.
- [14] J.F. Gillooly, J.R. Baylis, Reproductive success and the energetic cost of parental care in male smallmouth bass, *J. Fish Biol.* 54 (1999) 573–584.
- [15] R.W. Mackereth, D.L.G. Noakes, M.S. Ridgway, Size-based variation in somatic energy reserves and parental expenditure by male smallmouth bass, *Micropterus dolomieu*, *Environ. Biol. Fish* 56 (1999) 263–275.
- [16] M.A. Gravel, S.J. Cooke, Influence of inter-lake variation in natural nest predation pressure on the parental care behaviour of smallmouth bass (*Micropterus dolomieu*), *Ethology* 115 (2009) 608–616.
- [17] G.B. Steinhart, M.E. Sandrene, S. Weaver, R.A. Stein, E.A. Marschall, Increased parental care cost for nest-guarding fish in a lake with hyperabundant nest predators, *Behav. Ecol.* 16 (2004) 427–434.
- [18] Z.C. Zuckerman, C.D. Suski, Predator burden and past investment affect brood abandonment decisions in a parental care-providing teleost, *Funct. Ecol.* 27 (2013) 693–701.
- [19] S. Hinch, N. Collins, Importance of diurnal and nocturnal nest defense in the energy budget of male smallmouth bass: insights from direct video observations, *Trans. Am. Fish. Soc.* 120 (1991) 37–41.
- [20] K.C. Hanson, A. Abizaid, S.J. Cooke, Causes and consequences of voluntary anorexia during the parental care period of wild male smallmouth bass (*Micropterus dolomieu*), *Horm. Behav.* 56 (2009) 503–509.

- [21] K.C. Hanson, C.M. O'Connor, G. Van Der Kraak, S.J. Cooke, Paternal aggression towards a brood predator during parental care in wild smallmouth bass is not correlated with circulating testosterone and cortisol concentrations, *Horm. Behav.* 55 (2009) 495–499.
- [22] K.C. Hanson, C.M. O'Connor, G. Van Der Kraak, S.J. Cooke, The relative roles of parental investment and organismal condition in parental aggression in smallmouth bass, *Horm. Behav.* 55 (2009) 495–499.
- [23] C.D. Suski, J.H. Svec, J.B. Ludden, F.J.S. Phelan, D.P. Philipp, The effect of catch-and-release angling on the parental care behavior of male smallmouth bass, *Trans. Am. Fish. Soc.* 132 (2003) 210–218.
- [24] R.L. Trivers, Parental investment and sexual selection, in: B. Campbell (Ed.), *Sexual Selection and the Descent of Man*, Heinemann, London, 1972, pp. 139–179.
- [25] G.C. Williams, Natural selection, the costs of reproduction, and a refinement of Lack's principle, *Am. Nat.* 100 (1966) 687–690.
- [26] A.J. Gingerich, C.D. Suski, The role of progeny quality and male size in the nesting success of smallmouth bass: integrating field and laboratory studies, *Aquat. Ecol.* 45 (2011) 505–515.
- [27] Z.C. Zuckerman, D.P. Philipp, C.D. Suski, The influence of brood loss on nest abandonment decisions in largemouth bass *Micropterus salmoides*, *J. Fish Biol.* 84 (2014) 1863–1875.
- [28] S.J. Cooke, J. Steinmetz, J.F. Degner, E.C. Grant, D.P. Philipp, Metabolic fright responses of different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs, *Can. J. Zool.* 81 (2003) 699–709.
- [29] G. Steinhart, N.J. Leonard, R.A. Stein, E.A. Marschall, Effects of storms, angling, and nest predation during angling on smallmouth bass (*Micropterus dolomieu*) nest success, *Can. J. Fish. Aquat. Sci.* 62 (2005) 2649–2660.
- [30] T. Wagner, A.K. Jubar, M.T. Bremigan, Can habitat alteration and spring angling explain largemouth bass nest success? *Trans. Am. Fish. Soc.* 135 (2006) 843–852.
- [31] J.G. Foster, D.A. Algera, J.W. Brownscombe, A.J. Zolderdo, S.J. Cooke, Consequences of different types of littoral zone light pollution on the parental care behaviour of a freshwater teleost fish, *Water Air Soil Pollut.* 227 (2016) 404.
- [32] B. Nightingale, T. Longcore, C.A. Simenstead, Artificial night lighting and fishes, in: C. Rich, T. Longcore (Eds.), *Ecological Consequences of Artificial Night Lighting*, Island Press, Washington DC, 2006.
- [33] A.L. Graham, S.J. Cooke, The effects of noise disturbance from various recreational boating activities common to inland waters on the cardiac physiology of a freshwater fish, the largemouth bass (*Micropterus salmoides*), *Aquat. Conserv.* 18 (2009) 1315–1324.
- [34] D.P. Philipp, C.A. Toline, M.F. Kubacki, D.B.F. Philipp, F.J.S. Phelan, The impact of catch-and-release angling on the reproductive success of smallmouth bass and largemouth bass, *North Am. J. Fish. Manag.* 17 (1997) 557–567.
- [35] B.A. Barton, Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids, *Integr. Comp. Biol.* 42 (2002) 517–525.
- [36] N.M. Sopinka, M.R. Donaldson, C.M. O'Connor, C.D. Suski, S.J. Cooke, Stress indicators in fish, in: C.B. Schreck, L. Tort, A.P. Farrell, C.J. Brauner (Eds.), *Fish Physiology, Biology of Stress in Fish*, vol. 35, Academic Press, Amsterdam, 2016, pp. 406–436.
- [37] B.A. Barton, G.K. Iwama, Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids, *Annu. Rev. Fish Dis.* 1 (1991) 3–26.
- [38] M. Fuzzen, N.J. Bernier, G. Van Der Kraak, Stress and reproduction, *Horm. Reprod. Verteb.* 1 (2011) 103–117.
- [39] N.H. Fefferman, L.M. Romero, Can physiological stress alter population persistence? A model with conservation implications, *Cons. Phys.* 1 (2013) cot012.
- [40] T.P. Mommsen, M.M. Vijayan, T.W. Moon, Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation, *Rev. Fish Biol. Fish.* 9 (1999) 211–268.
- [41] S.E. Wendelaar Bonga, The stress response in fish, *Physiol. Rev.* 77 (1997) 591–625.
- [42] G.T. Crossin, O.P. Love, S.J. Cooke, T.D. Williams, Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context, and reproductive state, *Funct. Ecol.* 30 (2016) 116–125.
- [43] N.M. Sopinka, L.D. Patterson, J.C. Redfern, N.K. Pleizier, C.B. Belanger, J.D. Midwood, G.T. Crossin, S.J. Cooke, Manipulating glucocorticoids in wild animals: basic and applied perspectives, *Conserv. Physiol.* 3 (1) (2015) cov031, <http://dx.doi.org/10.1093/conphys/cov031>.
- [44] C.J. Dey, C.M. O'Connor, K.M. Gilmour, G. Van Der Kraak, S.J. Cooke, G. Van Der Kraak, Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care, *Horm. Behav.* 58 (2010) 599–605.
- [45] C.M. O'Connor, K.M. Gilmour, R. Arlinghaus, G. Van Der Kraak, S.J. Cooke, Stress and parental care in a wild teleost fish: insights from exogenous supraphysiological cortisol implants, *Physiol. Biochem. Zool.* 82 (2009) 709–719.
- [46] A.J. Zolderdo, D.A. Algera, M.J. Lawrence, K.M. Gilmour, M.D. Fast, J. Thuswaldner, W.G. Willmore, S.J. Cooke, Stress, nutrition and parental care in a teleost fish: exploring mechanisms with supplemental feeding and cortisol manipulation, *J. Exp. Biol.* 219 (2016) 1237–1248.
- [47] D.P. Struthers, A.J. Danylchuk, A.D.M. Wilson, S.J. Cooke, Action cameras: bringing aquatic and fisheries research into view, *Fisheries* (2015), <http://dx.doi.org/10.1080/03632415.2015.1082472>.
- [48] D.D. Brown, R. Kays, M. Wikelski, R. Wilson, A.P. Klimley, Observing the unwatchable through acceleration logging of animal behavior, *Anim. Biotele. J.* (2013) 20.
- [49] S.J. Cooke, J.W. Brownscombe, G.D. Raby, F. Broell, S.G. Hinch, T.D. Clark, J.M. Semmens, Remote bioenergetics measurements in wild fish: opportunities and challenges, *Comp. Biochem. Physiol. A* 202 (2016) 23–37.
- [50] M.A. Gravel, S.J. Cooke, Does nest predation pressure influence the energetic cost of nest guarding in a teleost fish? *Environ. Biol. Fish.* 96 (2013) 93–107.
- [51] M.A. Gravel, P. Couture, S.J. Cooke, Brood predation pressure during parental care does not influence parental enzyme activities related to swimming activity in a teleost fish, *Comp. Biochem. Phys. A* 155 (2010) 110–116.
- [52] J.W. Brownscombe, L.F.G. Gutowsky, A.J. Danylchuk, S.J. Cooke, Foraging behaviour and activity of a marine benthivorous fish estimated using tri-axial accelerometer biologists, *Mar. Ecol. Prog. Ser.* 505 (2014) 541–551.
- [53] J.D. DiBattista, H. Anisman, M. Whitehead, K.M. Gilmour, The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*, *J. Exp. Biol.* 208 (2005) 2707–2718.
- [54] M.R. Kubacki, The Effects of a Closed Season for Protecting Nesting Largemouth and Smallmouth Bass in Southern Ontario, Master's thesis University of Illinois, Urbana-Champaign, 1992.
- [55] R.S. Brown, S.J. Cooke, W.G. Anderson, R.S. McKinley, Evidence to challenge the “2% Rule” for biotelemetry, *N. Am. J. Fish. Manag.* 19 (1999) 867–871.
- [56] K.Q. Sakamoto, K. Sato, M. Ishizuka, Y. Watanuki, A. Takahashi, F. Daunt, S. Wanless, Can ethograms be automatically generated using body acceleration data from free-ranging birds? *PLoS One* 4 (2009) e5379.
- [57] J.W. Brownscombe, J.D. Thiem, C. Hatry, F. Cull, C.R. Haak, A.J. Danylchuk, S.J. Cooke, Recovery bags reduce post-release impairments in locomotory activity and behaviour of bonefish (*Albula* spp.) following exposure to angling-related stressors, *J. Exp. Mar. Biol. Ecol.* 440 (2013) 207–215.
- [58] S.J. Cooke, C.M. Bunt, J.F. Schreer, D.H. Wahl, Comparison of several techniques for mobility and activity estimates of smallmouth bass in lentic environments, *J. Fish Biol.* 58 (2001) 573–587.
- [59] L. Breiman, J. Friedman, R. Olshen, C. Stone, *Classification and Regression Trees*, Wadsworth, Belmont, CA, 1984.
- [60] M.B. Morrissey, C.D. Suski, K.R. Esseltine, B.L. Tufts, Incidence and physiological consequences of decompression in smallmouth bass after live-release angling tournaments, *Trans. Am. Fish. Soc.* 134 (2005) 1038–1047.
- [61] C.M. O'Connor, C.Y. Yick, K.M. Gilmour, G. Van Der Kraak, S.J. Cooke, The glucocorticoid stress response is attenuated but unrelated to reproductive investment during parental care in a teleost fish, *Gen. Comp. Endocrinol.* 170 (2011) 215–221.
- [62] N. Pankhurst, The endocrinology of stress in fish: an environmental perspective, *Gen. Comp. Endocrinol.* 170 (2011) 265–275.
- [63] J.F. Schreer, S.J. Cooke, R.S. McKinley, Cardiac response to variable forced exercise at different temperatures: an angling simulation for smallmouth bass, *Trans. Am. Fish. Soc.* 130 (2001) 783–795.
- [64] A.R. Emery, Preliminary comparisons of day and night habits of freshwater fish in Ontario lakes, *J. Fish. Res. Board Can.* 30 (1973) 761–774.
- [65] M.S. Ridgway, The parental response to brood size manipulation in smallmouth bass (*Micropterus dolomieu*), *Ethology* 80 (1989) 47–54.
- [66] A.F. Zuur, E.N. Ieno, N. Walker, A.A. Saveliev, G.M. Smith, *Mixed Effects Models and Extensions in Ecology with R*, Springer, New York, NY, 2009.
- [67] D. Bates, M. Maechler, B. Bolker, S. Walker, lme4: Linear Mixed-effects Models Using Eigen and S4, R Package Version 1, (2014), pp. 1–7.
- [68] A. Gelman, S. Yu-Sung, Y. Masanao, J. Hill, M. Grazia Pattau, J. Kerman, T. Zheng, V. Dorie, Package “arm”: Data Analysis Using Regression and Multilevel/Hierarchical Models. R Package v 1.9-3, (2016).
- [69] G.B. Steinhart, B.D. Lunn, When and why do smallmouth bass abandon their broods? The effects of brood and parental characteristics, *Fish. Manag. Ecol.* 18 (2011) 1–11.
- [70] D.A. Algera, L.F.G. Gutowsky, A.J. Zolderdo, S.J. Cooke, Parental care in a stressful world: experimentally elevated cortisol and brood size manipulation influence nest success probability and nest-tending behavior in a wild teleost fish, *Physiol. Biochem. Zool.* 90 (2017) 85–95.
- [71] T.R. Gregory, C.M. Wood, The effects of chronic plasma elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout, *Physiol. Biochem. Zool.* 72 (1999) 286–295.
- [72] H.J. Liew, D. Chiarella, A. Pelle, C. Faggio, R. Blust, G. De Boeck, Cortisol emphasizes the metabolic strategies employed by common carp, *Cyprinus carpio* at different feeding and swimming regimes, *Comp. Biochem. Physiol. A* 166 (2013) 449–464.
- [73] J.D. Morgan, G.K. Iwama, Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout (*Oncorhynchus clarki clarki*) parr, *Fish Physiol. Biochem.* 15 (1996) 385–394.
- [74] B.A. Barton, C.B. Schreck, L.D. Barton, Effects of chronic cortisol administration and daily acute stress on growth, physiological conditions, and stress responses in juvenile rainbow trout, *Dis. Aquat. Org.* 2 (1987) 173–185.
- [75] A.C. Gleiss, R.P. Wilson, E.L.C. Shepard, Making overall dynamic body acceleration work: on the theory of acceleration as a proxy for energy expenditure, *Methods Ecol. Evol.* 2 (2011) 22–33.
- [76] L.G. Halsey, E.L.C. Shepard, F. Quintana, A.G. Laich, J.A. Green, R.P. Wilson, The relationship between oxygen consumption and body acceleration in a range of species, *Comp. Biochem. Phys. A* 152 (2009) 197–202.
- [77] S. Wright, J.D. Metcalfe, S. Hetherington, R. Wilson, Estimating activity-specific energy expenditure in a teleost fish, using accelerometer loggers, *Mar. Ecol. Prog. Ser.* 496 (2014) 19–32.
- [78] A. Nagrodski, K.J. Murchie, K.M. Stamplecoskie, C.D. Suski, S.J. Cooke, Effects of an experimental short-term cortisol challenge on the behaviour of wild creek chub *Semotilus atromaculatus* in mesocosm and stream environments, *J. Fish Biol.* 82 (2013) 1138–1158.
- [79] C.M. O'Connor, K.M. Gilmour, R. Arlinghaus, C.T. Hasler, D.P. Philipp, S.J. Cooke, Seasonal carryover effects following the administration of cortisol to a wild teleost fish, *Physiol. Biochem. Zool.* 83 (2010) 950–957.
- [80] R.C. Sargent, M.R. Gross, Williams' principle: an explanation of parental care in

- teleost fishes, in: T.J. Pitcher (Ed.), Behaviour of Teleost Fishes, Croom Helm, London, 1986, pp. 275–293.
- [81] R.M. Coleman, M.R. Gross, R.C. Sargent, Parental investment decision rules: a test in bluegill sunfish, Behav. Ecol. Sociobiol. 18 (1985) 59–66.
- [82] J.D. Jeffrey, S.J. Cooke, K.M. Gilmour, Regulation of hypothalamic-pituitary-interrenal axis function in male smallmouth bass (*Micropterus dolomieu*) during parental care, Gen. Comp. Endocrinol. 204 (2014) 195–202.
- [83] A. Keast, Trophic and spatial interrelationships in the fish species of an Ontario temperate lake, Environ. Biol. Fish 3 (1978) 7–31.
- [84] J.F. Gillooly, J.H. Brown, G.B. West, V.M. Savage, E.L. Charnov, Effects of size and temperature on metabolic rates, Science 293 (2001) 2248–2251.
- [85] A. Keast, J. Harker, D. Turnbull, Nearshore fish habitat utilization of fish species associations in Lake Opinicon (Ontario, Canada), Environ. Biol. Fish 3 (1978) 173–184.
- [86] G. De Boeck, D. Aslop, C. Wood, Cortisol effects on aerobic and anaerobic metabolism, nitrogen excretion, and whole-body composition in juvenile rainbow trout, Physiol. Biochem. Zool. 74 (2001) 858–868.
- [87] S.H. McConnachie, C.M. O'Connor, K.M. Gilmour, G.K. Iwama, S.J. Cooke, Supraphysiological cortisol elevation alters the response of wild bluegill sunfish to subsequent stressors, J. Exp. Zool. A Ecol. Genet. Physiol. 317 (2012) 321–332.