

# Size-Dependent Consequences of Exogenous Cortisol Manipulation on Overwinter Survival and Condition of Largemouth Bass



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## ABSTRACT

Little is known about the size-dependent consequences of stressors on wild animals, which is particularly relevant during winter where size-specific trends in survival are common. Here, exogenous cortisol manipulation was used to investigate the effect of a physiological challenge on overwinter mortality and spring condition of largemouth bass (*Micropterus salmoides*) across a range of body sizes. Fish were wild-caught in the fall, assigned into either control or cortisol manipulated treatments, and held in replicated experimental ponds. For bass that survived the winter, length, mass, and health metrics (e.g., gonadosomatic index [GSI], hepatosomatic index [HSI], and water content) were determined in the spring. Winter survival was marginally lower for cortisol treated bass; however, there was no influence of initial length, mass, or condition on overwinter survival. When bass were grouped by size, survival was significantly higher for bass 300–350 mm in length compared to those <200 mm. The treatment did not strongly influence spring health metrics, suggesting that largemouth bass that survived the winter were able to recover from the effects of the cortisol elevation. Initial size and sex were linked to some spring health metrics, with large females having the highest GSI and HSI scores. Overall, results from this study do not support the notion that there are size-dependent responses to cortisol manipulation

Conflict of interest: None.

Grant sponsor: Natural Sciences and Engineering Research Council Discovery Grant; Grant sponsor: Canada Research Chairs Program; Grant sponsor: Illinois Natural History Survey.

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Received 1 November 2016; Revised 17 December 2016; Accepted 23 January 2017

DOI: 10.1002/jez.2064

Published online 23 February 2017 in Wiley Online Library (wileyonlinelibrary.com).

in a teleost fish. Rather, this type of physiological challenge may modulate the natural rates of winter mortality that are primarily driven by starvation and predation, independent of body size, in subadult and adult largemouth bass. *J. Exp. Zool.* 327A:5–17, 2017. © 2017 Wiley Periodicals, Inc.

*J. Exp. Zool.*  
327A:5–17,  
2017

How to cite this article: Midwood JD, Peiman KS, Burt AE, Sarker MY, Nannini MA, Wahl DH, Cooke SJ. 2017. Size-Dependent consequences of exogenous cortisol manipulation on overwinter survival and condition of largemouth bass. *J. Exp. Zool.* 327A:5–17.

## INTRODUCTION

All fish exhibit physiological responses (herein termed the stress response) when exposed to different types of environmental disturbances or negative stimuli (e.g., predation attempts, competition, drought, temperature change; Barton and Iwama, '91; Wendelaar Bonga, '97). These short-term stress responses are modulated by the hypothalamic–pituitary–interrenal (HPI) axis. Elevation of circulating cortisol (the primary response) is associated with a suite of physiological and behavioral changes (secondary and tertiary responses; Mazeaud et al., '77). While these physiological changes provide an immediate boost of energy that increases an individual's chances of surviving the abiotic or biotic stressor (Iwama, '98; Barton, 2002), when the stress axis is elevated for an extended period of time (i.e., chronic stress), this response can become harmful and maladaptive leading to decreases in somatic tissue growth, immune suppression, and reduced feeding (Gregory and Wood, '99; Edeline et al., 2009). Activation of the HPI axis can also have consequences long after the stress has been alleviated when an individual is exposed to a new, temporally separated stressful situation (e.g., McConnachie et al., 2012). This type of response, known as a carryover effect (Harrison et al., 2010; O'Connor et al., 2014), can influence subsequent growth and survival during important life-history transitions such as migration (e.g., Midwood et al., 2014; Schultner et al., 2015) or across seasons (O'Connor et al., 2010). Understanding inter- and intraspecific differences in physiological responses to stressors is critical for understanding the ability of individuals to survive and species to adapt to changing environments (Angelier and Wingfield, 2013).

For temperate fishes, winter is associated with reduced activity, lipid depletion, and in some situations increased mortality (Cunjak, '96; Hurst, 2007; Shuter et al., 2012). For centrarchids, due to reduced feeding activity in the winter, adequate prewinter energy stores are essential to meet energy demands over the winter (Garvey et al., 2004; Ostrand et al., 2005; Suski and Ridgway, 2009). Winter, therefore, represents an environmental challenge that has the potential to select against individuals that are in suboptimal condition as a result of a previous stressor. Indeed, O'Connor et al. (2010) observed earlier mortality in largemouth bass that had exogenous cortisol manipulations relative to control fish when exposed to under-ice hypoxia, demonstrating that winter can act to trigger a carryover effect. Largemouth bass

(*Micropterus salmoides*) included in that study were all of similar sizes (i.e., mid-sized adults) to limit variability among individuals; however, it is possible that size may interact with a stressor to ultimately determine an individual's fate. Therefore, while the existence of carryover effects is well established (Harrison et al., 2010; O'Connor et al., 2014), the extent to which such effects are manifested within a population is less well understood (Angelier and Wingfield, 2013), especially when linked to individual size.

Size-selective overwinter mortality is well documented for juvenile fishes, with the highest rates of mortality typically in the smallest size classes (reviewed in Sogard, '97). This is consistent in largemouth bass and thought to be linked to increased rates of lipid metabolism combined with smaller initial energy stores in smaller individuals (Miranda and Hubbard, '94; Garvey et al., 2004; Ostrand et al., 2005). In addition, depending on the availability of forage opportunities for smaller individuals, predation can also contribute to increased mortality for this size class if they are forced to forage more actively (Garvey et al., '98; Post et al., '98). Size-selective mortality in juveniles has important consequences for recruitment (DeVries et al., 2009); however, this same type of selection in larger subadult and adult largemouth bass may also influence recruitment (assuming a stock-recruit relationship; Ricker, '54), or at least the availability of fish for exploitation by anglers.

The potential exists for size-selective modulation of a stressor. Therefore the goal of this study was to determine whether there was an influence of body size on overwinter mortality and spring condition of largemouth bass following a fall physiological challenge. We used exogenous cortisol manipulation via intracoelomic injection, a method commonly used in fish, to elevate cortisol and mimic that aspect of the stress response recognizing that a "natural" stress response includes sensing a stressor and the initiation of a neuroendocrine cascade (Gamperl et al., '94; Crossin et al., 2016). Exogenous cortisol manipulations are still relatively rare among wild animals (Sopinka et al., 2015), but there is a rich laboratory-based literature focused on captive animals (often reared in captivity), which provides a basis for helping to interpret often complicated field-based observations (Crossin et al., 2016). Exogenous cortisol manipulations in largemouth bass result in cortisol titers that are elevated to supra-physiological levels for up to 6 days, and then stay around acute

response levels for over 2 weeks (O'Connor et al., 2009; Dey et al., 2010), thus representing an extreme semichronic physiological challenge. The health metrics assessed were gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K), and water content. GSI is the ratio of gonad weight to body weight and decreases with decreasing reproductive condition (Schreck and Moyle, '90). HSI is the ratio of liver weight to body weight and provides an indication of energy status. In poor environments, liver size decreases (less energy reserves) leading to smaller HSI values (O'Connor et al., 2013). Water content, however, is inversely proportional to lipid content, that is, maximum energy contents coincide with minimal water content values (Brett and Groves, '79; Jobling, '94; Barton et al., 2002). Based on the known relationships between these health metrics and fish condition, we predict that stressed individuals will have impaired health metrics (lower GSI, HSI, condition factor, and higher percent water), and a lower survival rate than controls. We also predict that smaller individuals will have a lower survival rate than larger individuals, and therefore, that smaller stressed individuals will exhibit the most impaired health and survival (e.g., a negative interaction between size and health metrics). The experimental approach used here serves as a test of the effect of body size on overwinter condition and survival of fish with temporarily elevated cortisol titers.

## MATERIALS AND METHODS

### Capture and Treatment

All experiments were conducted in accordance with the Canadian Council on Animal Care guidelines for use of fish in research (protocol number 315774-166). In late October and early November 2012, 180 largemouth bass were caught by electrofishing from lakes in central Illinois, and transferred to experimental ponds at the Sam Parr Biological Station (Kinmundy, IL). Cortisol levels in bass return to baseline within 1 day when hauled short distances (Carmichael et al., '84a). On November 14 and 15, 2012, the ponds were drained and all fish were moved to a holding tank. The timing of experimentation was intended to correspond with the period at which largemouth bass are known to become quiescent and exhibit reduced feeding and growth at this latitude (Adams et al., '82; reviewed in Suski and Ridgway, 2009). Fish were individually netted, wet mass was determined ( $\pm 1$  g), and they were placed with their ventral side exposed in a water-filled trough. Total length was measured ( $\pm 1$  mm) and a 12 mm Biomark FDX (Boise, ID) passive integrated transponder (PIT) tag was injected into the coelomic cavity. Anesthetic was not used given that the fish were fairly quiescent at the relatively low water temperatures combined with the speed ( $< 20$  sec) at which the procedure was completed. Cortisol levels in largemouth bass return to baseline within 12 hr of a stressful net confinement (Carmichael et al., '84b).

Fish were assigned to one of two treatment groups, control and cortisol-treated, in a quasi-random approach that attempted to ensure there was a similar distribution of sizes in each group. A  $5 \text{ mL kg}^{-1}$  dose of  $10 \text{ mg mL}^{-1}$  corticosterone suspended in liquid cocoa butter was injected into the coelomic cavity of treated fish. Control fish were handled in the same fashion but did not receive a cocoa butter injection. This method of cortisol elevation has been validated for use with largemouth bass (O'Connor et al., 2009). The dosage we used increases circulating cortisol to supraphysiological levels (approximately  $10\times$  baseline levels) for 6 days posttreatment, and from 9 to 19 days posttreatment results in circulating cortisol levels remaining in the acute-response range (O'Connor et al., 2009; Dey et al., 2010). Thus, this treatment has both an extreme and a long-term effect, which should exceed all other stressors (such as transport and handling) that the fish experienced. Sham treatments of only cocoa butter also elevate circulating cortisol (O'Connor et al., 2009). Thus, a sham treatment was not used as it represents an intermediate effect between cortisol-treated fish and controls and does not adequately test for the effects of cocoa butter alone (see also DiBattista et al., 2005).

After treatment, fish were released into drainable 0.4 ha research ponds ( $n = 60$  per pond), which were  $\sim 1.5$  m deep and had sparse aquatic vegetation. An attempt was made to keep a balanced distribution of size classes and experimental treatments in each pond. For the first week, ponds were checked daily for handling casualties, then fish were left over winter to forage naturally on an assortment of benthic invertebrates, crayfish, terrestrial vertebrates (mostly frogs), and insects. No other care was provided and fish were not observed again until the ponds were drained in Spring 2013. Overall weather conditions (mean temperature =  $2.03 \pm 5.96^\circ\text{C}$ ) during the study were similar to those recorded between 2005 and 2015 at the Sam Parr Biological Station, with slightly above average temperatures in December and January, slightly below average temperatures in March and overall less precipitation (M. Nannini, pers. obs.). Overwintering conditions in the present study therefore reflect the normal conditions largemouth bass are likely to experience in central Illinois.

### Recapture

Starting on March 12, 2013, one pond per day was drained and fish were moved to a raceway prior to processing. As before, fish were netted, wet mass was determined, and they were placed in a trough with the ventral side exposed. Total length was measured, and then fish were euthanized by cephalic blow and scanned using a PIT tag reader.

Fish were then dissected; the mass of the gonads, liver, and the eviscerated carcass was determined, and the sex was recorded. A sample of white muscle from the dorsal musculature of each fish was foil-wrapped and flash-frozen in liquid nitrogen and then transferred to an  $-80^\circ\text{C}$  freezer. In the laboratory, two replicates

**Table 1.** Size ranges for classes of largemouth bass and the number of individuals in each size class by treatment

Size class	Size range (mm)	Number in cortisol treatment	Number in control group
1	145–200	14	13
2	200–250	19	19
3	250–300	29	33
4	300–350	14	17
5	350–430	13	7

of each wet sample  $0.97 \pm 0.02$  g (mean  $\pm$  SE) were analyzed separately for water content (a surrogate for lipid content: Gravel et al., 2010). First, the mass of each replicate was determined, and then they were placed in a drying oven at 80°C for approximately 16–20 hr (or until the mass stabilized). After drying, the mass of the replicate was determined and water content was calculated as the difference in the mass of the replicate before and after drying, expressed as a percentage. GSI and HSI were calculated as tissue mass divided by eviscerated body mass (Schreck and Moyle, '90). Finally, Fulton's condition ( $K$ ) was calculated using the formula  $K = ((\text{mass}) \times (\text{length})^{-3}) \times 100$ .

#### Statistical Analysis

Prior to analysis, all continuous metrics were scaled by their standard deviations and centered by their means. In order to determine the influence of prewinter size or condition and treatment on overwinter survival of an individual bass, four mixed effect logistic regressions were run using the *lme4* package (Bates et al., 2015) in RStudio (RStudio: Integrated Development for R. RStudio, Inc., Boston, MA) with survival as the binary response (1 = survived, 0 = deceased) and pond included as a random effect. The first considered the influence of treatment (control or cortisol-treated), initial length, and their interaction. The second included treatment, initial mass, and their interaction. The third included treatment, initial  $K$ , and their interaction. The final model included treatment, size class (Table 1), and their interactions. Sex was not included as a dependent variable as fish were not sexed prior to overwintering. For the final model that included size class, a post hoc Tukey's HSD analysis was conducted to determine the source of the significant differences.

A series of mixed models (SAS v9.3; proc Mixed) were run for individuals that survived the winter to determine the influence of the treatment (control or cortisol-treated), sex (male or female), and initial size metric (separately analyzed as initial length, mass, and  $K$ ), and interaction terms on three health metrics: GSI, HSI, and water content as well as final length, mass,  $K$ , and the percent change in mass. Pond was included in the model as a random effect. Data were transformed when appropriate to meet assumptions of normality.

**Table 2.** Means  $\pm$  standard error of the means for spring size and health metrics measured in female and male cortisol-treated (treatment) and control largemouth bass

Physiological parameter	Sex	Treatment	Control
Sample size	Female	34	27
	Male	23	41
Spring mass (g)	Female	261.7 $\pm$ 30.2	324.5 $\pm$ 40.3
	Male	326.1 $\pm$ 41.7	269.3 $\pm$ 30.8
Spring length (mm)	Female	262.9 $\pm$ 11.1	281.4 $\pm$ 9.3
	Male	281.8 $\pm$ 10.2	268.6 $\pm$ 9.2
Spring condition	Female	1.26 $\pm$ 0.02	1.25 $\pm$ 0.02
	Male	1.26 $\pm$ 0.03	1.26 $\pm$ 0.02
Mass change (%)	Female	-4.7 $\pm$ 3.8	-6.0 $\pm$ 2.4
	Male	-12.0 $\pm$ 2.9	-9.7 $\pm$ 2.6
Gonadosomatic index	Female	2.5 $\pm$ 0.30	2.3 $\pm$ 0.30
	Male	0.3 $\pm$ 0.02	0.3 $\pm$ 0.02
Hepatosomatic index	Female	1.4 $\pm$ 0.07	1.5 $\pm$ 0.07
	Male	1.2 $\pm$ 0.06	1.1 $\pm$ 0.04
Water content (%)	Female	80.5 $\pm$ 2.2	80.3 $\pm$ 0.1
	Male	80.2 $\pm$ 0.2	80.3 $\pm$ 0.2

The mean of all nonscaled metrics were taken and tabulated for ease of comparison between sex and treatment (Table 2). All values are presented as mean  $\pm$  standard error of the mean. Analyses were performed using RStudio v0.98.945 (RStudio, Boston, MA) and SAS (v9.3). For all analyses  $\alpha = 0.05$ , but alpha between 0.05 and 0.1 are discussed as marginally significant.

## RESULTS

### Survival

Survival was evaluated for all 180 individuals; however, two individuals (one from each treatment) were excluded from the evaluation of health metrics (below) because final mass or length was recorded incorrectly. Overall, 69% of the largemouth bass survived the winter, and there were more survivors in the control group (75%) than the cortisol treatment (63%). Overwinter survival was marginally affected by treatment, but was not influenced by any size metric (length, mass, or condition) or by any interaction between size and treatment (Table 3). However, when fish were grouped into size classes based on initial lengths, overwinter survival was no longer affected by treatment, but was affected by size class: there was significantly lower survival in size class 1 (smallest fish) relative to size class 3 (medium-sized fish; Tukey's HSD,  $P = 0.039$ ,  $Z = 2.817$ ; Fig. 1), and marginally significantly greater survival in size class 2 and 4 relative to size class 1.

**Table 3.** Output from mixed-effect multiple logistic regressions of overwinter survival of largemouth bass. The table shows the degrees of freedom (DF), the model terms, model estimate, z-, and P-value

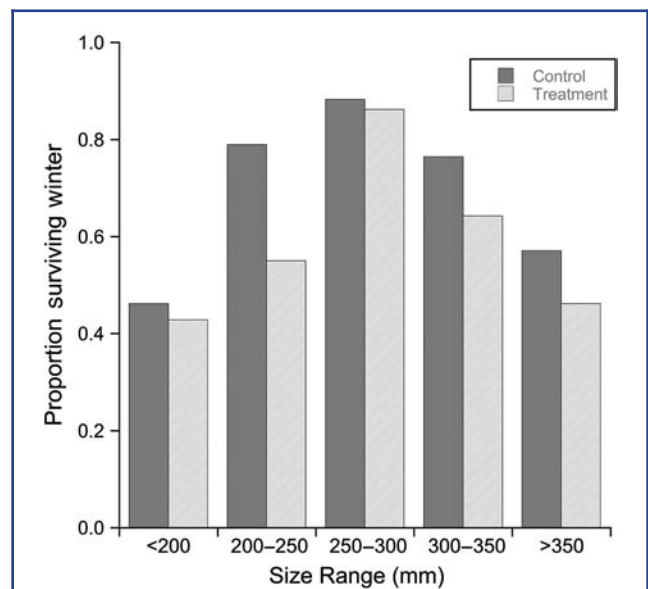
Model DF	Model term	Estimate	z-Value	P-value
3	<i>Treatment</i>	-0.63	-1.86	0.063
	Initial length	0.44	1.58	0.115
	Treatment × initial length	-0.28	-0.81	0.417
3	<i>Treatment</i>	-0.60	-1.81	0.070
	Initial mass	0.24	0.83	0.406
	Treatment × initial mass	-0.23	-0.64	0.524
3	<i>Treatment</i>	-0.59	1.76	0.079
	Initial condition	0.28	0.26	0.285
	Treatment × initial condition	-0.02	0.34	0.953
9	<i>Treatment</i>	-0.13	-0.17	0.863
	Size class 2	1.48	1.87	0.062
	Size class 3	2.17	2.82	0.005
	Size class 4	1.33	1.67	0.095
	Size class 5	0.44	0.47	0.640
	Treatment × size class 2	-0.99	-0.93	0.351
	Treatment × size class 3	-0.05	-0.05	0.964
	Treatment × size class 4	-0.46	-0.41	0.681
	Treatment × size class 5	-0.31	-0.25	0.801

Significant model values ( $\alpha = 0.05$ ) are bolded and values that can be considered marginally significant ( $\alpha = 0.05-0.10$ ) are italicized.

**Condition Metrics**

The initial size metrics were all significant predictors of final size metrics (Table 4). Initial length and mass were also strong predictors of the percent mass change; however, initial condition was not (Table 4). The effect of treatment on final mass/length interacted with sex and initial size metrics (Table 4; Fig. 2). For males, the relationship between initial size metric and final mass was stronger for control than cortisol fish, but for females was stronger for cortisol than control fish (Fig. 2). Initial mass had the opposite effect on final length: it was stronger in cortisol than control males, but was stronger for control than cortisol females (Fig. 2). Cortisol-treated males experienced the largest loss of mass over the winter (treatment × sex interaction, Tables 4; see means in Table 2). Changes in mass in males were not related to size metric, while larger females experienced larger changes in mass (size metric × sex interaction Table 4; Fig. 3).

The relationship between initial mass and GSI was stronger for females in the control treatment than the cortisol treatment while there was little relationship in males (sex × initial mass × treatment Table 4; Fig. 4). The relationship between initial condition and HSI was positive and stronger for females in the cortisol treatment than the control while males showed little relationship (sex × initial condition × treatment Table 4; Fig. 4). Females in general had a stronger relationship between initial size metrics and both GSI (Fig. 3) and HSI (Fig. 5) than males. Water content showed a more variable response: females showed no



**Figure 1.** Probability of survival based on size class as defined in Table 1 based on total length. Survival was significantly higher for size class 3 (250–300 mm) compared to size class 1 (<200 mm) when data from the two treatments were pooled. There was no interaction between size class and treatment in terms of predicting survival.

Table 4. Output from mixed models of largemouth bass size metrics by initial length, mass, and condition

Metric	Model term	Initial length		Initial mass		Initial condition	
		F-value	P-value	F-value	P-value	F-value	P-value
Final length	Treatment	0.43	0.581	0.10	0.777	0.00	0.958
	Sex	0.11	0.770	0.65	0.504	0.92	0.341
	Treatment × sex	0.06	0.828	0.14	0.746	3.01	0.086
	Size metric	<b>20,035.10</b>	<b>&lt;0.0001</b>	<b>647.16</b>	<b>&lt;0.0001</b>	<b>26.88</b>	<b>&lt;0.0001</b>
	Size metric × treatment	1.62	0.205	1.96	0.164	0.40	0.530
	Size metric × sex	0.42	0.520	0.00	0.980	0.04	0.851
	Size metric × treatment × sex	0.61	0.543	<b>10.50</b>	<b>&lt;0.0001</b>	<b>1.01</b>	<b>0.368</b>
Final condition	Treatment	0.05	0.818	0.23	0.633	0.06	0.823
	Sex	0.04	0.862	0.05	0.845	1.14	0.397
	Treatment × sex	0.55	0.458	1.20	0.275	1.14	0.398
	Size metric	<b>45.92</b>	<b>&lt;0.0001</b>	<b>69.63</b>	<b>&lt;0.0001</b>	<b>278.80</b>	<b>&lt;0.0001</b>
	Size metric × treatment	0.30	0.583	1.94	0.166	0.41	0.525
	Size metric × sex	1.15	0.286	<b>3.06</b>	<b>0.083</b>	0.20	0.656
	Size metric × treatment × sex	1.66	0.201	0.19	0.660	0.09	0.762
Final mass	Treatment	0.02	0.892	0.15	0.695	0.04	0.837
	Sex	0.15	0.734	2.65	0.106	0.66	0.418
	Treatment × sex	0.33	0.565	0.01	0.943	3.76	0.055
	Size metric	<b>996.66</b>	<b>&lt;0.0001</b>	<b>8,179.43</b>	<b>&lt;0.0001</b>	<b>48.66</b>	<b>&lt;0.0001</b>
	Size metric × treatment	2.14	0.147	0.21	0.646	0.13	0.715
	Size metric × sex	2.17	0.144	7.53	0.007	0.01	0.931
	Size metric × treatment × sex	<b>17.96</b>	<b>&lt;0.0001</b>	<b>10.54</b>	<b>0.002</b>	<b>6.24</b>	<b>0.014</b>
Percent mass change	Treatment	0.14	0.711	0.36	0.611	0.01	0.932
	Sex	0.42	0.583	0.89	0.446	0.63	0.510
	Treatment × sex	<b>5.32</b>	<b>0.023</b>	<b>4.86</b>	<b>0.030</b>	<b>6.58</b>	<b>0.012</b>
	Size metric	<b>22.36</b>	<b>&lt;0.0001</b>	<b>10.14</b>	<b>0.002</b>	<b>0.49</b>	<b>0.486</b>
	Size metric × treatment	1.07	0.303	1.34	0.250	0.02	0.899
	Size metric × sex	<b>9.27</b>	<b>0.003</b>	<b>9.69</b>	<b>0.002</b>	<b>4.21</b>	<b>0.043</b>
	Size metric × treatment × sex	0.20	0.659	0.32	0.571	0.71	0.402
GSI	Treatment	0.01	0.930	0.00	0.971	1.07	0.303
	Sex	<b>193.72</b>	<b>0.005</b>	<b>208.37</b>	<b>&lt;0.0001</b>	<b>130.11</b>	<b>&lt;0.0001</b>
	Treatment × sex	0.00	0.977	0.02	0.893	1.19	0.277
	Size metric	<b>91.10</b>	<b>&lt;0.0001</b>	<b>60.04</b>	<b>&lt;0.0001</b>	<b>18.70</b>	<b>&lt;0.0001</b>
	Size metric × treatment	0.38	0.538	1.66	0.201	2.17	0.143
	Size metric × sex	<b>72.60</b>	<b>&lt;0.0001</b>	<b>47.43</b>	<b>&lt;0.0001</b>	<b>15.82</b>	<b>0.0001</b>
	Size metric × treatment × sex	0.69	0.408	2.83	0.096	2.07	0.153
HSI	Treatment	0.00	0.980	0.01	0.924	0.23	0.681
	Sex	<b>35.86</b>	<b>0.027</b>	<b>26.18</b>	<b>&lt;0.0001</b>	<b>25.29</b>	<b>0.037</b>
	Treatment × sex	2.97	0.227	0.32	0.571	0.16	0.690
	Size metric	1.14	0.289	0.88	0.351	<b>8.25</b>	<b>0.005</b>
	Size metric × treatment	1.00	0.320	0.01	0.917	1.06	0.306
	Size metric × sex	<b>21.94</b>	<b>&lt;0.0001</b>	<b>15.92</b>	<b>0.0001</b>	<b>16.43</b>	<b>&lt;0.0001</b>
	Size metric × treatment × sex	2.65	0.106	2.54	0.114	3.29	0.072
Water content	Treatment	0.00	0.964	0.15	0.704	0.00	0.975
	Sex	0.33	0.625	0.66	0.418	0.00	0.947
	Treatment × sex	0.10	0.754	0.14	0.710	0.32	0.573
	Size metric	0.02	0.890	0.45	0.505	<b>9.53</b>	<b>0.003</b>



Table 4. Continued

Metric	Model term	Initial length		Initial mass		Initial condition	
		F-value	P-value	F-value	P-value	F-value	P-value
	Size metric × treatment	<b>4.20</b>	<b>0.043</b>	<i>3.86</i>	<i>0.052</i>	0.18	0.673
	Size metric × sex	2.15	0.146	<b>4.03</b>	<b>0.047</b>	3.53	<i>0.063</i>
	Size metric × treatment × sex	1.66	0.200	1.60	0.209	1.11	0.294

The table shows the response metric, the model terms, F-, and P-value. Significant model values ( $\alpha = 0.05$ ) are bolded and values that can be considered marginally significant ( $\alpha = 0.05-0.10$ ) are italicized.

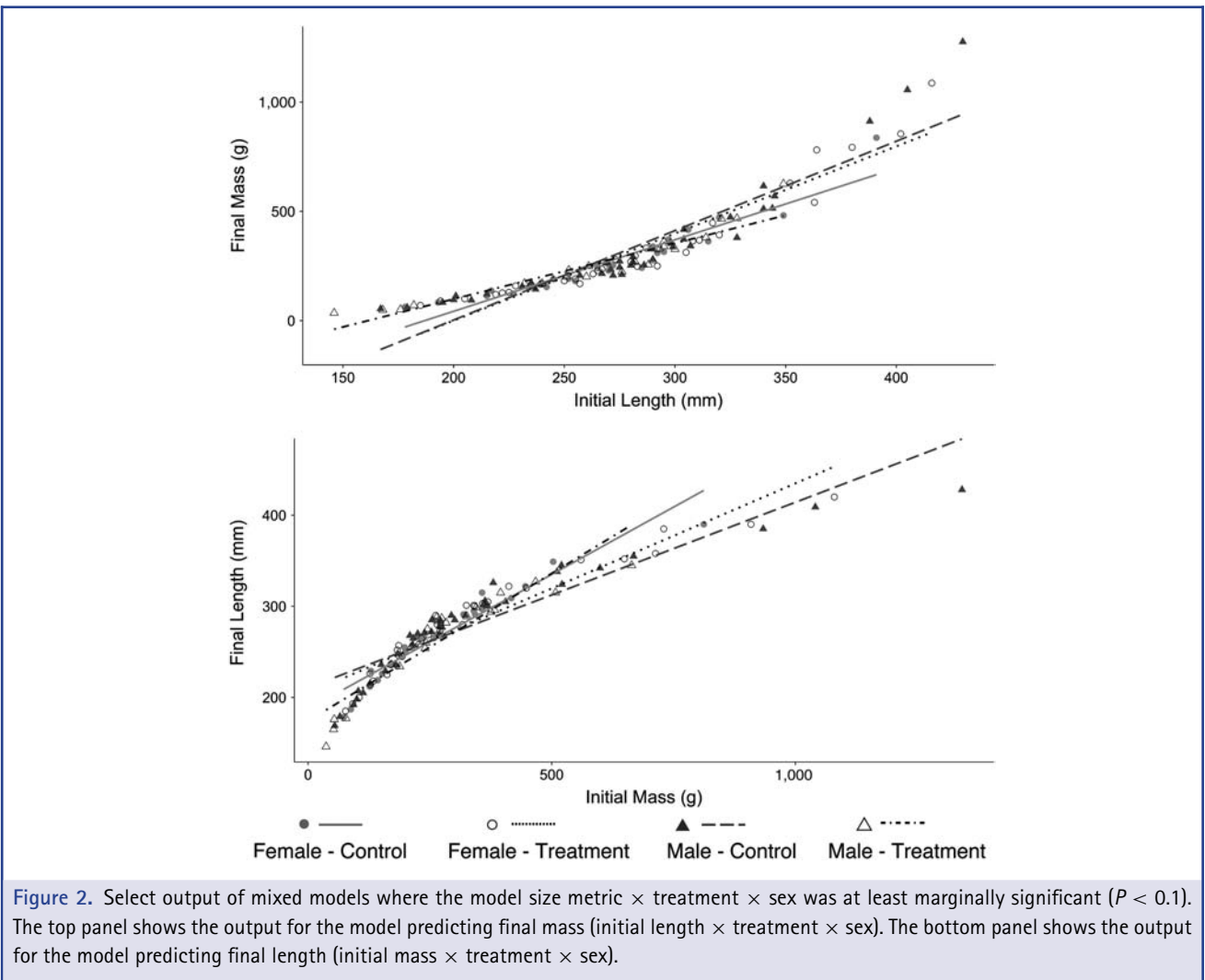
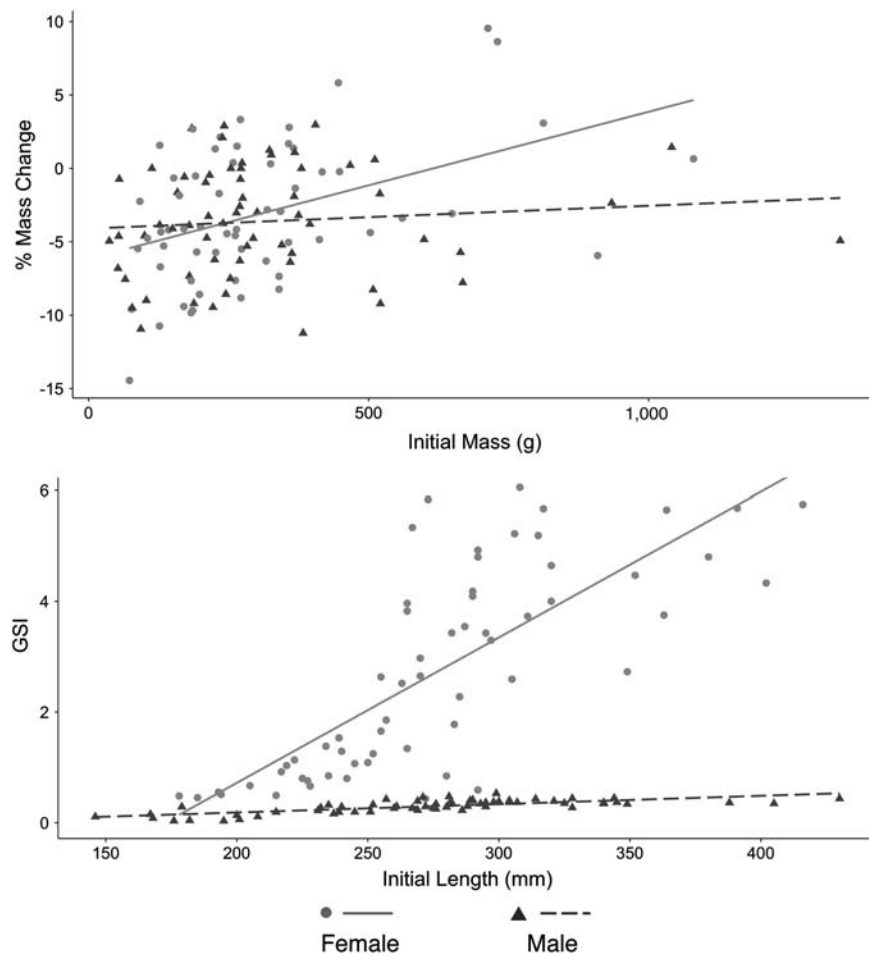


Figure 2. Select output of mixed models where the model size metric × treatment × sex was at least marginally significant ( $P < 0.1$ ). The top panel shows the output for the model predicting final mass (initial length × treatment × sex). The bottom panel shows the output for the model predicting final length (initial mass × treatment × sex).

relationship between initial mass/condition and water content and males exhibited a negative relationship (initial mass/condition × sex Table 4; Fig. 5). Finally, water content declined in larger individuals in the cortisol treatment relative to the control (initial length/mass × treatment Table 4; Fig. 6).

DISCUSSION

Size selective overwinter mortality is a common phenomenon in age-0 largemouth bass (Miranda and Hubbard, '94); however, the present study found only limited evidence to support size-selective mortality in larger subadult and adult largemouth bass.



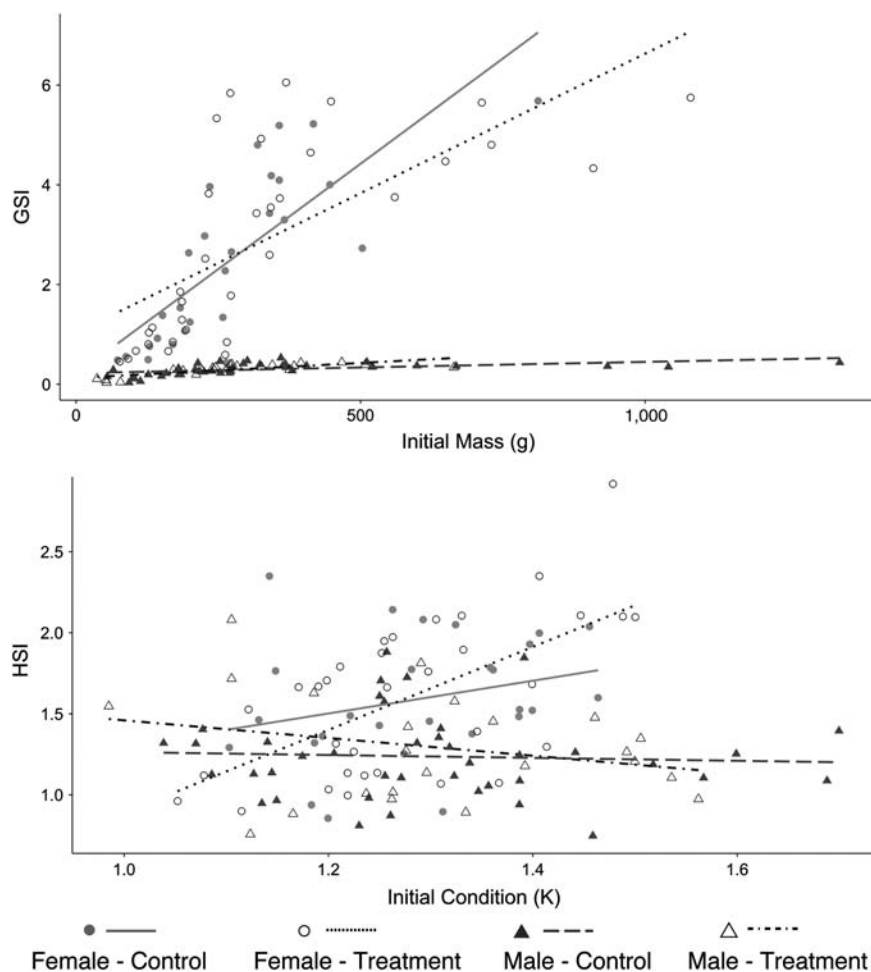
**Figure 3.** Select output of mixed models where the model size metric  $\times$  sex was marginally significant ( $P < 0.1$ ). The top panel shows the output for the model predicting percent mass change (initial mass  $\times$  sex). The bottom panel shows the output for the model predicting GSI (initial length  $\times$  sex).

Regardless of treatment, over 30% of all adult largemouth bass tagged and released during this study did not survive the winter. Previous studies of overwinter survival focused on age 0 (<150 mm) individuals, and so it is unclear whether the degree of mortality we observed for larger individuals is normal. Relative to the control group, the cortisol manipulation treatment reduced survival by approximately 12% suggesting that there was indeed a carryover effect of this physiological challenge. However, there was no evidence that this treatment had a variable impact on different-sized largemouth bass. In contrast, size alone was found to play a role in overwinter survival when largemouth bass were grouped into size categories, with survival of the smallest individuals almost half that of the medium-sized individuals. The observed range of survival among classes suggests that medium-sized individuals (250–300 mm in length)

have the highest probability of surviving the winter and this probability decreases for larger and smaller largemouth bass. Further examination of this relationship is warranted as there are potentially important implications from a management perspective such that unusually cold winters, which are known to affect survival of largemouth bass in general (O'Connor et al., 2010), may impact recruitment of future generations via both increased mortality of smaller bass (representing future recruitment potential) as well as larger bass that currently have higher reproductive potential.

In general, overwinter survival of bass is largely determined by two factors: starvation and predation (Garvey et al., 2004). The lower survival rate of the smallest size class of largemouth bass is potentially linked to both their predation by larger individuals as well as starvation due to smaller lipid reserves and



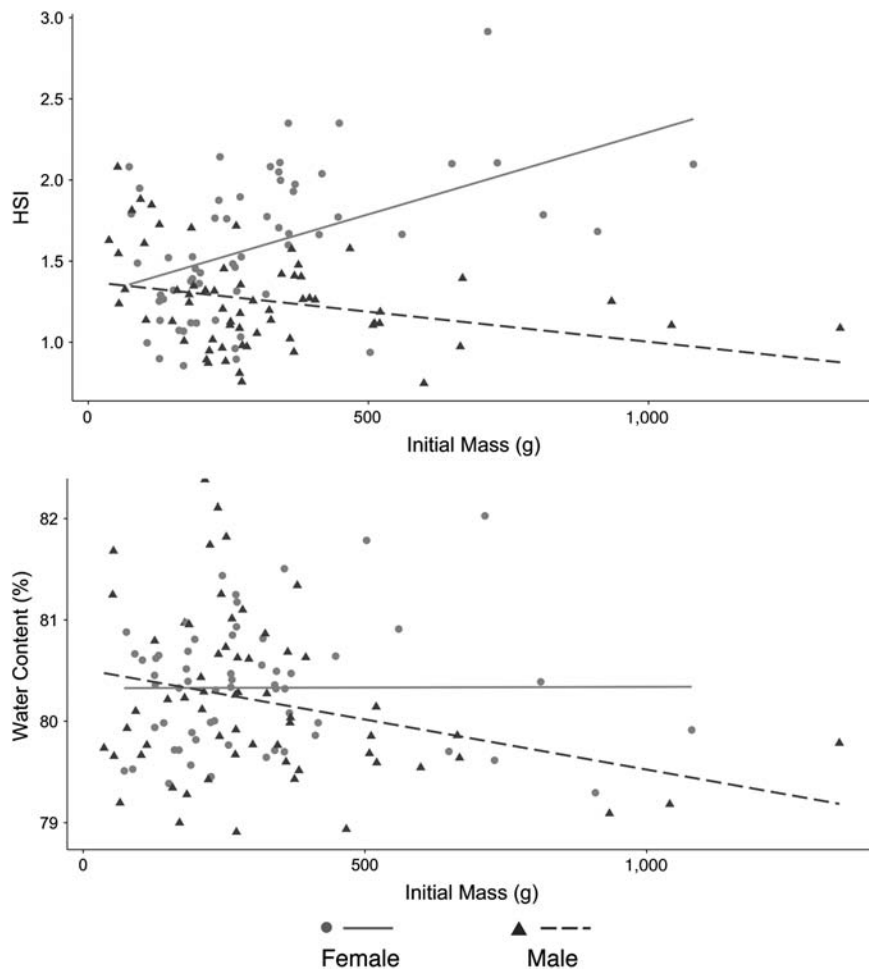


**Figure 4.** Select output of mixed models where the model size metric  $\times$  treatment  $\times$  sex was at least marginally significant ( $P < 0.1$ ). The top panel shows the output for the model predicting GSI (initial mass  $\times$  treatment  $\times$  sex). The bottom panel shows the output for the model predicting HSI (initial condition  $\times$  treatment  $\times$  sex).

proportionately higher metabolic rates (reviewed in Suski and Ridgway, 2009). Although the interaction between treatment and size class was not found to affect survival, survival of the cortisol treated largemouth bass was uniformly lower than the control (Fig. 1). By far the largest difference occurred in size class 2 with 30% lower survival for the treated group relative to the control; however, this difference was not found to be statistically significant. Largemouth bass greater than 200 mm are less likely to experience predation by conspecifics, but are still susceptible to winter mortality resulting from starvation. Artificial elevation of circulating cortisol levels could be acting to further reduce prewinter energy reserves in this size range, resulting in the observed lower winter survival. The exogenous cortisol manipulation occurred in mid-November during a time when fish would be quiescent and exhibit reduced food intake and growth

(e.g., Adams et al., '82; Suski and Ridgway, 2009) such that there would presumably be limited opportunities for experimental fish to attempt to replace any lost endogenous resources. That said, compensatory growth is known to occur in largemouth bass (e.g., Cline et al., 2012) and there is little detailed research on the overwinter feeding biology of largemouth bass in the wild (or in nature-like experimental ponds). Clearly there is a need for more research that involves examining detailed aspects of feeding after cortisol manipulation in the wild.

Larger female fish had the highest values for GSI, which is consistent with the notion that larger female fish have the greatest reproductive potential and that gonad investment is also greater in female fish relative to male fish (Brown and Murphy, 2004). Similarly, larger female largemouth bass also had the highest HSI values, an indicator of higher energy reserves.

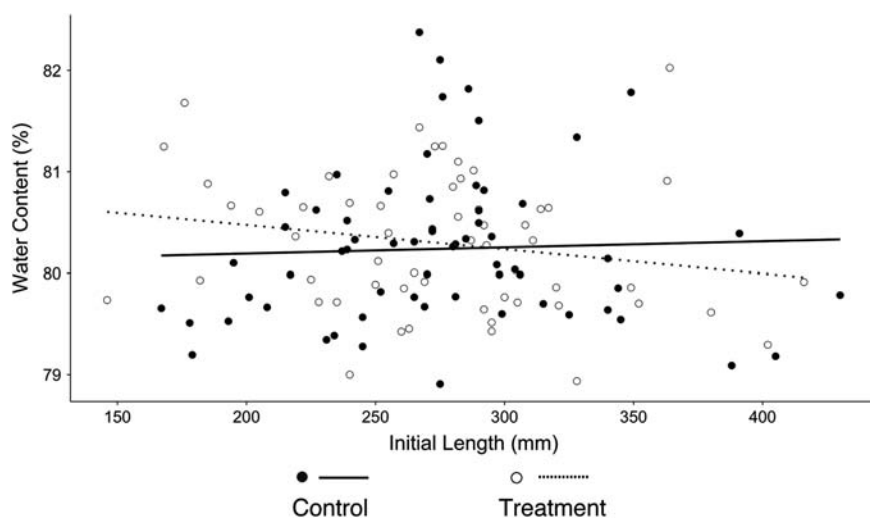


**Figure 5.** Select output of mixed models where the model size metric  $\times$  sex was marginally significant ( $P < 0.1$ ). The top panel shows the output for the model predicting HSI (initial mass  $\times$  sex). The bottom panel shows the output for the model predicting water content (initial mass  $\times$  sex).

Increased energy stores may indicate higher foraging rates or foraging success during the winter for females, or may indicate that females use less energy (through reduced activity or better energy efficiency) than males (Adams et al., '82). While we had predicted that exogenous cortisol manipulation would result in impaired health metrics, there was only limited evidence to support this prediction. Following a similar cortisol elevation, O'Connor et al. (2013) found a decrease in HSI in female largemouth bass immediately prior to reproduction. In our study, we noted that the percent water in muscle tissue, which is inversely proportional to the fat or energy content in the tissue, was inversely related to the prewinter condition of the largemouth bass, particularly for males. Therefore, individuals that went into winter in better condition had higher energy levels

(lower water content) at the end of winter. Similar to O'Connor et al. (2013), cortisol manipulation alone did not influence muscle lipid content.

Not surprisingly, the prewinter length, mass, and condition were all strong predictors of bass postwinter length, mass, and condition. However, all largemouth bass experienced a loss of mass over the winter, consistent with previous studies (e.g., Fullerton et al., 2000) and an indication of the energetically challenging nature of this season for all individuals. In general, males experienced higher rates of loss than females. This discrepancy was most apparent in male largemouth bass from the cortisol treatment group with an average loss of 12% of their initial mass. The influences of exogenous cortisol manipulation on growth and mass are well documented in fishes (e.g., Gregory



**Figure 6.** Select output of mixed models where the model size metric  $\times$  treatment was marginally significant ( $P < 0.1$ ). Specifically, the model for water content (initial length  $\times$  treatment) is shown.

and Wood, '99; O'Connor et al., 2010); however, it would appear that while female largemouth bass in the treatment group were able to compensate for losses associated with the cortisol manipulation, their male counterparts could not. Largemouth bass are generally quiescent during winter (Cooke et al., 2003) and there is growing evidence of some localized movements that might be indicative of feeding (Adams et al., '82; Hanson et al., 2007). Smaller changes in mass for female largemouth bass may suggest that they either forage more frequently or more successfully than males. Regardless, the impaired condition for male treatment fish in the spring could increase their mortality rate should winter conditions persist (e.g., following a winter kill event; O'Connor et al., 2010).

Results from the present study suggest that both size and exogenous cortisol elevation have a significant, albeit different, effect on overwinter mortality. With the exception of survival rates, the influence of overwintering itself appears to have a greater effect than experimental cortisol elevation. This was surprising as we elevated cortisol to supraphysiological levels for an extended period and so predicted a strong effect, which suggests that a more natural stressor would have even fewer effects. However, cortisol is only one component of the physiological response to a stressor; a natural stress response includes the psychological response to sensing the stressor and the release of additional hormones as part of the neuroendocrine cascade. Thus, the size-dependent consequences of stressors may involve more components of the stress response than cortisol alone. Additionally, it appears that for females, regardless of treatment, if they are able to survive the winter they can compensate for some of the negative consequence of a

physiological challenge, likely through continued foraging. In contrast, for males that survive the winter, a physiological challenge can act to reduce their overall growth. It remains unclear if there is potential for size-specific carryover effects to influence reproduction (e.g., gonad investment, decision to spawn, parental care investment) over the longer term, emphasizing the need for research on this topic that extends across multiple seasons or ideally years. The present study focused on evaluating postwinter health metrics; however, given the high rates of overwinter mortality for all largemouth bass as well as the increased mortality caused by experimental cortisol elevation (as a proxy for an extreme semichronic stressor), it would be beneficial to characterize individual prewinter energetic stores to determine whether these metrics are better predictors of overwinter survival. Since we were unable to sex bass prior to overwintering, the possibility also remains that cortisol treatment had sex-specific effects on mortality, and the prevalence of sex-specific effects in the physiological metrics of surviving bass makes the pattern of mortality an intriguing avenue for further study. In addition, given the observed lower rates of survival for the smallest size class, assessing the rate of conspecific predation (i.e., cannibalism) would help to determine the interconnectedness of starvation and predation on largemouth bass winter survival.

#### ACKNOWLEDGMENTS

We are grateful to the students and technicians who assisted us in the field, particularly Keith Stamplecoskie and Dan Schermerhorn. Funding for this project was provided by a Natural Sciences and Engineering Research Council Discovery Grant (awarded to S.J.C.).

## LITERATURE CITED

- Adams SM, McLean RB, Parrotta JA. 1982. Energy partitioning in Largemouth Bass under conditions of seasonally fluctuating prey availability. *Trans Am Fish Soc* 111:549–558.
- Angelier FDR, Wingfield JC. 2013. Importance of the glucocorticoid stress response in a changing world: theory, hypotheses and perspectives. *Gen Comp Endocr* 190:1–11.
- Barton BA. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr Comp Biol* 42:517–525.
- Barton BA, Iwama GK. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu Rev Fish Dis* 1:3–26.
- Barton BA, Morgan JD, Vijayan MM, Adams SM. 2002. Physiological and condition-related indicators of environmental stress in fish. In: Adams SM, editor. *Biological indicators of aquatic ecosystem stress*. Bethesda, MD: American Fisheries Society. p 111–148.
- Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *J Stats Software* 67:1–48.
- Brett JR, Groves TDD. 1979. Physiological energetics. In: Hoar WS, Randall DJ, Brett JR, editors. *Fish physiology: bioenergetic and growth*. New York, NY: Academic Press. p 279–352.
- Brown ML, Murphy BR. 2004. Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass *Micropterus salmoides* (Lacepede). *Ecol Fresh Fish* 13:23–36.
- Carmichael GJ, Tomasso JR, Simco BA, Davis KB. 1984a. Characterization and alleviation of stress associated with hauling largemouth bass. *Trans Am Fish Soc* 113:778–785.
- Carmichael GJ, Tomasso JR, Simco BA, Davis KB. 1984b. Confinement and water quality-induced stress in largemouth bass. *Trans Am Fish Soc* 113:767–777.
- Cline TJ, Weidel BC, Kitchell JF, Hodgson JR. 2012. Growth response of largemouth bass (*Micropterus salmoides*) to catch-and-release angling: a 27-year mark-recapture study. *Can J Fish Aquat Sci* 69:224–230.
- Cooke SJ, Grant EC, Schreer JF, Philipp DP, DeVries AL. 2003. Low temperature cardiac response to exhaustive exercise in fish with different levels of winter quiescence. *Comp Biochem Phys A* 134:157–165.
- Crossin GT, Love OP, Cooke SJ, Williams TD. 2016. Glucocorticoid manipulations in free-living animals: considerations of dose delivery, life-history context and reproductive state. *Funct Ecol* 30:116–125.
- Cunjak RA. 1996. Winter habitat of selected stream fishes and potential impacts from land-use activity. *Can J Fish Aquat Sci* 53:267–282.
- Dey CJ, O'Connor CM, Gilmour KM, Van Der Kraak G, Cooke SJ. 2010. Behavioral and physiological responses of a wild teleost fish to cortisol and androgen manipulation during parental care. *Horm Behav* 58:599–605.
- DeVries DR, Garvey JE, Wright RA. 2009. Early life history and recruitment. In: Cooke SJ, Philipp DP, editors. *Ecological diversity of centrarchid fishes: basic and applied perspectives*. Hoboken, NJ: Blackwell Scientific. p 105–133.
- DiBattista JD, Anisman H, Whitehead M, Gilmour KM. 2005. The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*. *J Exp Biology* 208:2707–2718.
- Edeline E, Haugen TO, Weltzien FA, et al. 2009. Body downsizing caused by non-consumptive social stress severely depresses population growth rate. *Proc R Soc B* 277:843–851.
- Fullerton AH, Garvey JE, Wright RA, Stein RA. 2000. Overwinter growth and survival of largemouth bass: interactions among size, food, origin, and winter severity. *Trans Am Fish Soc* 129:1–12.
- Gamperl A, Vijayan M, Boutilier R. 1994. Experimental control of stress hormone levels in fishes: techniques and applications. *Rev Fish Biol Fisher* 4:215–255.
- Garvey JE, Wright RA, Stein RA. 1998. Overwinter growth and survival of age-0 largemouth bass (*Micropterus salmoides*): revisiting the role of body size. *Can J Fish Aquat Sci* 55:2414–2424.
- Garvey JE, Ostrand KG, Wahl DH. 2004. Energetics, predation, and ration affect size-dependent growth and mortality of fish during winter. *Ecology* 85:2860–2871.
- Gravel MA, Couture P, Cooke SJ. 2010. Comparative energetics and physiology of parental care in smallmouth bass *Micropterus dolomieu* across a latitudinal gradient. *J Fish Biol* 76:280–300.
- Gregory TR, Wood CM. 1999. The effects of chronic plasma cortisol elevation on the feeding behaviour, growth, competitive ability, and swimming performance of juvenile rainbow trout. *Physiol Biochem Zool* 72:286–295.
- Hanson KC, Cooke SJ, Suski CD, et al. 2007. Assessment of largemouth bass (*Micropterus salmoides*) behaviour and activity at multiple spatial and temporal scales utilizing a whole-lake telemetry array. *Hydrobiologia* 582:243–256.
- Harrison XA, Blount JD, Inger R, Norris DR, Bearhop S. 2010. Carry-over effects as drivers of fitness differences in animals. *J Anim Ecol* 80:4–18.
- Hurst TP. 2007. Causes and consequences of winter mortality in fishes. *J Fish Biol* 71:315–345.
- Iwama GK. 1998. Stress in fish. *Ann NY Acad Sci* 851:304–310.
- Jobling M. 1994. *Fish Bioenergetics*. London:Chapman and Hall.
- Mazeaud MM, Mazeaud F, Donaldson EM. 1977. Primary and secondary effects of stress in fish: some new data with a general review. *Trans Am Fish Soc* 106:201–212.
- McConnachie SH, O'Connor CM, Gilmour KM, Iwama GK, Cooke SJ. 2012. Supraphysiological cortisol elevation alters the response of wild bluegill sunfish to subsequent stressors. *J Exp Zool A* 317:321–332.
- Midwood JD, Larsen MH, Boel M, et al. 2014. Does cortisol manipulation influence outmigration behaviour, survival and growth of sea

- trout? A field test of carryover effects in wild fish. *Mar Ecol Prog Ser* 496:135–144.
- Miranda LE, Hubbard WD. 1994. Length-dependent winter survival and lipid composition of age-0 Largemouth Bass in Bay Springs Reservoir, Mississippi. *Trans Am Fish Soc* 123:80–87.
- O'Connor CM, Gilmour KM, Arlinghaus R, Van Der Kraak G, Cooke SJ. 2009. Stress and parental care in a wild teleost fish: Insights from exogenous supraphysiological cortisol implants. *Physiol Biochem Zool* 82:709–719.
- O'Connor CM, Gilmour KM, Arlinghaus R, et al. 2010. Seasonal carryover effects following the administration of cortisol to a wild teleost fish. *Physiol Biochem Zool* 83:950–957.
- O'Connor CM, Nannini M, Wahl DH, et al. 2013. Sex-specific consequences of experimental cortisol elevation in pre-reproductive wild largemouth bass. *J Exp Zool A Ecol Genet Physiol* 319:23–31.
- O'Connor CM, Norris DR, Crossin GT, Cooke SJ. 2014. Biological carry-over effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* 5:1–11.
- Ostrand KG, Cooke SJ, Garvey JE, Wahl DH. 2005. The energetic impact of overwinter prey assemblages on age-0 largemouth bass, *Micropterus salmoides*. *Environ Biol Fishes* 72:305–311.
- Post DM, Kitchell JF, Hodgson JR. 1998. Interactions among adult demography, spawning date, growth rate, predation, overwinter mortality, and the recruitment of largemouth bass in a northern lake. *Can J Fish Aquat Sci* 55:2588–2600.
- Ricker WE. 1954. Stock and recruitment. *J Fish Res Board Can* 11:559–623.
- Schreck CB, Moyle PB. 1990. *Methods for fish biology*. Bethesda, MD: American Fisheries Society.
- Schultner J, Moe B, Chastel O, et al. 2015. Corticosterone mediates carry-over effects between breeding and migration in the kittiwake *Rissa tridactyla*. *Mar Ecol Prog Ser* 496:123–133.
- Shuter BJ, Finstad AG, Helland LP, Zweimüller I, Hölker F. 2012. The role of winter phenology in shaping the ecology of freshwater fish and their sensitivities to climate change. *Aquat Sci* 74:637–657.
- Sogard SM. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull Mar Sci* 60:1129–1157.
- Sopinka NM, Patterson LD, Redfern JC, et al. 2015. Manipulating glucocorticoids in wild animals: basic and applied perspectives. *Conserv Physiol* 3:1–16.
- Suski CD, Ridgway MS. 2009. Winter biology of centrarchid fishes. In: Cooke SJ, Philipp DP, editors. *Centrarchid fishes: diversity, biology, conservation*. West Sussex, United Kingdom: John Wiley & Sons Ltd. p 264–292.
- Wendelaar Bonga SE. 1997. The stress response in fish. *Physiol Rev* 77:591–625.