



Food deprived largemouth bass (*Micropterus salmoides*) are inactive and stressed, but do not show changes in lure inspections

Toniann D. Keiling*, Cory D. Suski

1102 S. Goodwin Avenue, Urbana, IL, 61820, USA

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ABSTRACT

Coping style traits, including physiology and behavior, can be used to predict if fish are vulnerable to capture by hook-and-line angling. Typically, fish with proactive coping styles are selectively captured, but effects of environmental influences, such as food availability, on the completion of each step leading to a successful angling capture (i.e., activity rates, encountering a lure, lure inspection, lure-striking, and ingestion) have not been quantified. Therefore, the objective of this study was to quantify the effects among activity behavior, stress (cortisol) responsiveness, and food availability on lure inspection behaviors of largemouth bass. No relationships were found between activity, stress responsiveness, and food availability to determine lure inspections. However, food deprivation decreased activity rates and increased baseline cortisol concentrations of largemouth bass. Additionally, after feeding treatments, fish with low baseline cortisol concentrations were more likely to inspect lures in both the fed and food deprived treatments. Results further discuss the implications of study findings to help fisheries managers predict the evolutionary impacts of angling.

1. Introduction

Coping styles are suites of correlated behavioral and physiological traits that can explain how individual animals, including fish, react to stressful situations (Koolhaas, 2008; Overli et al., 2007; Silva et al., 2010). Coping style traits fall along a continuum, and fish with proactive coping styles tend to express bold, aggressive, and active behaviors, while fish with reactive coping styles express shy, social, and inactive behaviors (Koolhaas, 2008; Koolhaas et al., 2010). From a physiological aspect, stress responsiveness, which can be quantified through plasma cortisol concentrations, is an indicator of an individual's reaction to acute or chronic stressors in their environment, and trigger either a fight or flight response in fish (Hollins et al., 2018; Koolhaas, 2008; Wendelaar Bonga, 1997). Generally, reactive fish have high stress responsiveness, shown through large differences between their baseline and maximum plasma cortisol concentrations, while proactive fish exhibit low stress responsiveness values (Koolhaas, 2008; Pottinger and Carrick, 2001). Because behavioral and physiological traits are closely linked within coping styles, consistent relationships have been shown between activity behaviors and cortisol responsiveness (Ricklefs and Wikelski, 2002; Thomson et al., 2012). Lastly, plasticity to changes in an individual's environment has been quantified as its own trait within coping styles. Proactive fish demonstrate lower plasticity in altered

environmental conditions and maintain rigid routines, while reactive fish are more likely to adjust to new conditions through modified behaviors (Benus et al., 1990; Koolhaas, 2008). Coping styles are therefore a way to categorize animals based on their behavioral and physiological reactions to stressors.

A fish's coping style can be used to predict interactions with fishing lures that may lead to capture by hook-and-line angling. For a fish to be captured by hook-and-line, the fish must encounter, inspect, strike, and ingest a presented fishing lure, and if any of these behaviors are not completed, a fish will not be captured (Lennox et al., 2017). Proactive fish have high activity rates, and these high activity rates can increase the likelihood of many fish species to be more likely to encounter fishing lures, in turn making proactive fish more vulnerable to angling (Alós et al., 2016; Lennox et al., 2017). In addition, proactive fish are bolder than reactive fish, leading individuals with proactive coping styles to be more likely to inspect novel objects, such as fishing lures (Lennox et al., 2017; Tix et al., 2017). However, behavioral traits alone, such as activity and boldness, have not been shown to predict capture vulnerability in largemouth bass (*Micropterus salmoides*) (Louison et al., 2017). Instead, cortisol responsiveness was the strongest predictor of capture for largemouth bass; proactive bass, with low cortisol responsiveness, were found to be more likely to strike fishing lures, leading to increased capture vulnerability when compared to their reactive

* Corresponding author.

E-mail addresses: toniannkeiling@outlook.com (T.D. Keiling), suski@illinois.edu (C.D. Suski).

conspecifics (Louison et al., 2017). Coping styles can therefore play a role in whether individual fish are vulnerable to hook-and-line angling.

Environmental stressors, such as food deprivation, can emphasize differences in individual coping styles of fish, thereby altering a fish's interactions with stimuli, such as fishing lures. Coping styles are expected to remain stable and repeatable across contexts, with activity rates remaining constant for periods of about 2 months (Kortet et al., 2014). However, phenotypes are plastic and can be modulated by environmental conditions, including food availability, and may alter both behavioral and physiological responses to perceived risk (Mittelbach et al., 2014; Ricklefs and Wikelski, 2002), including novel object interactions. For example, food deprived fish may alter their behaviors and inspect their surroundings more than satiated fish as they search for food resources while also minimizing unwanted interactions with predators (Härkönen et al., 2014a; Lima, 1998). Some behaviors are more repeatable than others, and a meta-analysis about repeatability of behaviors found that activity was the least repeatable behavior in contrast with other behaviors, such as habitat selection (Bell et al., 2009). Similar to behavior, cortisol responsiveness can be context-dependent based on environmental conditions. One example was seen in whitefish (*Prosopium williamsoni*), where cortisol responsiveness increased with changes to stream flows, and then leveled off again after fish adjusted to these new flow regimes (Taylor et al., 2012). Both behavior and cortisol responsiveness can be modulated by environmental factors, leading to shifts in coping styles in fish under stressful situations.

Many studies have identified coping styles in various fish species and the plasticity of coping style traits under changing environments. However, the combined effect of coping styles and food deprivation on novel object interactions have not been well defined. The combined effects of environmental conditions and coping styles is important for fisheries management, particularly when considered in the context of angling vulnerability. For example, in environments with low food availability, fish with proactive coping styles may be expected to increase activity rates (Beukema, 1968), thereby increasing the probability of encountering fishing lures, and increasing the likelihood of capture. However, fish with reactive coping styles may exhibit increased stress responsiveness due to the stressor of low food availability, then cortisol concentrations may increase, leading reactive fish to be less likely to inspect novel objects, and, in this case, not strike fishing lures (Louison et al., 2017), decreasing the chances of a successful capture. This information is valuable as the repeated capture and/or harvest of individuals of specific phenotypes (i.e., disproportionate capture/harvest of proactive individuals) can lead to genetic, heritable changes to fish populations, and can decrease angling vulnerability and alter population dynamics into the future (Hessenaue et al., 2016, 2015; Laugen et al., 2014; Mittelbach et al., 2014; Philipp et al., 2009a). Thus, defining how coping style and environmental context combine to influence lure inspection behaviors and the steps leading to capture by anglers is critical for the successful conservation and management of recreational fisheries.

Based on this background, the goal of this study was to identify how food availability and coping style interact to determine individual lure inspection behavior in largemouth bass, which may influence an individual's susceptibility to angling capture. To accomplish this goal, we completed behavior assays to define individual activity and risk-taking (novel object inspection) phenotypes and quantified individual cortisol responsiveness of largemouth bass. Following this, 2 food availability treatments were carried out as the environmental context, where largemouth bass we either fed or food deprived for 2 weeks. Then, behavior assays and cortisol responsiveness were measured a second time. Together, results from this study will allow conclusions to be made about the interaction of coping styles and food availability on lure inspection behaviors of largemouth bass.

2. Materials and methods

2.1. Study animals/study site

Largemouth bass naive to angling ($n = 75$) were acquired from Seven Springs Fish Hatchery in Evansville, Illinois and transferred to the Illinois Natural History Survey Aquatic Research Facility in Champaign, Illinois on 1 October 2018 (mean \pm standard deviation (SD) of total length = 280 ± 18.0 mm; mass = 284 ± 70.3 g; relative weight (a ratio of mass to length through the use of a species' standard weight; (Henson, 1991)) = 89.3 ± 8.74). The hatchery the study fish were acquired from had been raising fish for multiple generations. Fish were evenly divided and held in 8 circular 1135 L outdoor tanks supplied with water from a nearby 0.04 ha earthen pond via a flow-through system that flushed aerated water through the tanks about 8 times per day. Mean \pm SD of water temperature and dissolved oxygen in the outdoor tanks were 17.6 ± 4.9 °C and 9.7 ± 1.1 mg/L, respectively (YSI Inc. Professional Plus, Yellow Springs, Ohio). During holding, fish were fed ad libitum daily with Skretting high protein pellets (Tooole, Utah). Four days after arrival, fish were implanted with passive integrated transponder (PIT) tags (10 mm [length] \times 2 mm [diameter], HPT12, Biomark Inc., Boise, Idaho) for individual identification, and then held in the outdoor tanks for 4 additional days prior to the start of experiments.

2.2. Behavior assays

Fish were moved from outdoor tanks to indoor isolation tanks to acclimate overnight for 15–23 h directly before behavior assays began. Ten opaque 121 L aquaria were used for isolation, with an opaque perforated barrier in the middle separating each aquarium to allow for 2 fish to acclimate simultaneously. Aerated water was supplied from a reservoir tank and flowed through each aquarium via pumps (Outdoor air pump, Pentair, Cary, North Carolina). Mean \pm SD of water temperature and dissolved oxygen in the aquaria were 19.2 ± 2.1 °C and 8.2 ± 0.6 mg/L, respectively.

Behavior assays began on 9 October 2018 in one of 2 circular 94 L arenas (80 cm in diameter with a water depth of 24.5 cm). Fish behavior and location in the circular arenas were recorded with a video camera mounted to a PVC frame above the arenas (uEye Cockpit, IDS, Germany). Fish acclimated in the behavior arenas for 5 min before video recordings began, at which point fish had begun to settle into the new environment and explore the arena. For a 10 min period following this acclimation, the duration of time that largemouth bass spent swimming (s), as well as their total distance moved (cm) were calculated using commercially available animal tracking software (Lolitrak, Loligo Systems, Denmark). These acclimation (Bell and Stamps, 2004; Vainikka et al., 2016) and monitoring periods (Basic et al., 2012; Thomson et al., 2012) are similar to those used in other fish behavioral studies. Following this acclimation period, a barbless 1.8 g black Sink'n Jig (Northland Fishing Tackle, Bemidji, Minnesota) lure, commonly used in angling for largemouth bass, was dropped into the center of the tank, with the location of the lure identical for each fish regardless of the fish's position. This method of introducing a novel object into a behavioral arena from above is common for studies measuring behavioral phenotypes in fish, and a fish's reaction to a novel object presented in this manner can provide insights into an individual's risk-taking behaviors in the wild (Naslund and Johnsson, 2016; Tucker et al., 2018; Wilson et al., 1993). In addition, a fishing lure was specifically used as a novel object in the current study due to its relevance within the context of angling vulnerability. Following the addition of this fishing lure, the time largemouth bass spent swimming, as well as their distance moved, were again calculated with the tracking software for an additional 10 min. The number of times fish approached the fishing lure, and the duration of time spent within a 3.5 cm radius of the lure (to encompass the circular area around the largest lure used, which

was 7 cm in length, see below) were calculated, as well. Fish that spent the majority of the time swimming, with large distances moved, were considered to be active, and fish that approached the lure multiple times or spent longer periods of time in proximity to the lure were considered to be bold (Réale et al., 2007). In contrast, inactive fish moved shorter distances, and shy fish spent most of their time away from the lure, when compared to their conspecifics.

2.3. Cortisol responsiveness

Standardized stress protocols, commonly used for largemouth bass, were then used to measure individual cortisol responsiveness for all fish that completed the behavior assay (Cook et al., 2011; Louison et al., 2017). Immediately after completion of the behavior assay, largemouth bass were returned to indoor aquaria to repeat the overnight isolation period. The following day, fish were quickly netted from their isolation tanks and a blood sample (approximately 1 mL) was collected from the caudal vessel in under 3 min to define baseline cortisol concentrations (Asakawa et al., 2001; Cook et al., 2011). When blood samples are collected in under 3 min, baseline cortisol concentrations are not likely to be influenced by sampling because it takes between 4 and 8 min for cortisol concentrations to rise following the onset of a stressor (Lawrence et al., 2018; Romero and Reed, 2005). Regardless of how quickly the blood sample was collected, all fish remained out of water for a full 3 min as part of this blood collection to provide a standardized air exposure stressor. Following the initial blood draw and 3 min air exposure, fish were returned to the indoor aquaria and a second blood sample, with the same quantity and same blood draw procedure, was taken 25 min post-stressor to define maximum cortisol concentration (Cook et al., 2011). However, for this second blood draw, fish were immediately returned to the aquaria after the sample was collected to minimize further physiological effects of air exposure. Blood samples were stored temporarily on ice and later centrifuged on-site. Plasma was separated from red cells and then flash frozen under liquid nitrogen prior to transport to the laboratory, where samples were stored at -80°C . Concentrations of plasma cortisol, the primary stress hormone for fish, were assayed in the laboratory using a standard enzyme-linked immunosorbent assay (ELISA), verified for use with largemouth bass (Sink et al., 2008). These initial cortisol trials served to measure baseline stress responsiveness (maximum cortisol concentration – baseline concentration) of study fish.

2.4. Feeding treatments

Following the initial behavior assay and baseline cortisol responsiveness, fish were held in one of 2 circular 1135 L or one of 2 rectangular 180×65 cm, 379 L indoor holding tanks and randomly divided into 2 treatments (4 holding tanks total). Mean \pm SD of water temperature and dissolved oxygen of the indoor holding tanks were $15.6 \pm 1.1^{\circ}\text{C}$ and 9.4 ± 0.4 mg/L, respectively. Water quality was maintained through daily water exchanges with supply from a nearby 0.04 ha earthen pond. Fish in the ‘fed treatment’ received Skretting high protein pellets (Tooele, Utah) daily to satiation, while fish in the ‘food deprived treatment’ had food withheld (Gingerich et al., 2010). Feeding treatments lasted for approximately 2 weeks, which is sufficient to elicit behavioral and physiological responses in largemouth bass (Gingerich et al., 2010). Mean total length \pm SD, mean mass, and mean relative weight were all not significantly different between fish in the fed and food deprived treatments ($TL_{\text{food deprived}} = 278 \pm 13$ mm, $TL_{\text{fed}} = 282 \pm 22$ mm, $mass_{\text{food deprived}} = 270 \pm 36$ g, $mass_{\text{fed}} = 298 \pm 93$ g, $relative\ weight_{\text{food deprived}} = 88 \pm 6$, $relative\ weight_{\text{fed}} = 91 \pm 11$; t -tests, $t = -0.61$, -1.14 , and -0.99 , respectively, $p > .05$ for all 3 tests).

Following this 2 week period of feeding/food deprivation, a second round of behavior assays and cortisol responsiveness tests were carried out using procedures identical to those described above to define how food deprivation influences individual behavior and interactions with

fishing lures. For this second behavior assay, a different barbless lure, a 1.8 g white Sink'n Jig (Northland Fishing Tackle, Bemidji, Minnesota, USA) with a 7 cm ‘Canada Craw’ colored plastic worm (Z Man, Ladson, South Carolina, USA), was used to prevent habituation to the novel object (Thomson et al., 2012). Activity and novel object inspections have been shown to be repeatable over time (Bell et al., 2009; Kortet et al., 2014). Therefore, to minimize habituation of experimental conditions, behavior assays were only measured once before the feeding/feeding treatment and once afterward. All other aspects of the behavior assay were identical to those described above.

2.5. Statistics

Principal components analysis (PCA) was used to distill any collinearity between time spent swimming and distance moved both pre- and post-lure introduction to identify each individual's behavioral type. Two PCA analyses were performed: one for the behaviors prior to the feeding treatments (referred to as ‘pre-treatment’), and a second PCA for the behaviors performed following 2 weeks of largemouth bass being either fed or food deprived (referred to as ‘post-treatment’). Two PCAs were performed because the goal was to determine if there were behavioral changes due to the feeding/food deprivation treatments, and using a single PCA would have resulted in combinations of all measured behaviors, both pre- and post- feeding treatment, therefore not allowing for treatment comparisons. Principal components (PCs) with eigenvalues > 1 and factors with loadings > 0.50 after varimax rotation were included as primary factors for associated PCs (Budaev, 2010; Hair, 1998; Ho, 2006). Spearman correlation coefficients were used to discern collinearity between the number of visits to the fishing lure and time spent within close proximity to the lure both pre- and post-treatment to minimize model inflation by using only one of 2 correlated terms in subsequent statistical models. A Spearman correlation was also used to test for collinearity between time spent swimming and distance moved to ensure models were not inflated with the inclusion of 2 correlated activity metrics. Lastly, 2 separate Spearman correlations were used to test for the presence of true coping styles (correlations between the measured activity behaviors, represented by PC scores, and cortisol metrics) both pre- and post-feeding/food deprivation treatment.

Following the generation of principal components, three separate linear mixed effects models were used to define differences in cortisol metrics between feeding treatments both before and after the 2 week feeding/food deprivation period. For these 3 models, cortisol parameters were the dependent variables (baseline cortisol concentration, maximum cortisol concentration following the standardized stressor, and cortisol responsiveness (maximum – baseline)), and independent variables were treatment group (fed or food deprived), time point (pre- or post-treatment) and their interaction; fish ID was included as a random effect because fish were sampled twice during the study, and differences across sample times may not have been independent (Laird and Ware, 1982; Lindstrom and Bates, 1990).

A Spearman correlation found the time largemouth bass spent swimming and distance swam to be significantly correlated both pre- and post-lure introduction ($r_{\text{pre}} = 0.74$, $p_{\text{pre}} < 0.001$; $r_{\text{post}} = 0.81$, $p_{\text{post}} < 0.001$), so the term ‘activity’ in this study will therefore encompass both of these correlated variables, with only time spent swimming used in subsequent models. One additional linear mixed effects model was performed to determine differences in activity between treatments both pre- and post-treatment. Activity times were used in this because the PC scores from the 2 separate PCAs (1 pre-treatment and 1 post-treatment, which were completed to test for changes in behavior due to the feeding/food deprivation treatments) are not comparable within 1 model. For this model, activity was the response variable, with treatment group (fed or food deprived), time point (pre- vs. post-treatment) and their interaction as fixed effects; fish ID was added to this model as a random effect to account for the fact that the same fish were assayed twice.

A generalized linear mixed effects model with Poisson error distribution (intended for count data (Crawley, 2013)) was used to determine differences in the number of lure visits between treatments both pre- and post-treatment. For this model, the number of lure visits was the response variable, and treatment (fed or food deprived), time point (pre- vs. post-treatment) and their interaction were fixed effects, and fish ID and behavior arena were included as random effects. Lastly, 2 additional generalized linear mixed effects models with Poisson error distributions were carried out, 1 pre-treatment and 1 post-treatment, to define the effects of feeding treatments, cortisol responsiveness, and activity behaviors on the number of lure visits performed by each fish. Two separate models had to be used due to the PCA results, which had different outcomes for pre- and post-treatment behaviors (see below). For these models, the total number of lure visits performed was the dependent variable, and fixed effects were baseline cortisol, cortisol responsiveness, feeding treatment, and PC scores; fish identity was included as a random effect. The three generalized linear models described above initially included relative weight, water temperature, and behavior arena number as fixed effects. The lure visit and pre-treatment models including these additional fixed effects were compared to the non-parameterized lure visit and pre-treatment models with Akaike's Information Criteria (AIC), and the parameterized models better fit the data, indicated by lower AIC scores (Zuur et al., 2009; Crawley, 2013) (Table A.1). However, relative weight, water temperature, and behavior arena caused the AIC score of the post-treatment model to increase and were subsequently removed as they did not improve model fit (Zuur et al., 2010). The top models to determine lure visits using a Poisson distribution were overdispersed, defined as residual deviance larger than the residual degrees of freedom (Crawley, 2013). As such, the top models were re-run as negative binomial models with Poisson distributions, which accommodate for over-dispersion in count data regressions, within R package 'glmmADMB' (Fournier et al., 2012; Zeileis et al., 2008). All model parameters thought to influence lure inspections were identified as fixed effects a priori, and model selection was only used to compare models with and without extraneous variables (i.e., fish identity) to identify any influences of outside parameters and improve model fit. Individual model fit was assessed via visual examination of Pearson residuals (i.e., residual plots to define normality of residuals by predicted plots to define homogeneity of variances) (Zuur et al., 2009). All analyses were performed in R version 3.4.1, with 'tidyverse 1.2.1' (Wickham, 2017), 'irr 0.84.1' (Gamer et al., 2012), 'vegan 2.5-3' (Oksanen et al., 2018), 'Hmisc 4.2-0' (Harrell Jr., 2019), 'lme4 1.1-19' (Bates et al., 2015), 'lmerTest 3.1-0' (Kuznetsova et al., 2017), 'nlme 3.1-131' (Pinheiro et al., 2017), 'MuMIn 1.40.0' (Barton, 2017), 'languageR 1.5.0' (Baayen and Shafaei-Bajestan, 2019), 'lsmmeans 2.30-0' (Lenth, 2016), 'ggplot2 2.2.1' (Wickham, 2009), 'glmmADMB' (Fournier et al., 2012) and 'gridExtra 2.3' (Auguie, 2017) packages. The significance level for all tests was set at $\alpha = 0.05$.

3. Results

Largemouth bass displayed large inter-individual variation in time spent swimming, distance moved, number of lure visits, and time spent in proximity to the lure (Table A.2). Prior to the feeding/food deprivation treatments, the PCA analyses for the activity behaviors of largemouth bass generated a single principal component that explained 68% of the behavioral variation, with an eigenvalue of 2.7 (Table 1). Fish with high PC scores swam far distances and fish with low PC scores did not swim far distances, both before and after the introduction of the novel object (fishing lure), indicating that introduction of the novel object did not alter activity behaviors prior to the feeding treatment. The PCA performed after largemouth bass had been food deprived or fed for 2 weeks resulted in 2 principle components, with PC1 and PC2 accounting for 46% and 31% of the variance, respectively (Table 2). This second PCA separated activity behaviors performed pre- and post-novel object introduction, leading them to be grouped 'pre-lure

Table 1

Factor loadings from the first principal component analysis (PCA) to describe pre-treatment activity behaviors both pre- and post-lure introduction for largemouth bass. Loadings > 0.50 used for interpretations are shown in bold. Distance moved both pre- and post-lure loaded positively on PC1 to explain PC scores of each largemouth bass.

Factor	PC1 loadings
Pre-lure activity (s)	0.477
Post-lure activity (s)	0.434
Pre-lure distance moved (cm)	0.545
Post-lure distance moved (cm)	0.536
Eigenvalue	2.71
% variance explained	67.76

Table 2

Factor loadings from the second principal component analysis (PCA) to describe post-treatment activity behaviors both pre- and post-lure introduction for largemouth bass. Loadings > 0.50 used for interpretations are shown in bold. Activity and distance moved pre-lure introduction positively loaded on PC1, while activity and distance moved post-lure introduction positively loaded on PC2. Therefore, PC1 can be used to describe pre-lure behaviors and PC2 can be used to describe post-lure behaviors.

Factor	PC1 loadings	PC2 loadings
Pre-lure activity (s)	0.57	-0.42
Pre-lure distance moved (cm)	0.63	-0.30
Post-lure activity (s)	0.29	0.67
Post-lure distance moved (cm)	0.44	0.53
Eigenvalue	1.83	1.24
% variance explained	45.8	31.0

introduction' and 'post-lure introduction.' PC1 scores only explained activity prior to the introduction of the fishing lure, and fish with high PC1 scores were active and swam far distances before the lure was introduced. In contrast, PC2 only explained activity after the fishing lure was introduced, and largemouth bass with high PC2 scores were highly active and swam far distances after the lure was introduced into the arena.

Spearman correlations showed significant positive correlations between the number of visits to the lure and time spent in close proximity to the lure both pre- and post-treatment (Table 3). Therefore only number of lure visits was used in subsequent models to prevent model inflation. Spearman correlations determined that largemouth bass did not exhibit true coping styles as cortisol metrics did not correlate with PC scores either before or after 2 weeks of feeding/food deprivation (Table 4). In addition, maximum cortisol concentration was significantly correlated with both baseline cortisol and cortisol responsiveness pre- and post-treatment (Table 4), so only one of each of the correlated cortisol metrics was used within subsequent models to prevent model inflation.

Linear mixed effects models showed that largemouth bass in the food deprived treatment had significantly higher baseline cortisol concentrations following the 2 week period of food deprivation compared to pre-treatment concentrations (Table 5; Fig. 1). Both maximum cortisol concentrations and cortisol responsiveness were not different between treatment groups either pre- or post-treatment (Table 5; Fig. 1). Raw activity times (time spent swimming) were used to compare pre- and post-treatment activity behaviors; 2 weeks of food deprivation caused a 36% decrease in activity scores for largemouth bass in the food deprived treatment (Table 6; Fig. 2). The total number of lure visits was not significantly different between largemouth bass that had been fed or food deprived for 2 weeks, both pre- and post-feeding treatment (Table 6).

None of the predictor variables, including PC scores, cortisol metrics, behavior arena number, water temperature, and relative weight

Table 3

Results from Spearman correlation matrix testing for correlations between number of lure visits and time spent in proximity to the lure (novel object) in a behavior arena both pre- and post- 2 weeks of feeding/food deprivation treatments. Correlation coefficients (r) are shown in the top right section of the table and corresponding p -values are shown in the bottom left of the table. Significant correlations ($p < .05$) are in bold.

	Pre-treatment lure visits	Post-treatment lure visits	Pre-treatment time spent in proximity to lure (s)	Post-treatment time spent in proximity to lure (s)
Pre-treatment lure visits	–	0.06	0.94	0.11
Post-treatment lure visits	0.74	–	0.06	0.99
Pre-treatment time spent in proximity to lure (s)	< 0.01	0.76	–	0.10
Post-treatment time spent in proximity to lure (s)	0.53	< 0.01	0.60	–

had significant effects on the number of times fish visited lures prior to the feeding/food deprivation treatments (Table 7). However, baseline cortisol concentrations significantly influenced lure visits after largemouth bass had been fed or food deprived for 2 weeks, whereby fish with low baseline cortisol made more visits to the fishing lure than fish with high baseline cortisol (Table 7; Fig. 3).

4. Discussion

Two weeks of food deprivation had a significant effect on both activity rates and baseline cortisol concentrations in largemouth bass, but not maximum cortisol concentration or cortisol responsiveness following a standardized stressor. More specifically, 2 weeks of food deprivation caused a significant decrease in activity rates (either time spent swimming or distance moved as these metrics were correlated), and a significant increase in baseline cortisol concentration, relative to largemouth bass that had been fed over this same period. In nature, animals can experience natural variability in access to food, and periods of reduced food intake are common, such as during overwintering or while providing parental care (McCue, 2010; Navarro and Gutierrez, 1995; Wang et al., 2006). Therefore, to survive, fish need to be able to adjust to environments with low food availability, often through physiological and behavioral changes (McCue, 2010; Navarro and Gutierrez, 1995; Wang et al., 2006). Laboratory studies have shown that, during periods of restricted food access, animals experience a number of predictable changes in both behavior and physiology that include consumption of different fuel types, catabolism of different body constituents, declines in metabolism, and most closely linked to this study, reductions in activity (McCue, 2010; Navarro and Gutierrez, 1995; Wang et al., 2006). Twelve weeks of food deprivation, for

example, caused Atlantic cod (*Gadus morhua*) to demonstrate decreased sustained swimming activity (Martínez et al., 2002). Circulating gut hormones, including leptin and ghrelin, can be measured through blood plasma (Debris, 2013) and may cause decreases in swimming activity seen here (Killen, 2011; Volkoff, 2011), but no data were collected to support these relationships in the current study and should be investigated in the future. Past work with largemouth bass has shown that 2 weeks of food deprivation is sufficient to elicit a number of physiological changes, including a reduction in metabolic rates and decreased swimming performance relative to satiated conspecifics (Gingerich et al., 2010; Jobling, 2011; Mendez and Wieser, 1993). For largemouth bass in the food deprived treatment of the current study, baseline cortisol concentrations may have been higher than fed fish because food deprivation likely acted as a chronic stressor, thereby increasing baseline cortisol concentrations. One example of this was seen in largemouth bass during parental care, whereby more energy was used to protect broods than to forage, causing fish to have decreased food consumption and an increase in plasma glucose, another chronic stress indicator for fish (Hanson and Cooke, 2009). Interestingly, maximum cortisol and cortisol responsiveness concentrations in the current study were not different after the feeding/ food deprivation treatments. Another study also found mixed results of food deprivation on baseline, maximum, and cortisol responsiveness concentrations, where the effects of food deprivation on cortisol concentrations for rainbow trout (*Oncorhynchus mykiss*) were inconsistent (Pottinger, Rand-Weaver, & Sumpter, 2003). One reason maximum cortisol concentrations were not affected by food deprivation may be that when fish are already experiencing energetic, chronic stress due to food deprivation, their acute stress response in risky situations, such as air exposure and handling during blood draws, may be lower than in the absence of these chronic

Table 4

Results from Spearman correlation matrix testing for correlations between PC scores and cortisol metrics both pre- and post-treatment. Only PC1 score is shown pre-treatment, as the first PCA found only one principal component for the activity behaviors (Table 1), but both PC1 and PC2 scores are shown for post-treatment behaviors, as the second PCA determined 2 principal components (Table 2). Correlation coefficients (r) are shown in the top right section of the table and corresponding p -values are shown in the bottom left of the table. Significant correlations ($p < .05$) are in bold.

Pre-treatment					
	Baseline cortisol	Maximum cortisol	Cortisol responsiveness	PC1 score	
Baseline cortisol	–	0.37	0.10	0.14	
Maximum cortisol	0.04	–	0.92	–0.18	
Cortisol responsiveness	0.61	< 0.01	–	–0.13	
PC1 score	0.46	0.97	0.75	–	
Post-treatment					
	Baseline cortisol	Maximum cortisol	Cortisol responsiveness	PC1 score	PC2 score
Baseline cortisol	–	0.57	0.13	0.02	–0.09
Maximum cortisol	< 0.01	–	0.82	–0.01	0.11
Cortisol responsiveness	0.50	< 0.01	–	0.03	0.22
PC1 score	0.91	0.95	0.85	–	0.04
PC2 score	0.65	0.54	0.23	0.04	–

Table 5

Summary of mixed effects models explaining cortisol metrics of largemouth bass. Three separate models were used to account for feeding treatment (largemouth bass either fed or food deprived for 2 weeks), time period (pre- vs. post-feeding/food deprivation treatment), and the interaction between these treatment and time variables on baseline cortisol concentration, maximum cortisol concentration, and cortisol responsiveness (maximum – baseline concentrations). Fish ID was used as a random effect in all models because each fish was tested both pre- and post-feeding/food deprivation treatment. r^2_m is the marginal coefficient of determination, which represents the proportion of variance described by only the fixed factors, and r^2_c is the conditional coefficient of determination, which represents the proportion of variance that can be described by both the fixed and the random factors. Significant variables within each model ($p < .05$) are in bold.

Factors	Coefficient	SEM	df	t	p	r^2_m	r^2_c
Baseline cortisol							
Intercept	51,996.69	6367.50	57.81	8.17	< 0.001	0.20	0.25
Treatment	–18,887.47	9153.35	57.83	–2.06	0.043		
Time	–27,331.69	8888.82	29.42	–3.08	0.005		
Treatment × time	13,563.84	12,570.69	29.42	1.08	0.289		
Maximum cortisol							
Intercept	187,213.81	19,801.39	47.58	9.46	< 0.001	0.10	0.53
Treatment	–51,368.76	28,362.91	48.46	–1.81	0.076		
Time	–19,523.53	20,800.85	28.60	–0.94	0.356		
Treatment × time	6057.79	29,416.85	28.60	0.21	0.838		
Cortisol responsiveness							
Intercept	135,217.12	18,951.47	48.69	7.14	< 0.01	0.05	0.48
Treatment	–32,239.17	27,155.57	49.52	–1.19	0.241		
Time	6747.00	20,362.52	29.07	0.33	0.743		
Treatment × time	–6687.02	28,796.95	29.07	–0.23	0.818		

environmental stressors (Abrahams, 2011; Wendelaar Bonga, 1997). So, because food deprived fish were already experiencing a chronic stressor, they were less likely to result in high maximum cortisol concentrations and large cortisol responsiveness values during cortisol measurements, compared to fish in the fed treatment. This finding is in line with previous findings that when 2 stressors combine, the overall stress response may be negated through antagonism instead of enhanced through synergism (Folt et al., 1999; Schinegger et al., 2016; Teichert et al., 2016). In addition, wide individual variation in cortisol concentrations of largemouth bass made it more difficult to define the effects of feeding and food deprivation treatments. Food deprivation did indeed decrease activity behaviors and increase baseline cortisol concentrations of largemouth bass compared to fed conspecifics, but it did not alter maximum and cortisol responsiveness concentrations.

Activity behaviors and cortisol responsiveness did not influence lure inspection behaviors for largemouth bass, even after individuals had been food deprived for 2 weeks. During an angling event, there are a number of steps that occur prior to a fish being captured. More specifically, fish need to first encounter a lure, inspect it, and then decide to strike and ingest the lure (or not), thereby leading to a successful capture (Lennox et al., 2017). Relationships between activity and/or cortisol and novel object inspection have not been well studied, and work to date on this topic does not show consistent trends. However, some studies have addressed relationships between activity and cortisol with capture vulnerability. For example, asocial pumpkinseed sunfish (*Lepomis gibbosus*) that acclimate quickly to lab conditions (i.e., individuals that display reactive coping styles) were more likely to inspect novel objects, in this case fish traps, leading to increased capture vulnerability in traps (Wilson et al., 1993), suggesting that reactive individuals may be more prone to capture. In contrast, elevated activity consistent with proactive coping styles did not predict capture by anglers for either largemouth bass (Binder et al., 2012), or Eurasian perch (*Perca fluviatilis*) (Monk and Arlinghaus, 2018). It is possible that other traits, including sociability and environmental flexibility, are drivers of novel object (lure) inspections for largemouth bass, but these other traits were not measured in the current study, making links between these traits and novel object inspections speculative. A previous study found hatchery-reared rainbow trout to be more active than wild trout (Biro and Post, 2008), so future studies using wild largemouth bass, ideally without supplemental hatchery stocking, should be used to further explore relationships between behavioral phenotypes, physiological traits, and novel object inspections in wild populations. More

importantly, largemouth bass with high cortisol responsiveness (i.e., reactive stress coping styles) were previously shown to be less likely to be captured through hook-and-line angling (Louison et al., 2017), indicating that reactive fish are less likely to encounter, inspect, strike, and/or ingest novel objects, such as fishing lures, likely due to more ‘shy’ behavioral tendencies. Because results from the current study did not demonstrate a link between cortisol responsiveness and lure inspections, it is likely that cortisol responsiveness may not affect the lure inspection step leading to hook-and-line angling capture, but, instead, may affect other steps leading to capture, such as approaching, striking, and ingesting a lure. Together, results from this study demonstrate that neither cortisol responsiveness nor activity were significant predictors of the number of times largemouth bass inspected a novel object (fishing lure).

Baseline cortisol concentration influenced the number lure inspections performed by all study fish, but only after 2 weeks of feeding and food deprivation. More specifically, largemouth bass with low baseline cortisol concentrations were more likely to visit lures during a novel object assay relative to fish with high baseline cortisol concentrations, regardless of feeding/food deprivation treatment. Findings from Silva et al. (2010) showed that Senegalese sole with high baseline cortisol concentrations showed fewer escape attempts from a confined net. Results from the current study and Silva et al. (2010) both demonstrate negative relationships between baseline cortisol concentrations and proactive behaviors, including increased risk-taking and avoidance. One reason for a significant relationship between baseline cortisol concentrations and lure inspections in largemouth bass post-feeding/food deprivation treatment, but not pre-treatment, is that baseline cortisol concentrations are highly variable under differing environmental conditions (Cook et al., 2011). Therefore, a shift in the fishes' environment from the hatchery to long-term holding in tanks may have aided in eliciting the relationship between baseline cortisol concentrations and post-treatment lure inspections in both the fed and food deprived treatments of largemouth bass. Low baseline cortisol concentrations significantly increased the number of lure inspections of largemouth bass post-treatment, regardless if fish were fed or food deprived.

Although food deprivation influenced activity and baseline cortisol concentrations, food deprivation did not significantly predict a fish's likelihood to inspect fishing lures. The generalized linear mixed effects model used to predict post-treatment lure inspection did not find a significant effect of feeding treatment, such that the lure inspection

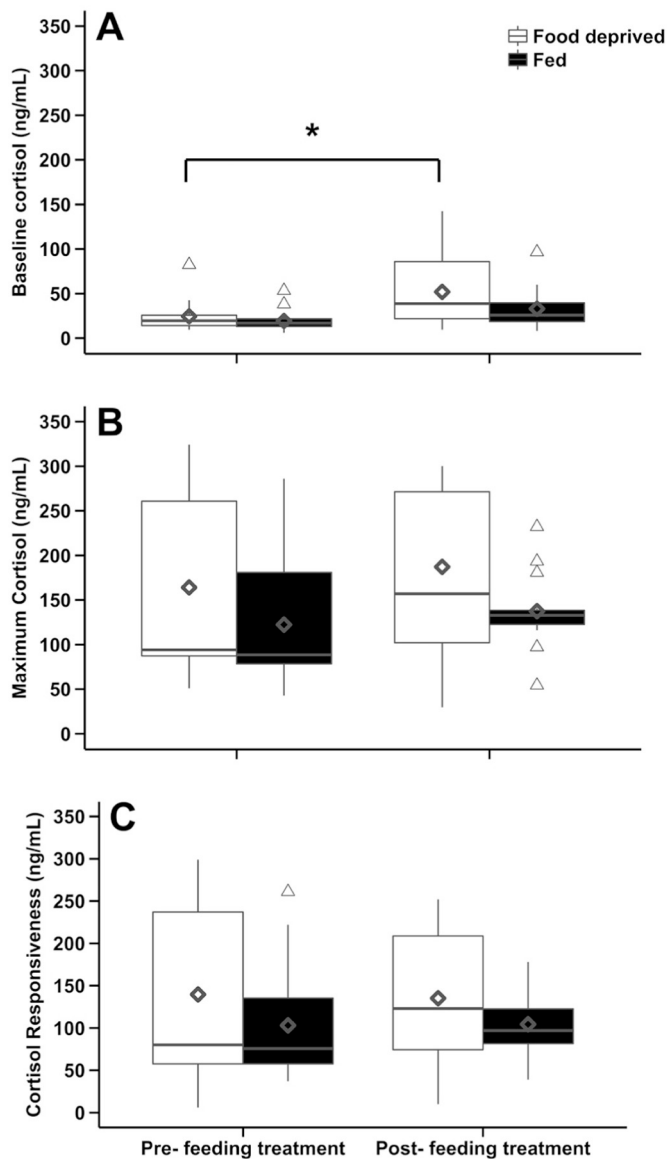


Fig. 1. Boxplots showing A) baseline cortisol, B) maximum cortisol, and C) cortisol responsiveness values both pre- and post-feeding treatment. White boxplots represent largemouth bass that were food deprived for 2 weeks, while black boxplots represent largemouth bass that were fed for 2 weeks. The lines in the boxes are medians and diamonds are means. The asterisk denotes a significant difference for baseline cortisol concentrations between pre-treatment and post-treatment food deprived largemouth bass ($p = .04$), and analyses are in Table 5.

behavior of fish was similar regardless if they had been fed or food deprived. Food deprivation can alter risk-taking behaviors in fish, whereby hunger increases an individual's likelihood to be involved in risky situations, such as in the presence of a predator, with the benefit of increased foraging success (Godin and Crossman, 1994; Härkönen et al., 2014b). If hunger had the same effect in the present study, food deprived largemouth bass would be expected to increase risky lure inspection behaviors, but this was not the case. Other studies found results to support the lack of relationship between food deprivation and novel object inspections. For example, food deprivation did not interact with cortisol responsiveness to explain risk-taking behaviors in rainbow trout (Thomson et al., 2012), and food deprivation did not influence novel object inspections in brown trout (*Salmo trutta*) (Naslund and Johnsson, 2016). Additionally, other studies found that reactive fish were more likely to adjust to changes in their environment compared to

Table 6

Summary of mixed effects models explaining activity (time spent swimming or distance moved during behavior assays) and the number lure (novel object) inspections performed by largemouth bass that were food deprived or that were fed for 2 weeks. Independent variables in the activity model include treatment (fed vs. food deprived), time (prior to feeding/food deprivation or after 2 weeks of feeding/food deprivation), and their interaction. Additional parameters relative weight and water temperature improved model fit for the lure visit model and were therefore included in the final model. Fish ID was used as a random effect in the activity model and fish ID and behavior arena were used as random effects in the lure visit model as the same fish were tested before and after the 2 week feeding/food deprivation period and were randomly assessed in one of two behavior arenas. A z-statistic is used in the model to describe lure inspections because this model had a Poisson error distribution as these were count data, while a t-statistic is used in the model to explain activity, as this variable had a Gaussian error distribution. Significant variables ($p < .05$) are in bold.

Factors	Coefficient	SEM	df	t/z	p
Activity					
Intercept	193.19	28.63	58.00	6.75	< 0.001
Treatment	18.08	41.16	58.00	0.44	0.662
Time	106.81	41.16	58.00	2.60	0.012
Treatment \times time	-50.45	58.20	58.00	-0.87	0.390
Lure visits					
Intercept	-5.20	3.21	-	-1.62	0.11
Treatment	0.49	0.54	-	0.92	0.36
Time	0.49	0.44	-	1.12	0.26
Relative weight	0.03	0.03	-	1.36	0.17
Water temperature	0.06	0.11	-	0.55	0.58
Treatment \times time	0.02	0.56	-	0.03	0.97

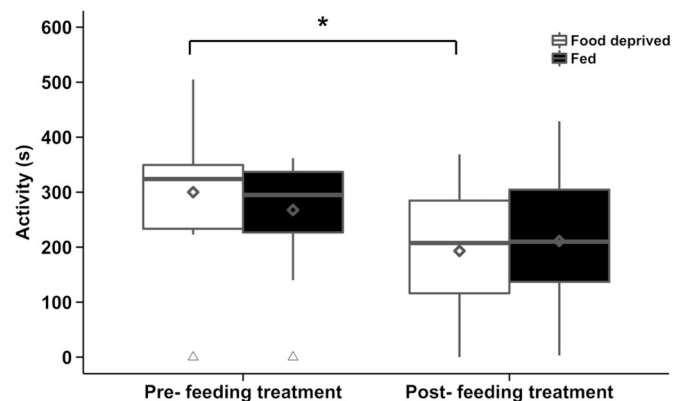


Fig. 2. Boxplots showing pre-lure introduction activity (which was correlated with post-lure introduction activity) in a behavior arena (time spent swimming, which was correlated with distance moved) for largemouth bass that had been fed or food deprived for 2 weeks. White boxplots represent largemouth bass that were food deprived, and black boxplots represent largemouth bass that had been fed for 2 weeks. Activity was measured prior to the onset of the feeding/food deprivation treatment (pre-feeding treatment) and again 2 weeks later (post-feeding treatment). Compared to the pre-treatment food deprived group, there was a significant decline in activity times for the post-treatment food deprived group, denoted by the asterisk ($p = .04$). Statistical tests are shown in Table 6.

proactive fish (Basic et al., 2012). Therefore, it was expected that food deprived proactive fish with low cortisol responsiveness would have been rigid in their behavioral responses and would have maintained similar novel lure inspections post-treatment, while food deprived reactive fish with high cortisol responsiveness would have adapted to the food deprivation and decreased their lure visits, but those risk-taking behavioral differences were not captured in the lure inspection data. One possibility for no observed response between behavioral and physiological traits and food deprivation may be that the 2 week period

Table 7

Summary of mixed effects models explaining the number of lure visits performed by largemouth bass prior to being fed/food deprived for 2 weeks (pre-treatment), or following 2 weeks of either feeding or food deprivation (post-treatment). Two separate mixed effects models were used due to different outputs from pre- vs. post-feeding treatment PCA scores given in Table 2. Independent variables in both models include treatment (fed or food deprived), PC scores (see Tables 1 and 2), baseline cortisol, and cortisol responsiveness (Table 4). Water temperature, behavior arena number, and relative weight were added as independent variables for the pre-treatment model to improve model fit. Fish ID was used as a random effect in both models. The models used in these analyses were selected based on model selection activities shown in Table A.1. Significant variables ($p < .05$) are in bold.

Factors	coefficient	SEM	z	p
Pre-treatment				
Intercept	-8.87	6.74	-1.32	0.188
Treatment	0.67	0.69	0.97	0.334
PC1	0.21	0.29	0.73	0.463
Baseline cortisol	< 0.01	< 0.01	0.01	0.993
Cortisol responsiveness	< 0.01	< 0.01	0.06	0.951
Water temperature	0.03	0.26	0.12	0.903
Behavior arena number	1.55	0.80	1.93	0.054
Relative weight	0.05	0.05	1.22	0.222
Post-treatment				
Intercept	-0.92	0.99	0.93	0.354
Treatment	0.37	0.69	0.54	0.586
PC1	< 0.01	0.28	0.03	0.973
PC2	0.01	0.29	-0.05	0.962
Baseline cortisol	< 0.01	< 0.01	-2.44	0.015
Cortisol responsiveness	< 0.01	< 0.01	-0.19	0.847

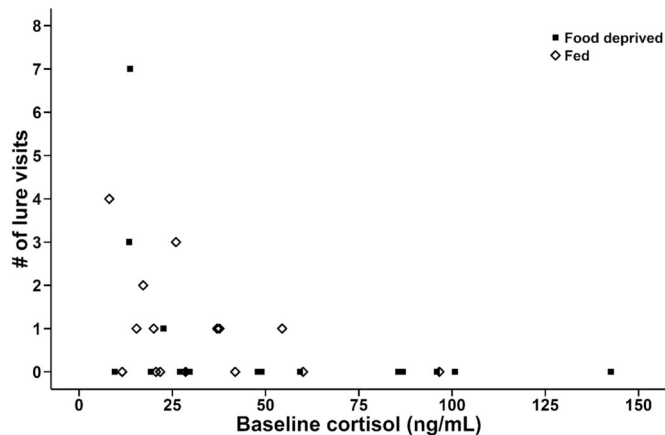


Fig. 3. Scatter plot showing the relationship between baseline cortisol concentration (ng/mL) and number of times largemouth bass visited the presented fishing lure (novel object inspection, which was correlated to time spent near the lure) in a behavior arena post-treatment (after 2 weeks of feeding or food deprivation; $p = .03$). Dark squares represent largemouth bass in the food deprived treatment and open diamonds represent largemouth bass in the fed treatment. The negative relationship between baseline cortisol concentration and number of lure visits held true for largemouth bass in both of the fed and food deprived treatments.

of food deprivation may have been too long in duration, potentially eliminating observation of changes in risk-taking behaviors. One of the consequences of food deprivation for fishes is a reduction in activity and metabolism, presumably to conserve energy through decreased energy expenditure (Jobling, 2011; Killen et al., 2011; Mendez and Wieser, 1993). A previous study with largemouth bass fasted for 16 days showed reduced body and liver mass as well as reduced metabolic rates compared to their force-fed conspecifics (Gingerich et al., 2010), demonstrating that food deprivation periods of this duration can have pronounced impacts on largemouth bass. So, fish in the current

study may have altered behaviors and became less active (i.e., reactive) to save energy and increase survival, which may have influenced lure inspection behaviors in unexpected ways (McCue, 2010; Navarro and Gutierrez, 1995; Tucker et al., 2018; Wang et al., 2006). Because activity did not influence lure inspections either pre- or post-feeding treatment, the mechanisms driving relationships between activity and novel object inspection remain unclear. Future studies may wish to ask similar questions, but use a shorter food deprivation period to better define how behaviors change over time. Regardless of the mechanism, 2 weeks of food deprivation did not elicit changes in lure inspection behaviors of largemouth bass.

Largemouth bass did not show distinctive coping styles when activity behaviors and cortisol metrics were considered together. Coping style refers to correlated behavioral and physiological traits that can predict how individuals react in stressful situations (Overli et al., 2007). For example, fish with reactive coping styles exhibit timid, inactive behaviors and high cortisol responsiveness during stressful situations, while individuals with proactive coping styles are bold, active, and have low cortisol responsiveness in stressful situations (Overli et al., 2007). Theoretically, activity behavior and cortisol metrics measured in this study could have been used in combination to explain a fish's coping style in response to novel objects, in this case, fishing lures. However, results did not indicate distinctive coping styles for activity behaviors and cortisol metrics in largemouth bass. Other studies support this finding; two with rainbow trout, which found no clear relationships between boldness behaviors and cortisol concentrations (Ruiz-Gomez et al., 2008; Thomson et al., 2012), as well as one with Senegalese sole (*Solea senegalensis*), which found that post-stressor cortisol concentration and behaviors (feeding latency and duration of escape attempts) were not correlated (Silva et al., 2010). One proposed explanation for uncoupled behaviors and stress responsiveness may be that fish can be highly plastic in their phenotypic response to stressful stimuli, whereby cortisol concentrations cannot always accurately indicate an individual's phenotypic response after stressful situations (Thomson et al., 2012). In other words, environmental and situational factors may have had a greater influence in the variation of activity behaviors than individual genotypes, leading to temporary environmental influences playing a larger role in the measured phenotype of individual fish (Sih and Bell, 2008). Activity behaviors and cortisol metrics of largemouth bass did not combine to exhibit distinct coping styles.

5. Conclusions

Results from this study can inform fisheries management in four important ways. First, previous work has demonstrated that cortisol responsiveness is a strong predictor of hook-and-line capture in largemouth bass (Louison et al., 2017), but, results from the current study indicate no relationship between cortisol responsiveness and lure inspections. This contrast reinforces the concept that there are many steps leading to the capture of fish by anglers, and that the importance of cortisol responsiveness may not be realized across all steps in the capture process (Lennox et al., 2017). Thus, while harvest by anglers has the potential to disproportionately remove largemouth bass with low cortisol responsiveness from a population leaving individuals less likely to strike fishing lures (Louison et al., 2017; Philipp et al., 2009b), data from the current study suggest that such harvest would not be expected to influence the frequency of lure inspection behaviors. Future studies should investigate links between additional traits to discern predictors that may influence encounter, inspection, lure-striking, and ingestion behaviors leading to capture, and that may be under selection from harvest. Second, food deprivation has previously been shown to decrease activity rates in fish (Gingerich et al., 2010; Martinez, 2004), and this study is one of the first to show that food deprivation can also increase baseline cortisol concentrations of largemouth bass, which may have negative ecological impacts on foraging and angling

vulnerability. More specifically, food deprivation may lead to stress (increased cortisol levels) and reduced swimming activity to save energy at the cost of decreased foraging success, and this stress may also reduce novel object inspection behaviors (i.e., lures) and subsequent reductions in capture rates by anglers (Jobling, 2011; Mendez and Wieser, 1993). Thus, managers should be aware of levels of prey abundance in the environment relative to largemouth bass abundance, and should consider adjusting management actions accordingly (i.e., stock fewer largemouth bass, stock supplemental prey) to ensure that stressors caused by food deprivation do not result in reduced catch rates and angler satisfaction (Hoxmeier and Wahl, 2002). Third, food deprivation alone was shown in the present study to have little influence on lure inspection behaviors. As such, any potential influence of selective harvest on behavioral and physiological traits through recreational angling would be expected to be similar across environmental contexts. Therefore, if largemouth bass in exploited populations are food deprived, managers would not expect to see enhanced evidence of selective fishing exploitation on lure inspection behaviors relative to individuals from populations with ample prey resources. Finally, because coping styles can predispose fish to angling capture through differences in interactions and inspection of fishing lures, it is important for fisheries managers to understand coping styles and how they relate to angling vulnerability to minimize effects of fisheries-induced evolution in fish populations. For example, activity behaviors and stress responsiveness, both of which are elements of coping styles, have previously been shown to influence capture, and can be influenced by selection and potentially result in reduced capture rates. Should managers suspect reduced capture rates through selection against coping styles, one possibility to restore capture rates might be to stock recreationally targeted fish from relic fish populations that have not been exposed to angling (Hessenauer et al., 2016). In addition, in situations where managers suspect that capture rates have been reduced through selection against coping styles, managers could implement a 'catch-and-release' regulation that mandates no fish be harvested to ensure that vulnerable phenotypes are not removed from a population (Arlinghaus et al., 2007). But, if behavior and stress responsiveness traits are uncoupled in certain species, including largemouth bass, these traits must be investigated independently to better predict hook-and-line vulnerability. Together, relationships determined from this study can be used to help fisheries managers further protect largemouth bass from potential negative consequences inflicted through angling exploitation.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cbpa.2019.110556>.

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