

INTERSECTION OF ENVIRONMENT, BEHAVIOR, AND PHYSIOLOGICAL TRAITS ON  
ANGLING VULNERABILITY

BY

TONIANN DANIELLE KEILING

THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science in Natural Resources and Environmental Science  
in the Graduate College of the  
University of Illinois at Urbana-Champaign, 2019

Urbana, Illinois

Master's Committee:

Associate Professor Cory Suski, co-chair  
Adjunct Assistant Professor Jeffrey A. Stein, co-chair  
Professor Alison Bell

## **ABSTRACT**

Recreational fishing can negatively impact fish populations through the removal of fish with specific heritable traits. The mechanism(s) defining why fish strike lures are unknown, as are how environmental factors influence capture, leaving knowledge gaps surrounding selection effects of capture and harvest. Objectives included how physiological and behavioral traits interacted with environmental contexts to influence angling vulnerability in three separate studies. Largemouth bass behavior assays and stress responsiveness were measured in a laboratory and followed by angling in ponds. Environmental contexts included food availability and the presence or absence of structure to test for variations in capture vulnerability. Results showed that behavior type did not influence capture, either on its own or across feeding or habitat contexts. Rather, prey availability only influenced capture rates across angling days. Results are further discussed within the framework of factors leading to fish captures, and how selective harvest may shape fish populations and aquatic ecosystems.

## **ACKNOWLEDGEMENTS**

I would like to acknowledge Dr. Jeffrey Stein for allowing us to use the INHS Aquatic Research Facility, where all fish holding, data collection, and experimental angling trials took place. I would also like to acknowledge Justin Rondón and Joey Nolan, who helped immensely at the ponds facility. A big thanks to Emi Tucker and Sarah Molinaro who helped with experimental set-up and data collection, and to Qihong Dai, who collected all of my blood samples for my cortisol analysis. Lastly, I'd like to thank Katey Strailey, Amaryllis Adey, and other graduate students I received writing and editing help from along the way. I'm thankful for the amount of support I received from colleagues in the NRES department and Suski lab, especially Dr. Cory Suski and Dr. Michael Louison, as well as other (very busy) graduate students.

## TABLE OF CONTENTS

CHAPTER 1: LITERATURE REVIEW .....	1
CHAPTER 2: BIG, HUNGRY FISH GET THE LURE: SIZE AND FOOD AVAILABILITY DETERMINE CAPTURE OVER BOLDNESS AND EXPLORATORY BEHAVIORS.....	9
CHAPTER 3: LARGEMOUTH BASS ARE NOT CHOOSY: CAPTURED FISH DO NOT SHOW HABITAT PREFERENCES, REGARDLESS OF ACTIVITY AND BOLDNESS BEHAVIORS.....	35
CHAPTER 4: FOOD DEPRIVED FISH ARE INACTIVE AND STRESSED, BUT DO NOT ALTER LURE INSPECTIONS.....	65
CHAPTER 5: SUMMARY AND BROADER CONTEXT.....	96
REFERENCES.....	99
APPENDIX A: SUPPLEMENTARY TABLES AND FIGURES.....	120

## **CHAPTER 1: LITERATURE REVIEW**

Human activities can influence animal populations on an evolutionary level, with many changes occurring rapidly over time (Sih 2013). Population changes, often caused by humans acting as predators, can fall under the umbrella of human-induced rapid environmental change, or HIREC (Sih 2013). Examples can be seen through human introduction of invasive species, climate change impacts, alterations of habitat (Sih 2013), and even the history of humans breeding domesticated dogs (Wang et al. 2013). All of these examples have caused artificial selection by favoring individuals with morphological traits that are well-suited for changing environments, and therefore indirectly cause the elimination of unfavorable traits from a species' phenotype (Sih 2013; Wang et al. 2013). An additional example of humans altering animal populations at an evolutionary level is the selection of specific animal morphologies through hunting (Festa-Bianchet 2016). Trophy hunting that targets individuals with large horns, antlers, or tusks, artificially selects for animals with smaller protective morphological traits, and can lead to reduced phenotypic diversity within a population (Festa-Bianchet 2016). For example, selective hunting removed the oldest rams with the largest horns, reducing the number of mature reproductive individuals, which lead to a population decline (Coltman et al. 2003). In addition, selective harvest through commercial fishing has led to smaller fish sizes as a result of the disproportionate removal of large, fecund individuals, which, in turn, can lead to maturation at smaller sizes and younger ages (Reznick and Ghalambor 2005). Small fish remaining in these populations have evolved to grow faster, reach maturity earlier, and therefore reproduce at earlier ages, a phenomenon commonly described as fisheries induced evolution (FIE) (Reznick and Ghalambor 2005; Kuparinen and Merilä 2007). FIE occurs because, from a life history perspective, it is more profitable to grow quickly and minimize susceptibility to predation (here,

through harvest) and reproduce as soon as possible to maintain high fitness (Reznick and Ghalambor 2005). Negative consequences of selection for small individuals include decreased survival (Olsen et al. 2004), declines in mean size and biomass (Conover et al. 2009), and increased adaptation to high fishing pressures, which can translate to low resilience to environmental change (Heino et al. 2015). HIREC is problematic because while the rate of change due to humans can occur in as little time as a few generations, the rate at which populations return to their original state is typically slow, which means humans have the ability to quickly cause negative ecological consequences with long-lasting impacts (Conover et al. 2009). Together, the disproportionate removal of specific phenotypes, and the rate at which this occurs due to human harvest, can lead to population-level changes for animals.

Freshwater recreational angling is a popular sport that has been shown to alter fish populations, likely through the selective removal of specific phenotypes from a population. Globally, recreational fishing involves millions of anglers capturing millions of fish, accrues billions of dollars for local economies annually, and has the potential to alter populations due to the sheer number of fish removed from aquatic ecosystems (Cooke and Cowx 2004). More importantly, the propensity of fish to be captured by anglers is a heritable trait, such that selective removal of fish that are vulnerable to angling can lead in a decline in catchability for a population (Philipp et al. 2009). Thus, the selective removal of fish by anglers may result in heritable changes to individual fish and alter populations, making vulnerability to angling important for managers to consider.

For fish to be captured by anglers, two conditions must be met: 1) the angler (represented by the location of the lure presentation) and the fish must overlap spatially, and, 2) fish that encounter a lure must be motivated to ingest it. Habitat use in fish is influenced by the trade-off

between predation risk and acquisition of energetic resources (Gallagher et al. 2017); this trade-off is demonstrated through non-random habitat use whereby fish typically have consistent, well-defined habitat preferences (Lennox et al. 2017; Monk and Arlinghaus 2017). For example, northern pike (*Esox lucius*) are typically found in shallow littoral habitats with dense aquatic vegetation, an ideal environment for foraging (Arlinghaus et al. 2017a), and freshwater cyprinids only coexist with predator species in a lake if complex refuges are available or otherwise occupy shallow waters not accessible to large predators (Jackson et al. 2001). Anglers are aware of the habitat requirements of their quarry and therefore prioritize fishing in specific areas preferred by their target species, with one instance of habitat overlap seen in a study by Weimer et al. (2014), where anglers in a study lake during the summer targeted the same vegetated habitats and depths that most bluegill sunfish (*Lepomis macrochirus*) preferred. Upon encountering a fishing lure, there are a number of reasons that fish may ingest it. For example, hungry fish have been shown to be less selective and more willing to eat prey items they are not familiar with, such as fishing lures (Lennox et al. 2017). Additional drivers of angling vulnerability include morphology (Klefoth et al. 2017), past experiences with fishing lures (Young and Hayes 2004), stomach content, appetite hormone levels (Volkoff 2011), and characteristics of the presented lure (Arlinghaus et al. 2017b). Clearly, there are many factors that determine whether or not fish ingest fishing lures, including habitat type, spatial overlap between anglers and fish, and lure characteristics.

The likelihood of an individual fish being captured through angling can also be associated with correlations between specific behaviors and physiological traits. Fish exhibit consistent, repeatable, and heritable differences in behaviors known as behavioral syndromes (Bell 2007). Behavioral science encompasses animal behavior on five different axes: shyness-

boldness, exploration-avoidance, activity, aggression, and sociability (Réale et al. 2007).

Individual fish fall along continua of these axes, which are used to describe behaviors between two extremes (Wilson et al. 1993). One example is from a study by Wilson et al. (1993) with pumpkinseed sunfish (*Lepomis gibbosus*), whereby the boldest individuals approached novel objects and the shyest individuals avoided them. These behavioral syndromes are often correlated with physiological traits; bold, exploratory, active individuals are associated with high metabolic rates and shy, neophobic, inactive individuals are associated with low metabolic rates (Killen 2011). Physiological links to behavior can also be seen through a fish's stress response to negative stimuli. For example, Louison et al. (2017) found that fish displaying high stress responsiveness had low probabilities to strike fishing lures, likely because animals with a high stress responsiveness tend to perform 'freeze' behaviors in situations with a perceived high threat risk (Bell and Stamps 2004). Both the physiology and behavior of individual fish must be considered simultaneously to fully understand drivers of angling vulnerability.

The terms pace-of-life syndromes (Reale et al. 2010) or stress-coping styles (Koolhaas et al. 2010) are used to describe correlations between consistent behaviors and physiological traits of individuals. Animals with reactive stress-coping styles, for example, exhibit characteristics such as low propensity to take risks under perceived threat, low activity rates, low metabolic rates, high stress responsiveness, and high behavioral plasticity in changing environments (Conrad et al. 2011). In contrast, proactive fish exhibit the opposite characteristics, including bold behaviors, high exploration in novel environments, and low stress responsiveness (Overli et al. 2007). Fish that are vulnerable to angling typically have elevated metabolic rates (Redpath et al. 2010), higher heart rates (Cooke et al. 2007), and increased reproductive fitness (Sutter et al. 2012) compared to fish with low angling vulnerability, all characteristics related to the proactive



stress-coping style. For example, largemouth bass (*Micropterus salmoides*) that experienced little to no angling pressure over approximately a century had higher metabolic rates than populations that had been exploited by anglers over the same time period (Hessenauer et al. 2015). Many wild populations contain a mix of individuals with different stress-coping styles (i.e. a mix of bold and shy with high and low metabolic rates, respectively) maintained through frequency dependent selection (Ayala and Campbell 1974). However, a fish's internal state, including appetite, can also influence its stress-coping style. Increased hunger due to high metabolic rates in proactive fish may increase encounter rates with fishing lures (Lennox et al. 2017). For example, hungry sticklebacks (*Gasterosteus aculeatus*) spent more time swimming while searching for prey items than those that were satiated (Ringler 1983), and increased activity can lead to increased encounter rates with fishing lures. Therefore, a fish's hunger levels, combined with behavior and hormonal status (i.e. within the fish's gut) based on the amount of food in the fish's stomach (Volkoff 2011), can influence the likelihood of a fish interacting with fishing lures. Thus, individual stress-coping styles influence both encounter rates and the likelihood fishing lure ingestion, which combine to determine individual angling vulnerability.

The study of angling vulnerability becomes interesting when the interaction of stress-coping styles and environment is considered. Food availability and habitat preference can both influence a fish's behavior and physiology (Killen et al. 2013), with changes in behavior type extending to encounter rates with fishing lures (Härkönen et al. 2014). Because habitat and habitat choice are key components of angling vulnerability (Matthias et al. 2014; Lennox et al. 2017; Monk and Arlinghaus 2018), habitat shifts due to natural or anthropogenic variation have the potential to create changes in individual angling vulnerability. For example, fish with

different stress-coping styles have been shown to occupy different habitat types, with proactive Eurasian perch (*Perca fluviatilis*) more likely to spend time in open water than reactive conspecifics, which typically occupy sheltered areas to minimize predation risk (Härkönen et al. 2016). These habitat preferences extend to catchability, whereby proactive fish are more likely to be captured by anglers compared to their reactive conspecifics (Härkönen et al. 2016). Similarly, timid, reactive bluegill sunfish (*Lepomis macrochirus*) occupy highly sheltered habitats (Wilson et al. 2011a). Continuous fishing pressure in specific habitats can lead to fish avoidance in these habitats (Pauli and Sih 2017). Food availability can also influence angling vulnerability. This influence was shown in a study by Mogensen et al. (2014) where catch rates in a lake with low seasonal prey abundance were higher than those in a lake with high prey abundance, highlighting the increase in overall population vulnerability in the context of low prey resources due to increased hunger. Together, it has been clearly demonstrated that different environmental contexts, including habitat alterations, can interact with stress-coping styles to alter individual angling vulnerability.

While a number of potential mechanisms explaining why fish strike lures have been proposed (Lennox et al. 2017), the reality is that work explicitly linking these mechanisms to the actual angling response of fishes is relatively scarce – most work on this topic focuses on stress-coping styles and angling vulnerability within stable environments and ignores the dynamic nature of both abiotic and biotic environmental factors (Sih and Bell 2008). More importantly, of the work performed on this topic to date, the majority of studies have been completed under static, isolated conditions with factors typically quantified independently and often at a group level (i.e., how do bold vs. shy fish respond to fishing lures?), with integrated studies lacking, especially around individual variation in different environmental settings (Sih and Bell 2008).

Therefore, there is a critical need to better define the factors that drive angling vulnerability, and how those factors may change across differing environmental conditions. Research on angling vulnerability needs to incorporate environmental contexts because fish habitats are rather dynamic both over the short term (i.e., successional changes in macrophytes, seasonal differences in temperature; Fraser et al. 1993) and over the long term (i.e., human impacts on ecosystems such as urbanization or habitat destruction) (Killen et al. 2016). The ability to predict the factors that predispose a fish to capture by anglers has important benefits for defining which segments of a population may be vulnerable to exploitation, as well as the potential population-level outcomes that may occur through FIE if a population of fish experiences extended periods of targeted harvest. For example, in ecosystems with high prey abundance, if periods of prolonged angling harvest are imposed, and if characteristics of a proactive stress-coping style are drivers of angling vulnerability, that population could experience a disproportionate removal of proactive fish (and therefore selection for reactive fish to remain in a population). This removal could alter the population, whereby the entire suite of behavioral and physiological traits associated with the reactive stress-coping style dominate, such as high stress responsiveness and neophobic behaviors (Heino et al. 2013), as seen through most individuals exhibiting low metabolic rates and quick learning under changing environments. In addition, angling that artificially selects for reactive fish could lead to an increase of individuals with traits less likely to elicit a response to fishing lures. This lack of response to lures can translate to a reduction in the number of individuals vulnerable to angling and a reduction in angler catch rates and satisfaction, which, in turn, could negatively impact local economies (Philipp et al. 2009; Reale et al. 2010; Hessenauer et al. 2015; Killen et al. 2016). Conversely, in conditions of low prey abundance, proactive and reactive individuals may be equally vulnerable to fishing lures,

whereby decreased prey availability leads to increases in hunger levels for all fish, increased boldness and activity levels, and all fish become more likely to encounter and strike lures. Understanding why and when fish are vulnerable to fishing lures has widespread ecological impacts for entire populations, as well as economic impacts for the recreational fishing industry.

Based on this background, the objective of my thesis was to define how stress-coping styles and environmental contexts interact to drive individual angling vulnerability. To accomplish this, I conducted three separate yet complementary studies (chapters), asking questions related to how behavior, physiology, and environment combine to drive angling vulnerability in largemouth bass. The first chapter determined the interaction of food availability and behavioral type to see how the two interact to predict individual angling vulnerability. The second chapter combined habitat selection and individual behavioral types to see how the two intersected to determine angling vulnerability. The third chapter identified possible changes in individual behavior and stress responsiveness due to food deprivation, and how these changes affect novel object (lure) inspections. Together, this research provides pertinent information on the effect of HIREC through recreational fishing, and can inform management decisions to maintain stable sportfish populations and angler satisfaction.

## **CHAPTER 2: BIG, HUNGRY FISH GET THE LURE: SIZE AND FOOD AVAILABILITY DETERMINE CAPTURE OVER BOLDNESS AND EXPLORATORY BEHAVIORS**

### **2.1 Introduction**

Fish have previously been found to demonstrate consistent, repeatable and heritable differences in behaviors known as behavioral syndromes (Bell, 2007), with behaviors being grouped into five axes (boldness, exploration, sociability, activity and aggression; Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Conrad, Weinersmith, Brodin, Saltz, & Sih, 2011). Research has also found that these syndromes can be influenced by environmental conditions (Ruiz-Gomez et al., 2008; Dingemanse & Wolf, 2010; Killen, Marras, Metcalfe, McKenzie, & Domenici, 2013). More specifically, environmental context can influence a fish's behavior, resulting in behavioral shifts often referred to as behavioral plasticity (Killen et al., 2013). One example of behavioral plasticity due to environmental context relates to food availability, where food shortages created increased variability in boldness behaviors of European sea bass, (*Dicentrarchus labrax*) during foraging (Killen, Adriaenssens, Marras, Claireaux, & Cooke, 2016). An additional example of behavioral plasticity due to environmental context is a 2.5- to 6-fold increase in boldness behaviors of speckled damselfish, (*Pomacentrus bankanensis*) when temperatures were raised from 24 °C to 27 °C (Biro, Beckmann, & Stamps, 2010). Clearly, individual fish behavior can change with environmental context.

Behavior has also been shown to play a large role in influencing vulnerability to hook-and-line angling. Simply encountering a lure is not sufficient to cause a fish to strike (Monk & Arlinghaus, 2017), and lure-striking decisions by fish result from the combination of a number of factors that include encountering angling gear, the interaction of the fish with the angling gear,

and internal characteristics of the fish, including aspects of behavioral syndromes and correlated traits, such as metabolic rates (Stoner 2004; Lennox et al. 2017). More specifically, work has shown that bold and exploratory behavioral phenotypes can be more vulnerable to angling than shy behavioral phenotypes (Biro & Post, 2008; Biro & Sampson, 2015), though this finding does not occur in all species, including bluegill (*Lepomis macrochirus*) (Wilson et al., 2011; Mittelbach, Ballew, & Kjelson, 2014). In addition, hunger from reduced food intake can increase risk-taking and exploratory behaviors (Beukema 1968), which can lead to increased capture rates (Härkönen et al. 2014; Lennox et al. 2017). Larger fish are often behaviorally dominant to smaller fish (Krause 1994), which could lead to increased capture of larger fish. Importantly, the repeated removal of fish with specific, heritable behavioral traits by anglers (i.e., bold or active individuals; [Biro & Post, 2008]) has the potential to result in behavioral changes to a population over the long term (Cooke, Suski, Ostrand, Wahl, & Philipp, 2007; Heino et al., 2013; Alós, Palmer, Rosselló, & Arlinghaus, 2016), often referred to as the timidity-syndrome (Arlinghaus et al. 2017b). Thus, if angling preferentially selects and removes bold phenotypes from a population, over many generations due to the heritability of angling vulnerability (Philipp et al. 2009), it may be that only shy individuals with lower angling vulnerability remain. Angling therefore has the potential to render fish populations less catchable overall (Philipp et al. 2009) due to timidity-syndromes (Arlinghaus et al. 2017b), making mechanisms of vulnerability an important concept for managers to consider to conserve, protect, and enhance recreational fish stocks.

While a number of potential mechanisms explaining angling vulnerability in the context of behavior have been proposed (Stoner 2004; Lennox et al. 2017), findings and trends across studies have been variable and inconsistent, and many factors predicting angling vulnerability

have not been explored, limiting our ability to predict how harvest by anglers can shape populations. For example, most studies to date that focus on angling vulnerability have used only a single behavioral axis (typically boldness), limiting the ability to define the relative importance of different behavioral axes on vulnerability. In addition, most work on this topic has focused on behavioral phenotypes and angling vulnerability within a single, stable environment, ignoring the dynamic nature of both abiotic and biotic environmental factors, precluding the ability to define the role of environmental conditions and behavioral plasticity on vulnerability (Sih & Bell, 2008; Lennox et al., 2017). But, certain behavioral phenotypes may be highly vulnerable to angling under certain environmental conditions and not others. If this is the case, then the evolutionary outcomes of angling selection could differ greatly between water bodies, or even within water bodies at different times, depending on the environmental conditions present in that system. Therefore, there is a critical need to better define the factors that influence angling vulnerability and, in particular, how these factors change across contexts to successfully predict the evolutionary consequences of angling.

To address this knowledge gap, the goal of this study was to identify how behavioral phenotype and food availability interact to drive vulnerability to angling of individual largemouth bass (*Micropterus salmoides*). To accomplish this, the behavioral phenotypes of individual largemouth bass were first determined by a series of behavior assays targeting two different axes of behavior (boldness and exploration). Following this, angling sessions were carried out in ponds with and without prey fish. It was predicted that bold, explorative individuals would be most vulnerable to angling in the pond with prey available, and due to increased hunger, both shy, non-exploratory and bold, exploratory phenotypes would be equally vulnerable to capture in the pond with no prey.

## 2.2 Methods

All described procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (IACUC), protocol no. 17160.

### 2.2.1 Study Animals

Largemouth bass ( $n=250$ ) were acquired from Keystone Fish Hatchery, Richmond, IL and transported to Illinois Natural History Survey (INHS) Aquatic Research Facility near Champaign, IL on 19 September 2017. These hatchery-reared fish were one year old and naïve to fishing lures at the start of the experiment. The population had been at the hatchery for about 11 generations and the brood stock was initially collected from wild populations in southern Illinois. Mean total length (TL)  $\pm$  standard deviation (SD) of fish was  $180 \pm 16$  mm and mean relative weight was  $102 \pm 0.1$  (Table 2.1), based on standard weight calculations for largemouth bass (Murphy et al. 1991). This size of fish has been previously shown to be catchable by anglers in both wild populations and in angling simulation studies (Murphy et al., 1991; Hessenauer, Vokoun, Davis, Jacobs, & O'Donnell, 2016; Sass, Gaeta, Allen, Suski, & Shaw, 2018). Upon arrival at the Research Facility, all fish were divided and held among 12 circular 1,135 L outdoor tanks supplied with continuous flow-through aerated water from a nearby 0.04 ha earthen pond at a rate of  $\sim 8$  water exchanges per day. Mean water temperature during holding was  $21.8 \pm 2.6$  °C and mean dissolved oxygen concentration was  $9.2 \pm 0.8$  mg/L. Fish had been feed trained since a young age, and were fed Skretting high protein pellets (Tooele, Utah) *ad libitum* daily during holding at the INHS Aquatic Research Facility. Forty-eight hours after transport, all fish were implanted with passive integrated transponder (PIT) tags (10 mm length  $\times$  2 mm diameter, HPT12, Biomark Inc., Boise, Idaho) for individual identification. Fish



were allowed to acclimate to holding conditions for 3 additional days before behavior assays began on 25 September 2017.

### *2.2.2 Behavior Assays*

A total of 143 largemouth bass were randomly selected for behavior assays. Prior to behavior assays, fish were moved from outdoor tanks to indoor aquaria to acclimate to lab conditions for 17-23 hours, during which time no feeding occurred. Indoor aquaria (121 L opaque plastic holding tanks) were each divided by an opaque plastic barrier with holes to allow for water flow between two chambers. Each aquarium held 2 fish, one on either side of the barrier to minimize interactions between individuals. A re-circulating supply of aerated water was provided *via* a pump from a reservoir tank equipped with an aerator (Outdoor air pump, Pentair, Cary, North Carolina). The temperature in the indoor aquaria was maintained between 23 and 24 ° C with a TK 500 Heater-Chiller (Teco, Revenna, Italy) and dissolved oxygen concentrations remained above 8.0 mg/L, verified with a dissolved oxygen probe (YSI Inc. Professional Plus, Yellow Springs, OH).

Behavior assays were conducted from 25 September 2017 to 5 October 2017 and between 0830 and 1430 hours in one of four identical arenas. Arenas consisted of a 180 × 65 cm opaque rectangular tank filled with water to a depth of 35 cm (410 L). A video camera (GoPro Hero 3 or Session 4, San Mateo, California) suspended above the arena from a PVC frame was used to record fish location and behavior. The arena had a refuge zone and an open zone that were separated by an opaque 6 mm -thick Plexiglas divider. The refuge zone occupied about a quarter of the tank and had a natural gravel bottom with plastic aquarium plants for shelter, and the open zone was the remaining three-quarters of the tank and had no bottom substrate or

vegetation, similar to behavioral arenas used in previous studies (Dingemanse, Barber, Wright, & Brommer, 2012; Killen, Marras, Ryan, Domenici, & McKenzie, 2012; Figure A.1).

Immediately before behavior assays commenced, largemouth bass were netted from indoor aquaria and placed into the refuge zone of an arena and left to acclimate for 10-min. An acclimation time of ~10 min is common for behavior assays (Vainikka, Tammela, & Hyvärinen, 2016; Louison, Adhikari, Stein, & Suski, 2017), and preliminary trials revealed that largemouth bass typically began slowly moving around the refuge zone within 5-6 min after transfer. Following the 10-min acclimation period and immediately after video cameras began recording, the divider was removed using an overhead pulley system, allowing fish to emerge from the refuge zone and explore the open zone, similar to behavior assays from other studies (Jenjan et al. 2013; Louison et al. 2017). After a 15-min period where fish were allowed to explore the arena, a simulated predator attack was imposed. For this, a model great blue heron, (*Ardea herodias*) measuring 73.7 cm in height was used (United Aquatics LLC, Marlton, New Jersey), as great blue herons are a common predator of largemouth bass and have been used as simulated predators in other behavior studies with fish (Cooke et al. 2003; Bell & Stamps, 2004). For the simulated predator attack, an observer held the heron model over the behavioral arena and struck the water four times in a square pattern at the far end opposite the refuge with the heron's beak. The location of the heron strikes was the same across trials and did not vary with the position of the fish within the arena. After striking the water, the observer placed the entire heron model into the arena at the end opposite the refuge for 30 min (Huntingford & Wright, 1993; Godin & Crossman, 1994; Fedele, 2017), during which time fish location continued to be monitored. No feeding occurred in the behavior assay tanks. Following this 30-min period, the behavior assay was considered complete, video recording stopped and all fish were returned to the outdoor tank

system to continue feeding until being divided and stocked into pond treatments prior to the start of angling trials (see below). Each fish was tested only once for behaviors because previous studies using similar arenas have shown these behaviors to be repeatable (Hart et al. 2009; Kortet et al. 2014; Mazué et al. 2015). Additionally, repeated tests were avoided because they can encourage habituation (Réale et al. 2007).

Four metrics were used to score fish behavior observed in the arena: 1) latency to emerge from the refuge after the divider was initially raised (termed ‘initial latency to emerge’), 2) duration of the ‘freeze response’ behavior after the simulated predator attack (termed ‘freeze time’), 3) latency to re-emerge from the refuge following the simulated predator attack (termed ‘latency to re-emerge’) and 4) time to approach the predator (termed ‘approach time’) (Pauli et al. 2015). Behavior prior to the simulated predator attack, initial latency to emerge, was considered to reflect a fish’s exploratory tendency, while behaviors following the attack (and the imposition of risk), freeze time, re-emergence time, and approach time, were considered to reflect a fish’s boldness (Réale et al. 2007). Emergence from the refuge occurred when the entire body length of the fish crossed a PVC pipe separating the refuge area from the open area of the arena (Louison et al. 2017). Nearly all largemouth bass returned to the refuge and exhibited the ‘freeze response’ after the simulated predator attack (Bell & Stamps, 2004); thus, ‘freeze time’ and ‘latency to re-emerge’ from the refuge post simulated predator attack were also included as behavioral metrics. Fish that did not immediately return to the refuge after the simulated predator attack (n=3) were removed from further analyses. ‘Freeze time’ was the recorded time (seconds) between when the fish initiated the freeze behavior following the simulated predator attack until the fish moved again; a movement was deemed to have occurred when the fish completed a half-body length displacement, or performed a 90-degree lateral turn. The same

behavior for ‘initial latency to emerge’ was used for ‘latency to re-emerge’. Time to approach the predator (seconds) was determined as the time between the simulated predator attack and when the fish approached within one body length of the heron’s feet. If a fish did not perform any of the expected behaviors before the behavior assay was complete, they received the maximum scores of 900 seconds for the initial latency to emerge behavior and 1,800 seconds for the freeze time, latency to re-emerge and approach behaviors (Killen, Marras, & McKenzie, 2011).

### 2.2.3 Angling Trials

After all sets of behavior assays were complete; largemouth bass were randomly stocked into one of two 0.04 ha ponds (n=70 per pond) of the same shape and the same ~ 2 m depth. One pond was designated as the ‘fed’ pond and was stocked with approximately 8,000 fathead minnows, (*Pimphales promelas*) for forage 6 days prior to receiving largemouth bass. Fathead minnows are commonly used as prey items in predation experiments (Chivers, Zhao, & Ferrari, 2007; Ahrens, Walters, & Christensen, 2012), and a previous study in a laboratory setting showed that largemouth bass prey on fathead minnows almost immediately once presented with them (Midway et al. 2017). The second pond was designated as the ‘fasted’ pond and had no fathead minnows. Both experimental ponds were drained to allow for sediment to air-dry for 7 days before refilling and fish stocking, thereby minimizing the abundance of aquatic plants and benthic invertebrates. Ponds were then refilled, stocked with fathead minnows and/or largemouth bass, and angling began one week later. As such, submerged and emergent vegetation was minimal and, although not specifically quantified, vegetative cover was similar across the ponds. Mean turbidity values measured as Secchi depth were  $161.1 \pm 3.1$  cm and  $133.1 \pm 17.5$  cm, mean dissolved oxygen concentrations were  $6.3 \pm 1.3$  mg/L and  $7.0 \pm 0.7$

mg/L, and mean temperatures were  $17.6 \pm 1.4$  °C and  $17.3 \pm 1.4$  °C for the fed and fasted ponds, respectively (YSI Inc., Professional Plus, Yellow Springs, OH) (measurements collected 13 October 2017 to 20 October 2017; Welch's two sample t-tests;  $p_{\text{turbidity}} = 0.03$ ;  $p_{\text{DO}} > 0.05$ ;  $p_{\text{temperature}} > 0.05$ ). Temperature and dissolved oxygen were not significantly different between fed and fasted ponds ( $p > 0.05$ ). The fasted pond was significantly more turbid than the fed pond ( $p = 0.03$ ). Turbidity has the potential to decrease foraging success due to low visibility (Sloman 2011), but the fact that Secchi depth readings were over 1.5 m, differed by only 30 cm and were close to the maximum depths of the pond (approximately 2 m), the impacts of turbidity on prey capture were presumed to be low. The stocked largemouth bass remained in these ponds for a total of 15 days, with 7 days for acclimation and 8 days of angling. This period of food deprivation in the fasted pond is long in duration compared to some previous fasting studies that withheld food for only 6-7 days (Pettersson & Brönmark, 1993; Killen et al., 2011; Fedele, 2017).

Daily angling sessions began on 13 October 2017 and continued for 8 consecutive days. Each daily session consisted of 30-min of angling in each pond, including handling time for all captured fish. All sessions took place between 0900 and 1000 hours and were completed by the same experienced angler each day. The order in which ponds were fished alternated each day. Angling gear consisted of a medium-action spinning rod and reel spooled with clear, 2.7 kg test monofilament fishing line. Two lures were used: a 2 g orange jig baited with a 5 cm pumpkinseed colored plastic grub and a size 0 Aglia in-line spinnerbait, both of which are appropriately sized for the capture of largemouth bass of the size used in this study. The jig + grub lure was used for all sessions across both treatments during the first four days of angling, and the spinnerbait was used for all sessions across both treatments during the last four days of

angling. Different lure types were used to maximize catch rates because fish with different behavioral phenotypes may prefer to strike different types of lures (Wilson et al. 2015), and the lure types used here include two presentation speeds (the plastic grub is retrieved slowly, several cm below the water's surface and the spinnerbait is retrieved quickly, very close to the water's surface). During angling sessions, the angler was free to move around the pond during the sessions, casted from all areas around the perimeter of the ponds and attempted to pass the lure through all areas of the pond, thereby ensuring that all fish would be presented with the lure. Upon capture, each largemouth bass was identified by PIT tag before being released back into the pond in less than one minute; no bleeding or other injury was noted for any captured fish. At the conclusion of the angling trials, ponds were drained and 63 live fish were recovered from each of the fed and fasted ponds (N = 126 total), and TL (mm) and mass (g) was recorded for each fish. Fish that were found dead or were not recovered during pond draining and therefore presumed dead, were excluded from subsequent analyses.

#### *2.2.4 Data Analysis*

Principal components analysis (PCA) could not be used to simplify behavior metrics because the Kaiser-Meyer-Olkin test value was  $< 0.6$ , deeming PCA inappropriate (Budaev 2010). Rather, a Spearman correlation matrix was first used to identify correlated behavioral responses (Table 2.2). Freeze time and latency to re-emerge, latency to re-emerge and approach time and TL and relative weight were significantly correlated precluding the use of all variables in common models (Table 2.2). Freeze time was therefore selected for use in models below from among these correlated variables because it maximized model fit compared to other correlated metrics and because latency to re-emerge and approach time were simply reflections of freeze time (Zuur et al. 2010). Neither TL nor relative weight were related to behavioral metrics ( $p >$

0.39 for all relationships; Table 2.2). Spearman correlations were also used to quantify whether TL and body condition (relative weight) were related to boldness.

A model comparison approach (Akaike's Information Criterion) based on permutations of logistic regression models was used to define the factors that predicted capture during angling trials. The dependent variable for each model was capture during angling trials (yes or no), and predictor variables were initial latency to emerge, freeze time, feeding treatment, relative weight and TL. Two-way interactions included feeding treatment  $\times$  initial latency to emerge, feeding treatment  $\times$  freeze time, TL  $\times$  initial latency to emerge and TL  $\times$  freeze time. These interactions were chosen because the goal of the study was to identify potential interactions between behavioral phenotypes and feeding treatments, and TL is known to be a predictor of angling capture. Model weight was calculated for every possible combination of fixed effects and included interactions. Total length and relative weight were selected for use in the models because these individual factors have been associated with exploratory and boldness behaviors (Krause et al. 1998) and can therefore potentially influence angling vulnerability; feeding treatment was included as per the goal of the study. Models including combinations of these predictor variables and all two-way interactions were compared against the null model, with top models ranked based on the Akaike Information Criterion adjusted for small sample sizes ( $AIC_c$ ). Full-model averaging, the use of all possible combinations of predictor variables in models, was then used to determine the predictor variables with the greatest influences on whether a fish was captured (Arnold 2010; Symonds and Moussalli 2011). Full-model averaging was used in place of model averaging because of high model selection uncertainty (Symonds and Moussalli 2011). For this, the sum of the Akaike weights for each parameter included in all the models where they appeared were calculated to quantify relative importance (Arnold 2010;

Symonds and Moussalli 2011). Relative importance values close to one represent predictors that strongly influence fish capture and low relative importance values, those close to zero, represent predictors that have little influence on capture (Arnold 2010).

Poisson regression was used to compare the total number of captures between ponds across the 8 angling sessions and whether catch rates within each session were different between the fed and fasted ponds. This regression model included the number of captures as the dependent variable (a count), with feeding treatment, session number (nested within lure type) and their interaction as fixed effects. Session was nested within lure type to account for a possible change in catch rate starting with session 5, resulting from switching to a lure that was unfamiliar to the fish (Lennox et al. 2017).

For the 10 models with the lowest  $\Delta AIC$  scores, individual model fit was assessed via visual examination of Pearson residuals (i.e., quantile-quantile plots to define normality of residuals and residuals by predicted plots to define homogeneity of variances), as well as an inspection of outlier observations (Table 2.3) (Menard 2002; Zhang 2016). Results from the model fit analyses, as well as outlier analyses, indicated the absence of influential data points or outliers, so all data were included in the statistical models. All analyses were conducted in R version 3.4.1, with use of the packages “car” (Fox and Weisberg 2011), “ggplot2” (Wickham 2009), “Hmisc” (Harrell Jr. 2019), “gridExtra” (Auguie 2017), “xlsx” (Dragulescu and Arendt 2018), and “MuMIn” (Barton 2017) (R Core Team, Vienna, Austria); the significance level ( $\alpha$ ) was set at 0.05.

## **2.3 Results**

During behavior assays to determine behavioral phenotype of each individual, the time required for largemouth bass to initially emerge from the refuge averaged approximately  $116 \pm$



195.5 seconds (Table 2.1). Once in the refuge after the simulated predator attack, largemouth bass remained motionless for approximately  $91 \pm 181$  seconds, returned to the open zone after approximately  $257 \pm 258$  seconds and required, on average,  $841 \pm 695$  seconds to approach the heron model (Table 2.1, Figure A.2). The variation of individual fish behavior in these data are similar to the behavioral ranges found in previous studies that used similar protocols for behavioral assays of this kind (Killen et al., 2011, 2012).

Seventy largemouth bass were initially stocked into each pond;  $TL \pm SD$  did not differ between ponds ( $TL_{fed} = 176 \pm 17$  mm,  $TL_{fasted} = 175 \pm 15$  mm; Welch two-sample t-test,  $t_{134.86} = 0.61$ ,  $p=0.541$ ; Figure 1a). In addition, initial latency to emerge and freeze times did not differ significantly between the fed and fasted ponds (mean initial latency to emerge in fasted pond =  $110.7 \pm 185.7$  s, mean initial latency to emerge in fed pond =  $122.2 \pm 206.1$  s, Welch two sample t-test,  $t_{122.22} = -0.33$ ,  $p=0.741$ ; mean freeze time for fasted pond =  $105.0 \pm 220.1$  s, mean freeze time in fed pond =  $76.4 \pm 132.3$  s, Welch two sample t-test,  $t_{101.64} = 0.88$ ,  $p=0.379$ ; Figures 2.1b and 2.1c).

At the conclusion of the angling trials, 14 fish were captured from the fed pond (20% of individuals stocked), with 12 fish captured once and two fish captured twice. Twenty-three fish were captured from the fasted pond (33% of individuals stocked), with 19 fish captured once and four captured twice. Seven models relating the effects of behavioral metrics and experimental treatments on capture probability had  $\Delta AIC_c$  values  $< 2$  and all had  $\Delta AIC_c$  values lower than either the full model with all possible predictor variables or the null model (Table 2.3). The strongest variable predicting fish capture was total length with a relative importance of 1.00. This finding indicates larger fish were more likely to be captured than smaller fish, despite the fact that mean lengths of captured and uncaptured fish differed by only 12 mm (Figure 2.1a).

Feeding treatment was the second strongest predictor of fish capture, with a relative importance value of 0.69, indicating that fish in the fasted pond were more likely to be captured than were fish in the fed pond. Initial latency to emerge, freeze time, and relative weight had smaller relative importance values at 0.55, 0.42 and 0.36, respectively. The two-way interaction with the strongest influence on capture likelihood was total length  $\times$  initial latency to emerge, with a relative importance of 0.31 and the two-way interactions feeding treatment  $\times$  initial latency to emerge and feeding treatment  $\times$  freeze time had weaker influences on capture likelihood, with relative importance values of 0.11 and 0.09, respectively.

Overall, for both fed and fasted ponds, there was a significant negative relationship between number of fish captured and angling session, with the highest number of fish captured at the start of the angling trials and capture rates decreasing thereafter (Table 2.4; Figure 2.2). While the total number of captures across all angling sessions did not differ by feeding treatment alone, the interaction between treatment and session (nested within lure type) was a significant factor predicting the number of fish captured (Table 2.4). This interaction resulted from catch rates in the fasted pond remaining higher into later sessions than catch rates in the fed pond, which remained low (Figure 2.2). Additionally, catch rates increased in session 5 in the fasted pond immediately after the lure switch but remained relatively low in the fed pond.

## **2.4 Discussion**

Behavioral phenotype was not a strong predictor of capture for largemouth bass, with behavioral phenotypes equally likely to be captured in both feeding treatments. The relative importance of initial latency to emerge, a measure of exploration, and freeze time, a measure of boldness, in the model predicting angling vulnerability were 0.55 and 0.42, respectively, indicating only weak links between lab-measured behaviors and angling vulnerability. Fish have

previously been shown to have heritable differences in behaviors that are consistent within contexts, known as behavioral syndromes (Bell, 2007). The behaviors identified in the laboratory are measures of 2 different behavioral axes, as the time required for largemouth bass to emerge from the refuge is referred to as exploratory behavior, while the 3 remaining behaviors recorded post-predator simulation were all measures of behavior under threat (Réale et al. 2007). Long freeze times post-threat typically indicate shy behavioral phenotypes, while short freeze times are typical for bolder individuals (Pauli et al. 2015). Several studies have linked behavioral phenotypes and correlated metabolic rates with angling vulnerability (Biro & Post, 2008; Biro & Sampson, 2015; Lennox et al., 2017; Mittelbach et al., 2014; Wilson et al. 1993), though this conclusion has not been consistent. For example, studies of rainbow trout, (*Onchorynchus mykiss*), found that bold fish exhibiting high exploration rates were more susceptible to angling relative to shy fish with low exploration rates (Biro & Post, 2008), while a study with angling targeted at nesting largemouth and smallmouth bass (*Micropterus dolomieu*) found that the most aggressive male bass were the most vulnerable to capture (Suski and Philipp 2004; Sutter et al. 2012). The lack of a relationship between behavioral phenotype and capture rates in the current study may be due to species-specific differences between largemouth bass and other fish examined previously. More specifically, largemouth bass have been identified as sit-and-wait predators (Demers et al. 1996), and behavioral metrics related to exploration may not factor into their foraging style or interaction with lures (Lennox et al. 2017). Indeed, Louison et al. (2017) found that laboratory-based boldness behaviors did not predict angling vulnerability for largemouth bass, noting that capture was better predicted by stress hormone responsiveness. The traditional framework built around angling vulnerability has been based off of carp and trout species, which have more active foraging and movement ecology, and therefore

activity rates may have stronger relationships with the likelihood of these fish species to encounter fishing lures, providing a stronger link between foraging activities and angling vulnerability (Lennox et al. 2017). Alternatively, largemouth bass may not associate fishing lures with food and may strike lures for reasons other than hunger, such as aggression or territory defense, behaviors not quantified in the current study (Suski and Philipp 2004; Sutter et al. 2012). Finally, behaviors are often reflections of immediate environmental stimuli, and the stimuli presented to largemouth bass in the behavioral arena likely differed from stimuli in ponds, such that laboratory-derived behaviors may not carry over into field situations (Toms et al. 2010). Data from this study show that boldness and exploratory behavioral phenotypes are not drivers of angling vulnerability for largemouth bass.

In contrast to expectations, food availability did not impact which behavioral phenotype was most vulnerable to angling. The relative importance values of the interactions between behavioral metrics and feeding treatment to predict capture were weak, indicating that behavioral phenotypes were captured at similar rates across feeding treatments. Previous studies have documented behavioral changes across environmental contexts (Pettersson & Brönmark, 1993; Naslund & Johnsson, 2016), including a study by Beukema (1968) who found that hungry three-spined sticklebacks (*Gasterosteus aculeatus*), had higher rates of activity and prey encounters while foraging. One potential reason for not observing a difference in catchability across behavioral phenotypes is that capture vulnerability may be better predicted from behavioral axes other than boldness and exploratory behaviors. For example, capture by largemouth bass has previously been shown to be influenced by aggression (Suski and Philipp 2004; Sutter et al. 2012). However, because aggression was not measured in the current study, the relationship between food availability and other behavioral phenotypes cannot be quantified. Regardless of

the mechanism, results from the current study clearly show that food availability did not affect which behavioral phenotypes were captured by anglers.

Feeding treatment had an impact on catch rates, with largemouth bass from the pond with no minnows more likely to be captured than fish from the pond with minnows. Several past studies have also found that food availability can influence capture rate, with anglers more likely to have higher capture rates of walleyes (*Sander vitreus*) when abundance of prey was low (VanDeValk et al. 2005). However, this conclusion is not universal, as Fedele et al. (2017) found that food availability for juvenile rock bass, (*Ambloplites rupestris*), had no effect on capture. A possible reason for increased susceptibility of fasted largemouth bass to angling capture could have been due to higher engagement in foraging activities. This proposed foraging mechanism is consistent with results from a study in which fasted crucian carp (*Carassius carassius*) spent more time foraging relative to fed crucian carp (Pettersson and Brönmark 1993). The number of largemouth bass captured was higher in the pond with no minnows compared to the pond with minnows, a relationship between food availability and capture that was found in other studies, as well.

Across both the fed and fasted treatments, fish size was the strongest predictor of capture, with larger fish more likely to strike lures than smaller fish, even with a relatively small mean difference in length between captured and uncaptured fish. Previous studies have supported the positive relationship between total length of fish and capture probability (Biro & Post, 2008; Klefoth et al., 2017; Monk & Arlinghaus, 2018), and there are a number of possible explanations for why larger fish were more likely to strike lures in the current study. For example, all largemouth bass in this study were hatchery-raised and the same age, so it is likely that the largest individuals were the fastest growers (Klefoth et al., 2017), though data on individual

growth rates were not generated. It is therefore possible that these large, fast-growing largemouth bass increased their activity, a behavioral axis separate from exploration and boldness, to obtain food, which may have provided a greater chance of encountering fishing lures thereby making them more vulnerable to angling (Biro & Sampson, 2015; Biro & Post, 2008). Additionally, previous studies have found fish in exploited populations have lower metabolic rates than unfished populations, suggesting that the fish with high metabolic rates were vulnerable to angling and were removed from these systems (Redpath et al. 2010; Hessenauer et al. 2015). Larger fish may also be behaviorally dominant than smaller fish, leading to large fish outcompeting small fish to strike fishing lures (Krause 1994). It should be noted that the mean size of largemouth bass used in this study (180 mm) falls below the minimum total length threshold for capture of 200 mm seen in some studies (Wegener et al. 2018), but is still within the range of sizes captured in other studies (Anderson and Heman 1969; Nannini et al. 2011). Owing to the fact that the hatchery-reared fish used here may be bolder and more active than wild fish (Biro & Post, 2008), future studies using largemouth bass across a greater range of sizes, and from wild populations that have not received supplemental hatchery stocking, should be useful to further explore relationships between fish size, behavioral phenotypes and angling vulnerability. Data from the current study show that larger largemouth bass were more likely to be captured over smaller conspecifics, regardless of feeding treatments.

Capture rates declined over angling sessions in both the fed and fasted treatments. The largest number of captures for both fed and fasted treatments was in angling session one and the number of captures declined in each session until session 5 after lures were changed, when the decline was repeated. Declines in hook-and-line capture rates over time have been demonstrated many times and across multiple species (VanDeValk et al., 2005; Askey, Richards, Post, &

Parkinson, 2006; Hessenauer et al., 2016). For example, highly vulnerable rainbow trout avoided fishing lures after about 10 days (Askey et al. 2006), while catch rates of largemouth bass in an experimental pond also declined over time (Hessenauer et al. 2016). There are a number of reasons why capture rates of fishes decline over time, including declines in population density (VanDeValk et al. 2005) and shifts toward more timid phenotypes in exploited populations as bold individuals are harvested, known as the timidity-syndrome (Arlinghaus et al. 2017b). The timidity-syndrome occurs when fish populations experience long-term fishing exploitation, increasing the “landscape of fear” effect, leading to lower activity rates, lower exploration, and lower willingness to approach and ingest fishing lures (Arlinghaus et al. 2017b). More importantly, because behaviors have a genetic component, the effects of long-term disproportionate harvest can be more concrete and alter mean population behaviors on a genotypic level (Arlinghaus et al. 2017b). Learning to avoid hooks is also common in fisheries and has previously been identified as a factor contributing to catch rate declines over time (Fernö and Huse 1983; Laugen et al. 2014; Lennox et al. 2016; Fedele 2017). Additionally, largemouth bass can exhibit “angler avoidance” to negative stimuli including the presence of boats and people, instead of lures, where the catch rate of fish in small impoundments declined over a 6-month period when a total of 11 fishing lure types were used and with the absence of harvest (Wegener et al. 2018). Regardless of the mechanism, the current study showed a decline in capture rate of largemouth bass across successive angling sessions.

Findings from this study have three main implications for the ecology and management of recreational fish populations. First, results suggest that angler-induced changes to the boldness and exploratory behaviors of largemouth bass are not likely as different behavioral phenotypes were captured at similar rates, regardless of food availability. Second, results

suggest that the largest, fastest-growing largemouth bass can be disproportionately removed from aquatic systems through harvest. Over time, this selective removal of fast-growing individuals could lead to declines in both catch rates and angler satisfaction as growth rates (and therefore size) and behavioral traits such as exploration are often correlated (Alós et al. 2016; Arlinghaus et al. 2017b). Finally, because fish in ponds with low prey abundance were more vulnerable to capture, environmental factors, such as prey abundance, must be considered when managing populations of largemouth bass. While one aspect of angler satisfaction relates to the size of fish captured (Dotson et al. 2013), another aspect of angler satisfaction relates to catch rates and the capture of large numbers of fish (Young and Hayes 2004), which is likely to occur in systems with low prey available to the target species. Results suggest that an overabundance of prey in a system may create declines in catch rates (VanDeValk et al. 2005), suggesting that managers be aware of prey availability for largemouth bass fisheries. Obviously, if prey abundance falls too low, growth and abundance of the targeted predator species can plummet, emphasizing the need for an ideal amount of prey to be available to the predator species. To summarize, results from this study do not suggest selection against exploratory and boldness behavioral traits, but size and growth rates may be selected upon through harvest; and environmental conditions, including food availability, may alter the strength of selection. The ecological impacts of selective harvest should be considered in future management plans and policies to minimize effects of artificial selection and maintain balanced recreational fish populations.



## 2.5 Tables

Table 2.1. Summary statistics for behavior scores, size and condition of largemouth bass. Data were collected at pond draining following the conclusion of the study; n=126.

	Range	Mean $\pm$ SD	Median
Initial latency to emerge (s)	1-900	116 $\pm$ 195.5	46
Freeze duration (s)	3-1540	91 $\pm$ 181.4	50
Re-emergence time (s)	4-1541	257 $\pm$ 258.5	188
Approach time (s)	15-1800	841 $\pm$ 695.2	466
Total length (mm)	147-234	180 $\pm$ 15.9	180
Relative weight	85.2- 131.7	101.7 $\pm$ 8.7	100.9

Table 2.2. Spearman correlations matrix showing relationships between predictor variables of capture for largemouth bass. Correlation coefficients (r) between pairs of predictors are given in the top right section of the table and p-values for correlations are shown in the bottom left section. Significant correlations between predictors (p-values < 0.05) are shown in bold text.

	Initial latency to emerge (s)	Freeze time (s)	Re-emergence time (s)	Approach time (s)	Relative weight	Total length (mm)
Initial latency to emerge (s)	-	0.13	-0.05	-0.16	0.06	0.01
Freeze time (s)	0.15	-	<b>0.26</b>	-0.01	0.02	-0.03
Re-emergence time (s)	0.60	<b>0.002</b>	-	<b>0.35</b>	0.00	0.04
Approach time (s)	0.08	0.95	<b>&lt;0.001</b>	-	-0.07	-0.08
Relative weight	0.50	0.81	0.96	0.41	-	<b>0.21</b>
Total length (mm)	0.92	0.71	0.62	0.39	<b>0.02</b>	-

Table 2.3. Top 10, null and full logistic regression models for largemouth bass capture during experimental angling trials. Sample size was 126 individuals.  $W_i$  is model weight.

Model	AIC <sub>C</sub>	$\Delta$ AIC <sub>C</sub>	-2 log likelihood	$W_i$
Total length + feeding treatment	127.86	0.00	121.67	0.10
Total length + feeding treatment+ freeze + relative weight	128.90	1.03	120.57	0.06
Total length	129.00	1.14	121.68	0.06
Total length + initial latency to emerge + feeding treatment + total length $\times$ initial latency to emerge	129.10	1.23	118.60	0.06
Total length + feeding treatment + freeze	129.50	1.64	121.17	0.05
Total length + initial latency to emerge + total length $\times$ initial latency to emerge	129.65	1.79	121.32	0.04
Total length +initial latency to emerge + feeding treatment	129.68	1.18	121.35	0.04
Total length + relative weight	130.10	2.23	123.90	0.03
Total length + initial latency to emerge + feeding treatment + relative weight +total length $\times$ initial latency to emerge	130.22	2.36	117.52	0.03
Total length + freeze	130.59	2.72	124.39	0.03
Null	140.35	12.48	138.32	0.0002
Total length + initial latency to emerge + feeding treatment + freeze + relative weight + total length $\times$ initial latency to emerge + total length $\times$ freeze + initial latency to emerge $\times$ feeding treatment + freeze $\times$ feeding treatment	137.79	9.93	115.88	0.0007

Table 2.4. Model output for Poisson regression with the total number of captures of largemouth bass as the dependent variable and feeding treatment, lure type nested within angling session and their interactions as fixed effects. Significant predictors of capture within angling sessions ( $p < 0.05$ ) are shown in bold text.

	Parameter Estimate	SE	z-score	p
<b>Angling session</b>	<b>-0.92</b>	<b>0.33</b>	<b>-2.83</b>	<b>0.005</b>
Feeding treatment	1.04	1.02	1.02	0.31
Feeding treatment $\times$ angling session	-0.95	0.75	-1.28	0.20
<b>Fasted treatment <math>\times</math> angling session (lure type)</b>	<b>0.60</b>	<b>0.26</b>	<b>2.32</b>	<b>0.02</b>
<b>Fed treatment <math>\times</math> angling session (lure type)</b>	<b>1.21</b>	<b>0.55</b>	<b>2.21</b>	<b>0.03</b>

## 2.6 Figures

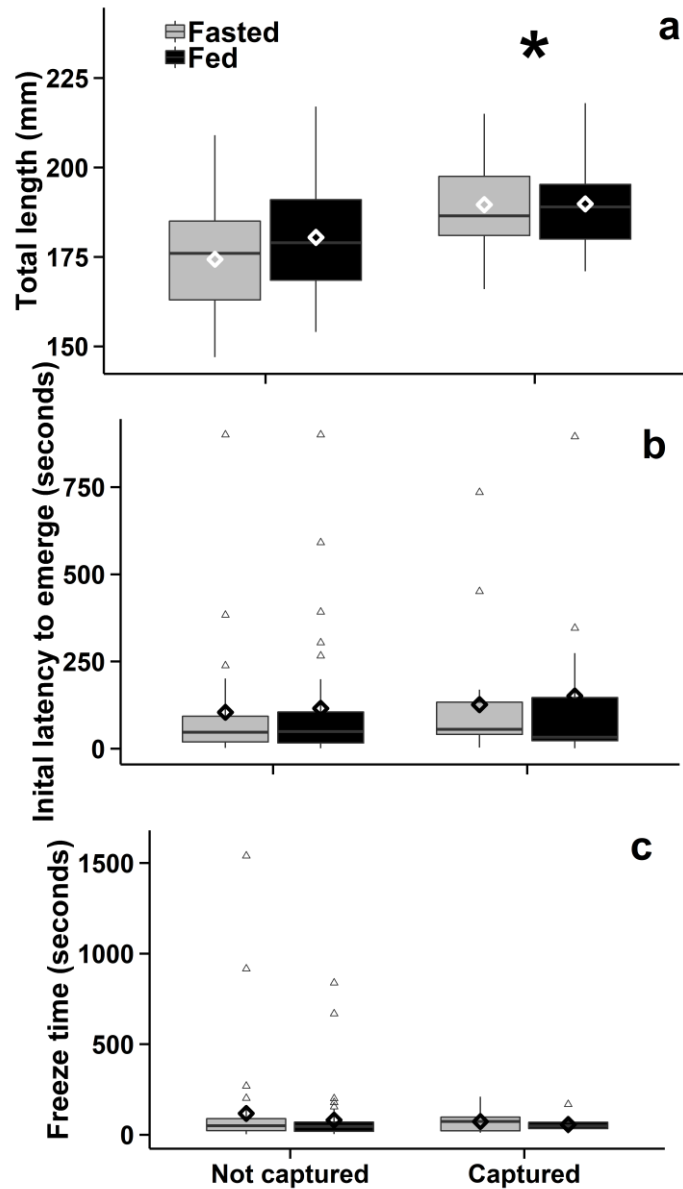


Figure 2.1. Boxplots of (a) the total length (b) initial latency to emerge and (c) freeze time for largemouth bass captured and not captured during experimental angling trials. Asterisk denotes a significant ( $p < 0.05$ ) difference between captured and uncaptured fish. The lines in the boxes are the median, diamonds are the mean, whiskers represent the upper and lower quartiles  $\pm$  the interquartile range, and open triangles are outliers that fall outside of the interquartile range (below 25<sup>th</sup> percentile or above 75<sup>th</sup> percentile).

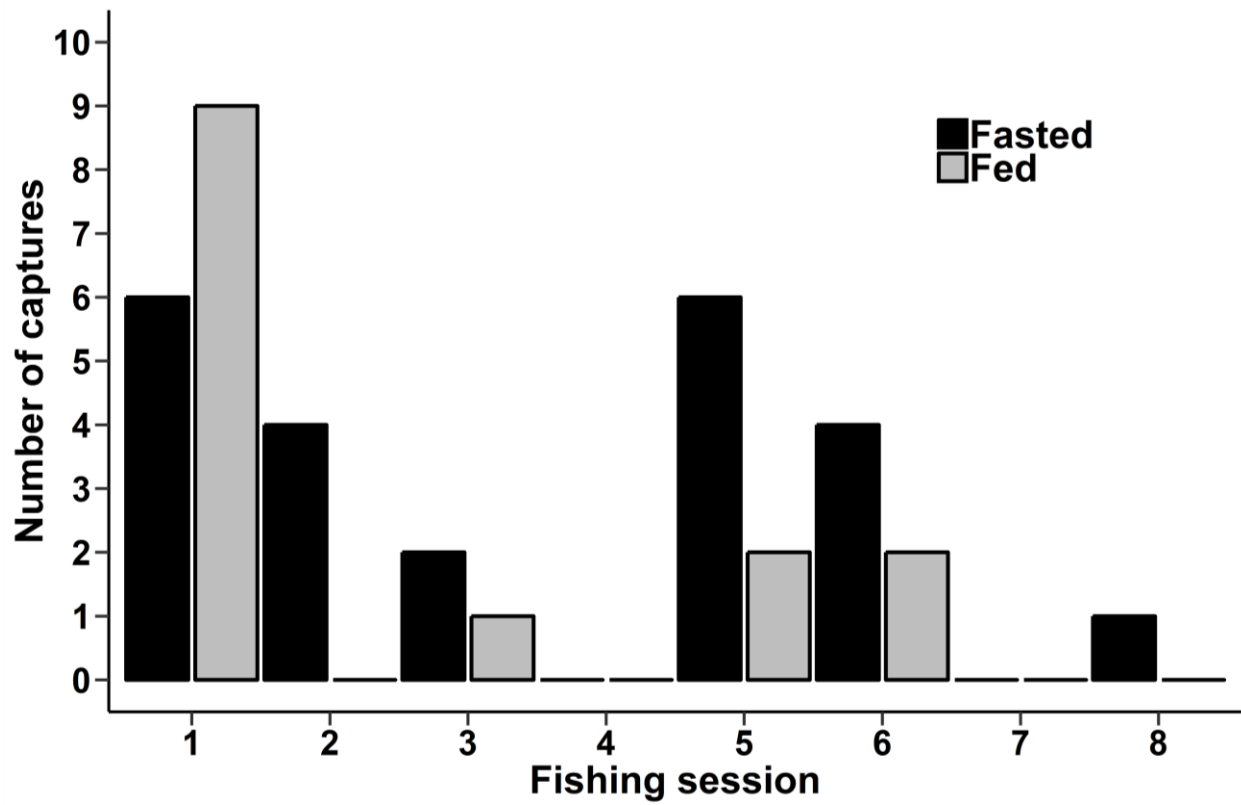


Figure 2.2. Number of unique individual largemouth bass captured in each fishing session in fasted and fed ponds.

# **CHAPTER 3: LARGEMOUTH BASS ARE NOT CHOOSY: CAPTURED FISH DO NOT SHOW HABITAT PREFERENCES, REGARDLESS OF ACTIVITY AND BOLDNESS BEHAVIORS**

## **3.1 Introduction**

Habitat is a key element aiding the survival of all fish species. Essential habitat provides food, predator protection, and often suitable conditions for spawning (Werner et al. 1983). As a result of its importance, fisheries managers allocate millions of dollars to restore fish habitats with the expectation of increased fish abundances, densities, carrying capacities, and survival (Baumann et al. 2016; Roni 2019). Often, management goals for habitat restoration also includes societal factors, such as increases in satisfaction for recreational anglers, which can be achieved through increased catch rates (Bolding et al. 2004; Smokorowski and Pratt 2007). Habitat conservation and restoration are therefore important for the stability of fish populations, and to enhance recreational angling.

While increased fish capture by recreational anglers can result in increased satisfaction, it can also increase negative impacts on fish populations through human-induced evolution. For example, recreational fishing has the potential to affect fish populations directly through mechanisms such as harvest and/or overharvest, as well as through sub-lethal injuries and/or delayed mortality within catch-and-release fisheries, both of which can reduce population sizes, impair recruitment, and lead to population collapse (Post et al. 2002; Cooke and Cowx 2004). In addition, anglers can negatively influence populations through more subtle means, including the disproportionate capture or harvest of fish with suites of correlated, repeatable traits, such as “fast” life histories with bold behavioral phenotypes, commonly referred to as fisheries-induced evolution (FIE) (Heino et al. 2013). For example, should anglers disproportionately harvest fish

with “fast” life history traits, this can lead to the predominance of timid individuals in a population that are less likely to encounter, approach, inspect, and strike fishing lures, (referred to as the ‘timidity syndrome’) possibly leading to declines in catch rates and angler satisfaction (Arlinghaus et al. 2017b). Selection of “fast” life history individuals through FIE can also extend to other correlated traits, leading to selection against high metabolic rates and fast reproduction (Reale et al. 2010). Through the removal of certain traits, FIE can reduce genetic and behavioral phenotypic diversity of a stock and threaten population stability much faster than through natural selection, in as short as a few generations (Reznick and Ghalambor 2001; Laugen et al. 2014; Uusi-Heikkilä et al. 2015). Selective fishing pressure can lead to negative effects for fish populations, from genetic to ecological scales.

Individual behaviors, however, are not always repeatable across environmental contexts, owing to behavioral plasticity, and this plasticity can affect how fish interact with fishing lures across different habitats. Some behaviors co-vary across environmental conditions or throughout ontogeny (Bell & Stamps, 2004) and fish may adjust behaviors in varying habitats to benefit from current conditions (Mazué et al. 2015), often displayed through behavioral flexibility (Mittelbach et al. 2014). For example, activity and predator avoidance behaviors of fathead minnows (*Pimephales promelas*) changed across varying carbon dioxide concentrations (Tix et al. 2017). Individual boldness behaviors have been linked to habitat use by fish, with timid fish more likely to associate with shelter compared to their bold conspecifics (Hollins et al. 2018). These changes in microhabitat use between individuals of different behavioral phenotypes can influence the capture vulnerability of fish across these habitat types. For example, as seen in a study with Eurasian perch (*Perca fluviatilis*), food shortage during the autumn months increased



the food demand, decreased body condition, and increased capture rates (Heermann et al. 2013). Fish behaviors can therefore co-vary with altered environments, including changes in habitat.

Although it is known that habitat is essential to fish survival, that recreational angling has the potential to select for fish of specific behavioral traits, and that behaviors can change across habitat types, it is still unknown how behavior and angling vulnerability interact across habitat types. Habitats supplemented with artificial structures likely make fish more vulnerable to angling, as structures typically aggregate fish and are highly sought out by anglers (Matthias et al. 2014). However, if vulnerable fish have certain behavioral characteristics in association with habitats (i.e., if all shy fish hide in structure as a form of refuge, or if all bold fish outcompete their conspecifics and dominate structure for survival benefits), then the presence of structures could alter vulnerability of behavioral phenotypes and negatively change fish populations through FIE. Thus, it is important that we define how structures, behavior, and vulnerability interact so we can manage fish populations over long temporal scales.

Based on this background, this study's objective was to quantify the combined influence of behavioral phenotype and habitat preference on largemouth bass (*Micropterus salmoides*) angling vulnerability. To accomplish this objective, we designed a combination laboratory- and pond-based study that first quantified activity and boldness behavioral types in largemouth bass, and then subjected these fish to experimental angling trials in ponds with and without supplemental habitat. We predicted that within the zone of the ponds where no supplemental habitat was added, anglers would catch few fish of only bold and active phenotypes while, in the area of the ponds with supplemental habitat, we predicted anglers to have many captures with mostly timid, inactive fish captured. Together, results from this study will help define factors that influence angling vulnerability in largemouth bass, and help improve our understanding of

how supplemental habitat can alter the evolutionary trajectory of fish populations through exploitation.

## **3.2 Methods**

### *3.2.1 Study Animals*

Largemouth bass (n=250) were acquired from Seven Springs Fish Farm, Evansville, IL and transported to Illinois Natural History Survey Aquatic Research Facility near Champaign, IL on 16 May 2018. Mean total length  $\pm$  standard deviation (SD) of fish was  $281 \pm 20.9$  mm, mean mass  $\pm$  SD was  $313 \pm 90.0$  g, and mean relative weight was  $96.3 \pm 7.8$ , based on standard weight calculations for largemouth bass (Murphy et al. 1991). This size of fish has previously been shown to be vulnerable to angling in both laboratory simulation studies, as well as in wild populations (Murphy et al. 1991; Hessenauer et al. 2016; Sass et al. 2018). Once at the Research Facility, all largemouth bass were divided and held among 12 circular 1,135 L outdoor tanks supplied with continuous flow-through, aerated water from an adjacent 0.04 ha earthen pond, at a rate of  $\sim 8$  water exchanges per day. Mean water temperature was  $26 \pm 2.1^\circ$  C and mean dissolved oxygen concentration was  $8.6 \pm 0.9$  mg/L (Professional Plus dissolved oxygen and temperature meter, YSI Inc., Yellow Springs, OH) during holding. Fish were fed Skretting high protein pellets (Tooele, Utah) *ad libitum* daily. All fish were implanted with a passive integrated transponder (PIT) tag (10 mm length  $\times$  2 mm diameter, HPT12, Biomark Inc., Boise, Idaho) for individual identification three days after transport. Following tagging, fish acclimated to holding conditions for an additional six days before behavior assays began on 25 May 2018.

### 3.2.2 Behavior Assays

One hundred thirty-two largemouth bass were randomly selected to complete behavior assays. For this, fish were first moved from outdoor tanks to indoor aquaria to acclimate to lab conditions for between 17 and 23 hours prior to the start of assays. Indoor aquaria (121 L opaque plastic holding tanks) were each divided into two chambers by an opaque plastic barrier with holes to allow for water flow between the chambers. Each aquarium held 2 fish, one on either side of the barrier, to keep individuals isolated. A reservoir tank, equipped with an aerator, provided a re-circulating supply of water to all aquaria *via* a pump (Outdoor air pump, Pentair, Cary, North Carolina). Indoor aquaria temperature was maintained at  $23.8 \pm 0.7^{\circ}\text{C}$  with a TK 500 Heater-Chiller (Teco, Revenna, Italy) and dissolved oxygen concentrations remained above  $7.51 \pm 0.5\text{ mg/L}$ , verified daily with a hand-held probe (YSI Inc. Professional Plus, Yellow Springs, OH).

Behavior assays were conducted between 0830 and 1430 hours, from 25 May 2017 to 7 June 2017, in one of two identical arenas. Arenas were opaque 94 L circular tanks (80 cm diameter) filled to a water depth of 24.5 cm with water from a nearby pond. Video cameras (GoPro Hero 3 or Session 4, San Mateo, California) were used to record fish location and behavior and were suspended above the arenas from a frame made from polyvinyl chloride (PVC) pipes. The arena consisted of two zones: a refuge zone and an open zone. The refuge zone occupied the perimeter of the tank and had a natural gravel bottom with plastic aquarium plants for shelter, and the open zone occupied the center of the tank and had no bottom substrate or vegetation. The open zone was 40 cm in diameter and the refuge zone had a radius of 20 cm when measured from the tank perimeter to the outside of the open zone. The behavioral arena was similar to others used to measure habitat preference (Hart et al. 2009).

For the behavior assay, largemouth bass were netted from the indoor aquaria, identified to individual using a PIT tag reader, and placed into the center of the open zone of an arena. Fish were left to acclimate for 10-min immediately before behavior assays commenced, during which time movement was unrestricted, and fish could move freely around the arena. A 10-min acclimation time is common for behavior assays (Vainikka et al. 2016; Louison et al. 2017), and preliminary trials revealed that fish began moving around the arena typically within 5-6 min after the transfer. Video cameras recorded baseline behavior data for a 10-min period following the acclimation period to generate baseline data.

Following this generation of baseline data, fish were subjected to a simulated predator attack. For this attack, a model great blue heron (*Ardea herodias*) measuring 73.7 cm in height was used (United Aquatics LLC, Marlton, New Jersey), as great blue herons are a common largemouth bass predator and have been used as predator models in other fish behavior studies (Bell & Stamps, 2004; Cooke, Steinmetz, Degner, Grant, & Philipp, 2003). The simulated predator attack involved an observer that held the heron model over the behavioral arena and struck the water 4 times in a square pattern in the center of the open zone with the heron's beak. The heron strike locations were the same across trials and did not vary with the fish's position in the arena. Fish behavior was then monitored for an additional 10-min after conclusion of the simulated predator attack, following which, behavior assays were considered to be complete and video recording ended.

After this 7-day recovery period, the entire behavioral assay was conducted a second time in its entirety for all fish, and this second test allowed for the calculation of repeatability metrics for the measured behaviors (Bell, 2007). Following this second behavioral assay, largemouth

bass were again returned to the outdoor tank system to continue feeding until being divided and stocked into pond treatments before the start of angling trials (see below).

Five metrics were used to score fish behavior observed during the behavior assay: 1) time spent swimming (s) before the simulated predator attack (termed ‘pre-predator swimming activity’), 2) time spent in the open zone (s) before the simulated predator attack (termed ‘pre-predator open zone’), 3) duration of the freeze response (s) after the simulated predator attack (termed ‘freeze time’), 4) time spent swimming (s) after the simulated predator attack (termed ‘post-predator swimming activity’), and 5) time spent in the open zone (s) after the simulated predator attack (termed ‘post-predator open zone’). A fish was considered to be swimming when horizontal displacement in the water column was observed. Time spent in the open zone was recorded if more than half of the fish’s body length was within the open zone boundary. ‘Freeze time’ was the recorded time (s) between when the fish initiated the freeze response after the simulated predator attack until the fish resumed movement; a movement was deemed to occur when the fish completed a half-body length displacement, or performed a 90-degree lateral turn. Time spent swimming was a metric of activity, while freeze time and time spent in the open zone of the tank were boldness behaviors (Réale et al. 2007). Durations of all performed behaviors were recorded using Solomon Coder (<https://solomoncoder.com/>) version beta 17.03.22.

### *3.2.3 Angling Trials*

After all behavior assays were complete, largemouth bass were randomly stocked into one of three 0.04 ha ponds, with approximately a 2 m depth, 15 m length, and 6 m width, on 8 June 2018 (n = 44 per pond). Experimental ponds were drained prior to stocking to allow benthic sediment to air-dry for 7-days, thereby minimizing the abundance of aquatic plants and benthic invertebrates. Ponds were then refilled and angling began 10-days later. As such,

submerged and emergent vegetation was minimal and vegetative cover was similar across the ponds, although not specifically quantified.

Each pond was divided into three sections: one section, termed the ‘structure zone,’ that comprised approximately 45 % of the pond area that contained artificial structure, a second section, termed the ‘no structure zone,’ of approximately 45 % of the pond area that contained no supplemental habitat, and a third section that contained approximately 10 % of the total area that was a ‘neutral zone’ between the two other sections. The structure zone contained eight evenly-spaced ‘porcupine attractors’ in the benthic zone (Figure 3.1). Each porcupine attractor was made with eight 1.3 cm-diameter PVC pipes secured within a cement-filled plastic tote as the base. These porcupine attractors mimic the design and complexity of evergreen trees, and have been shown to increase largemouth bass abundance at similar rates to evergreen trees and other manufactured fish attractors (Baumann et al. 2016). In addition, artificial structures such as these have previously been used to attract fish, increase fish density, and increase angling catch rates (Rogers and Bergersen 1999; Bolding et al. 2004; Smokorowski and Pratt 2007). Benthic vegetation was allowed to grow freely within the structure zone, although vegetation was limited due to the dry-out period prior to the start of the study and the relatively brief duration of the angling portion of the study (15-days total). The no structure zone had multiple 3 m × 15 m black Lake Bottom Blanket (Wayne, NJ, <https://lakebottomblanket.com/>) benthic liners atop the substrate to prevent aquatic plant growth and provided no refuge for the largemouth bass. The liner covered approximately 90 % of the pond’s no structure zone. The neutral zone between the two treatment zones had no artificial structure and no benthic liner, allowing natural vegetation to grow, but was not targeted by anglers during angling trials, thereby acting as a buffer to increase the confidence of assigning an angled fish to either the structure or no structure zones of

the pond. Together, this experimental design for the pond study contained areas of structure and no structure, and thus mimicked the arenas where behavioral assays were performed (i.e., a refuge zone and open zone), increasing the likelihood of having inter-individual lab-based behaviors carry over into field trials (Mazué et al. 2015).

All ponds were stocked with approximately 2,000 forage fish each, made up of a combination of fathead minnows (subset mean total length=  $51 \pm 5.0$  mm) and golden shiners (*Notemigonus crysoleucas*), subset mean total length=  $65 \pm 5.4$  mm) one day prior to stocking largemouth bass. Fathead minnows and similar species are commonly used as prey items in predation experiments (Chivers et al. 2007; Ahrens et al. 2012), and previous studies in laboratory settings showed that largemouth bass of the size used in the current study can consume minnows of this size (Midway et al. 2017). Mean temperature was  $27.0 \pm 2.3$  °C across all three ponds, mean dissolved oxygen concentration was  $6.2 \pm 2.8$  mg/L, and mean turbidity (measured with a Secchi disk) was  $99.5 \pm 20.6$  cm. Mean total length (mm) of stocked largemouth bass was not different across the ponds ( $TL_A = 282 \pm 19$  mm,  $TL_B = 282 \pm 23$  mm,  $TL_C = 280 \pm 21$  mm; one-way analysis of variance (ANOVA),  $F_2=0.121$ ,  $p=0.887$ ). Stocked largemouth bass remained in these ponds for a total of 15-days, with 9-days for acclimation and 6-days during which angling occurred.

Daily angling sessions began on 17 June 2018 and concluded on 22 June 2018, with one day where no angling occurred, on 21 June 2018. Each daily session consisted of 20-min of angling in each pond, including handling time for all captured fish. All sessions took place between 0815 and 0945 hours and were completed by the same two experienced anglers (MJL and CDS). Angling gear consisted of a medium-action spinning rod and reel spooled with clear, 2.7 kg test monofilament fishing line. One lure type was used for the entire study: a size two

weedless hook with a 7.6 cm watermelon/ red flake colored plastic Yum dinger worm, rigged “wacky style,” appropriate for the size of largemouth bass used in this study. The order in which the three ponds were fished each day was decided with a random number generator, both anglers fished concurrently in the same pond, and only one angler at a time fished the structure or no structure sections. Upon arriving at the designated starting pond, a coin flip was used to assign anglers to a section of the pond (structure zone or no structure zone), and anglers were free to choose a starting location around the perimeter of the pond. Anglers started fishing at the same time, and were free to move around the perimeter of their assigned section, cast, and attempt to pass their lures through all areas of their section, thereby ensuring that all fish within the pond would be presented with the lure. Anglers fished in a way that was common when targeting largemouth bass with lures of this kind (i.e., lures casted and retrieved slowly, a ‘hook set’ occurred when the angler believed a fish had ingested the lure), and care was taken to attempt to have anglers fish in a similar, consistent fashion throughout the study. Each captured largemouth bass was identified by PIT tag before being released back into the pond in less than one minute after capture, allowing for recaptures to occur. Records indicated instances where minor bleeding occurred or deeply-hooked fish were released with the hook in place ( $n = 6$  captures) (Stein et al. 2012). The occurrence of “misses” was also recorded when anglers hooked a fish, but the fish rejected the hook and was ultimately uncaptured. Altogether, each pond received 5 days of angling during the study.

The same day angling trials concluded, ponds were drained and between 38 and 42 of the 44 originally-stocked fish were recovered from each of the study ponds ( $n=121$  recovered fish from a total of 132 total stocked, 92 % recovered). Fish found dead, or that were not recovered during pond draining, were excluded from subsequent analyses. All methodology was approved



by the University of Illinois Institutional Animal Care and Use Committee (IACUC), protocol no. 17160.

### *3.2.4 Statistical Analysis*

Intraclass correlation coefficient (ICC) analysis, with a two-way ANOVA design suited for a single rater, was first used to determine repeatability of the five recorded behaviors quantified during behavioral assays in the arenas (Bell, Hankison, & Laskowski, 2009; Shrout & Fleiss, 1979). Principal components analysis (PCA) was then used to distill repeatable behaviors (Budaev 2010). Principal components with eigenvalues  $> 1$  were extracted and rotated, and factor loadings above 0.4 were used for interpretation (Hair 1998; Ho 2006; Budaev 2010).

One-way ANOVAs, one with PC scores as the response variable and pond as the main effect, and a second with the number of times individual fish were captured as the response variable and pond as the main effect, were used to test for differences in behavior distributions and number of times individuals were captured across the three study ponds. To define differences in the proportion of captures and misses between anglers across the study ponds, a logistic regression model with binomial error distribution was used (Crawley 2013). A Poisson regression, recommended for count data, was used to quantify differences in the total number of captures across angling sessions (Bolker et al. 2008). To quantify any effects of angler and pond on the behaviors of captured fish, a two-way ANOVA was used that included the PC scores of captured fish as the response variable, and angler, pond and their interaction as fixed effects.

A logistic regression with a binomial error distribution was used to test for differences in the proportion of fish captured between the structure and no structure zones across the study ponds (Crawley 2013). A multinomial regression, defined as a logistic regression with three or more categorical outcomes, was used to test for the influence of pond, angler, and PC score on

the pond zone where captures occurred (Bolker et al. 2008). To discern differences of behavior of captured fish, a linear mixed effects model was used that included the interaction between angling day and the pond zone where capture occurred as fixed effects, and angler and pond as random effects (Bolker et al. 2008). Lastly, a generalized linear mixed effects model was used to define the effects of behavior and habitat on the number of times individual fish were captured, with PC scores and habitat as fixed effects and angler, pond, and fish ID as random effects. All analyses were conducted in R (version 3.4.1), including “Tidyverse 1.2.1” (Wickham 2017), “irr 0.84” (Gamer et al. 2012), “psych 1.8.12” (Revelle 2018), “vegan 2.5-3” (Oksanen et al. 2018), “readr 1.2.1” (Wickham et al. 2018), “lme4 1.1-15” (Bates et al. 2015), “nnet 7.3-12” (Venables and Ripley 2002), “car 2.1-6” (Fox and Weisberg 2011), “coefplot 1.2.6” (Lander 2018), “lsmeans 2.30-0” (Lenth 2016), “ggplot2 3.1.0” (Wickham 2009), and “gridExtra 2.3” (Auguie 2017) packages. Statistical significance was determined at  $\alpha = 0.05$ .

### **3.3 Results**

Of the five behavioral variables measured, ICC analyses confirmed that pre-predator swimming activity, pre-predator open zone, post-predator swimming activity, and post-predator open zone were repeatable, with repeatability values above 0.2 and confidence intervals that did not include zero (Table 3.1). Freeze times were not repeatable, and were therefore not included in PCA analyses (Table 3.1). One principal component, PC1, was extracted from the repeatable behaviors (Table 3.2). This lone PC (PC1) explained 57% of the behavior variation and had an eigenvalue of 2.28 (Table 3.2). Largemouth bass with high PC1 scores were inactive pre-predator simulation and spent a lot of time in the open zone (bold) during both pre- and post-predator simulation, while fish with low PC1 scores were highly active and spent very little time in the open zone (shy).

Across the five angling sessions, a total of 112 captures occurred, with 51 fish captured only once, 26 fish captured twice, and 3 fish captured three times. There was no significant difference of the proportion of total captures between the structure zone ( $n = 55$  captures, 49%) versus the no structure zone ( $n = 57$  captures, 51 %) across all ponds (logistic regression,  $z$ -value =  $-0.433$ ,  $df = 2$ ,  $p = 0.665$ ). Mean PC1 score and total number of times individual fish were captured both did not differ significantly across ponds ( $PC1_A = -0.117 \pm 1.0$ ,  $PC1_B = 0.184 \pm 1.8$ ,  $PC1_C = -0.041 \pm 1.6$ , one-way ANOVA,  $F_2 = 0.392$ ,  $p = 0.677$ ; mean number of times individual fish were captured in ponds:  $A = 0.975 \pm 0.77$ ,  $B = 0.857 \pm 0.85$ , and  $C = 0.860 \pm 0.80$ , one-way ANOVA,  $F_2 = 0.275$ ,  $p = 0.76$ ). The proportion of total misses, as well as the proportion of total captures, did not differ significantly across the two anglers, accounting for the three different ponds used in the study; CDS had a total of 12 misses and 56 captures and MJL had a total of 18 misses and 56 captures (logistic regressions for misses:  $z$ -value =  $0.327$ ,  $df = 2$ ,  $p = 0.744$ ; logistic regression for captures:  $z$ -value =  $-0.835$ ,  $df = 2$ ,  $p = 0.403$ ). The total number of captures was significantly different across angling sessions, with 43 captures during session one, 30 captures during session two, and then between eight and 18 captures in each of sessions three through five (Table A.1). Angler had a significant effect on the behavior types that were captured, whereby the PC1 scores of the CDS captures were significantly lower than MJL, meaning that CDS captured highly active, shy fish compared to MJL's captures (Table 3.3).

The PC1 score of captured fish did not differ significantly across angling sessions (Table 3.4; Figure 3.2), and there was no difference in the PC1 scores of largemouth bass captured in either pond zone, or captured in both pond zones, relative to uncaptured fish (Table 3.4; Figure 3.3). Angler had a significant influence on the pond zone where fish were captured, with fish captured by both anglers (MJL and CDS) more likely to be captured in both the structure zone

and the no structure zone when compared to fish captured only by MJL or only by CDS (Table 3.5; Figure 3.4A). The pond zone in which a fish was captured significantly influenced the number of times it was captured (Table A.2; Figure 3.4A). Specifically, fish captured once were equally likely to be captured in either the structure or no structure zone, but fish captured more than once were significantly likely to have been captured in both the structure and no structure zones, rather than having multiple captures within a single pond zone. PC1 scores did not significantly predict number of individual captures, or differ between captured and uncaptured fish (Table A.2; Figure 3.4B).

### **3.4 Discussion**

Activity and boldness behaviors did not predict any aspect of angling vulnerability for largemouth bass in this study. Repeatable laboratory-derived behaviors formed a single principal component that consisted of measures of activity and boldness, and this PC score did not significantly predict the number of times fish were captured, the PC score of captured fish did not differ from that of uncaptured fish, and the PC score of captured fish did not change across the 5 angling sessions. Not all fish strike fishing lures, and there are many factors known to influence angling vulnerability including the probability of a fish encountering a lure, the metabolic rate of the fish, and behavioral phenotype (i.e., individual boldness or activity) (Binder et al. 2012; Härkönen et al. 2014; Arlinghaus et al. 2017a; Monk and Arlinghaus 2017). The five established animal behavior axes are aggression, sociability, activity, exploration, and boldness (Réale et al. 2007), and these behaviors differ between individual fish and have a heritable, genetic component (Mazué et al. 2015). Behavioral phenotypes are particularly important for fisheries as past studies have shown that behavioral phenotype can cause some fish to be predisposed to capture through angling (Biro & Post, 2008; Biro & Sampson, 2015; Lennox et

al., 2017; Stoner, 2004), and, more importantly, anglers have the potential to artificially select for fish of specific behavioral phenotypes (Cooke et al. 2007), decreasing genotypic diversity (Uusi-Heikkilä et al. 2015), and reducing capture rates (Kuparinen and Merilä 2007). Previous work linking behavioral metrics to angling vulnerability has been inconsistent; patterns emerging from some studies are not repeated in other work. For example, activity within ponds, measured by acoustic telemetry, did not predict angling vulnerability of Eurasian perch (Monk & Arlinghaus, 2018), while work measuring activity in hatchery-reared brown trout (*Salmo trutta*) in a laboratory setting found that the most active fish were the most vulnerable to angling (Härkönen et al. 2014). For boldness, bolder rainbow trout (*Oncorhynchus mykiss*; Biro & Post, 2008) and common carp (*Cyprinus carpio*; Klefoth, Skov, Kuparinen, & Arlinghaus, 2017) were both shown to be more vulnerable to capture than their shy conspecifics, while the opposite was found for bluegill (*Lepomis macrochirus*), however, with shy bluegill more vulnerable to angling (Wilson et al., 2011b). But, studies relating behavioral traits to capture in largemouth bass specifically seem to be consistent, as several studies have failed to demonstrate a link between behavior and capture (present study; Louison et al., 2017), with angling vulnerability more strongly linked to cortisol responsiveness and learning (Louison et al. 2017, 2019). Because largemouth bass are sit-and-wait ambush predators that remain stationary and wait for prey items to appear before them (Fu et al. 2009; Conrad et al. 2011), activity and boldness may not play a role in their foraging strategy, and therefore may not influence encounters and strikes with fishing lures. Rather, for largemouth bass, upon having a lure appear in front of them, their decision to strike appears to be more strongly influenced by cortisol responsiveness and aggression (Suski and Philipp 2004; Louison et al. 2017), rather than boldness or activity

metrics. Together, results from this study indicate that activity and boldness behavioral traits do not predict capture in largemouth bass.

The total proportion of largemouth bass captured, as well as the number of times individuals were captured, were similar between the no structure and structure zones of the ponds. Habitat is a key aspect of fish growth and survival, with many fish commonly found in association with habitat (Lima 1998; Hollins et al. 2018). As a result, many management programs are predicated on building, restoring, or enhancing habitat, often with artificial structures, in an effort to enhance fish populations, encourage growth and improve angler catch rates (Smokorowski and Pratt 2007; Baumann et al. 2016). Previous work with largemouth bass have found that artificial habitat can attract fish, increase densities, and also increase angler catch rates (Bolding et al. 2004). In addition, work by Baumann et al., (2016) showed that the addition of porcupine attractors increased largemouth bass abundance compared to control sites, but angling was not used to link this increased abundance to influences on catch rates. There are three possible explanations as to why we did not see differences in capture metrics between the structure and no structure zones of our ponds. First, it is possible that the habitat we used (PVC tubes) was not sufficiently structurally complex and did not have small interstitial spaces, which are known to be more effective at aggregating largemouth bass (Bolding et al. 2004), such that largemouth bass did not associate with these structures. However, porcupine attractors are commonly used to successfully aggregate fish, including largemouth bass, and enhance fisheries when compared to control environments with no habitat additions (Baumann et al. 2016), suggesting that the association of largemouth bass with structure of this type is possible. Second, the small pond size (approximately 0.04 ha) and lack of predators may have allowed largemouth bass to utilize the entire pond for foraging without the perceived risk of being preyed upon,

making it difficult to detect differences in habitat use through angling (Lima 1998). Finally, it is possible that the addition of habitat could have resulted in an increase in fish abundance in the structure zone, but abundance was not measured (only capture). Fish abundance and angler catch rates are not always related (Wegener et al. 2018) and catch rates in some fisheries remain high even when abundance of fish is low (Erisman et al. 2011), meaning that largemouth bass densities may have been different across the 2 sections of our ponds, but this difference may not have translated into noticeable differences in catch rates. Future work on this topic should explore how different habitat types can influence habitat selection in largemouth bass, ideally working in larger environments to increase knowledge about space, habitat use, and possible influences of conspecific competition for this species. Regardless of the mechanism, results from this study demonstrate that the proportion of largemouth bass captured by anglers did not differ between areas of the pond that received supplemental additions of porcupine attractors relative to habitats where no artificial structures were added.

Unexpectedly, the behavioral phenotypes of captured largemouth bass was significantly different between the two anglers. More specifically, MJL captured fish with significantly higher PC scores, indicating more inactive and bold laboratory-based behaviors compared to CDS, and this difference was consistent across the three replicate ponds. This study was designed in a way to minimize differences in angler effects, including care taken to ensure both anglers fished in the same systematic and standardized way, both anglers using a single identical lure, and the use of randomized angling assignments during each session. In addition, based on the fact that there were no differences in either proportion of captures or proportion of misses, it is reasonable to assume that both anglers were similar in terms of skill. Other studies have found positive relationships between angler skill level, size of fish captured, and hooking depth, where

skilled anglers captured larger Eurasian perch (Monk & Arlinghaus, 2018) and hooked Eurasian perch deeper than unskilled anglers (Dunmall et al. 2001), indicating that different types of anglers can result in differences in fishing outcomes. In the current study, one possible explanation for why anglers of similar skill levels captured fish with different behavioral phenotypes may be related to subtle differences in angler fishing techniques or reaction times, measured between the time fish strike lures and when the anglers set their hooks (Gutowsky et al. 2017). Fish differ in their willingness to taste, bite, and reject fishing lures (Monk & Arlinghaus, 2017), as well as their approach to a fishing lure (aggressive vs. passive), and anglers can have different reaction times or fishing styles, such that this interaction can lead to capture of different behavioral phenotypes between anglers. For example, CDS may have had longer delays before setting the hook, or had longer reaction times, leading to the capture of only active fish that continued swimming after striking or aggressively ingesting the lure. In contrast, MJL may have allowed less time to pass before setting his hook following the detection of a strike, resulting in him capturing more inactive fish that did not strike forcefully. However, the direct relationship between angler skill level and behavior traits of captured fish was not explicitly quantified in the current study, making conclusions of this nature somewhat speculative. Overall, this difference in behavioral score across anglers suggests that selection pressures on fisheries may be more complex than previously thought, owing to behavioral differences of captured fish between individual anglers, even of the same skill level.

The behavioral phenotype of largemouth bass did not predict the pond zones where captures occurred, and largemouth bass captured by anglers did not exhibit habitat preferences across behavioral phenotypes. The behavioral phenotype of largemouth bass did not differ between fish captured in either the no structure or structure zones, fish captured multiple times



across zones (rather than being caught in only one zone), and uncaptured fish. Previous work has shown that behavioral phenotypes of fish, such as boldness and activity, are consistent within individuals, and that shy fish tend to spend more time in sheltered habitats than their bolder conspecifics (Hollins et al. 2018). As such, prior to starting this study, we predicted that fish of certain behavioral phenotypes would have associated with different habitat types, which would have resulted in PC scores for captured fish differing across capture locations. Previous work with bluegill, for example, found a relationship between habitat use, behavior, and vulnerability, with bold bluegill being more vulnerable to angling while spending time in open areas away from refuges when compared to their shy conspecifics (Wilson et al., 2011b). A study with Eurasian perch found a similar result, in which habitat choice strongly influenced capture vulnerability, whereby fish were more vulnerable to capture in specific spatial locations of their study lake (Monk & Arlinghaus, 2018). However, the most highly vulnerable perch with the most captures were not repeatedly captured in the same habitat types (Monk & Arlinghaus, 2018), similar to results shown here. In the current study, potential reasons for not finding a relationship between behavioral phenotypes and habitat types as predicted are likely due to the small size of the ponds or lack of direct translation from boldness behaviors measured in the laboratory to habitat selection behaviors within the study ponds. It is also possible that the interaction between behaviors and habitat selection do not exist and therefore cannot determine angling vulnerability of largemouth bass, which warrants further investigation in future studies. Together, results show that largemouth bass of all behavioral phenotypes were utilizing all available habitats (both no structure and structure zones), and that habitat selection is likely not a product of behavioral phenotypes.

Results from this study have three main implications for fisheries management. First, findings indicate that there will be minimal selection on activity and boldness behaviors of largemouth bass by anglers, with all behavioral phenotypes equally vulnerable to capture regardless of habitat type. Previous work has suggested that angling has the potential to disproportionately remove bold individuals from the population leaving only shy fish, a condition often referred to as the ‘timidity syndrome’ (Arlinghaus, Laskowski, et al., 2017; Biro & Post, 2008). Results from the current study do not support this hypothesis, however, and concur with previous work in largemouth bass showing that behavior has little impact on angling vulnerability for this species (Louison et al. 2017), indicating that the disproportionate removal of bold individuals by anglers would not be expected. It is possible that selection on other factors could occur (i.e., cortisol responsiveness, learning (Louison et al. 2017, 2019)) that could lead to reduced capture rates and/or FIE, and future work should quantify additional behavioral axes not measured here (i.e., sociability, conspecific aggression) while also incorporating other environmental contexts not addressed here. Second, this study did not find capture rates to be higher in the structure zone of the ponds with porcupine attractors present. Because the goal of many management projects is to introduce artificial structures to supplement habitat restoration, attract fish, and increase catch rates, management activities aimed at increasing capture rates of largemouth bass should consider structures other than porcupine attractors, including those with more interstitial space (Bolding et al. 2004; Baumann et al. 2016). Lastly, individual anglers may be influencing capture of fish with certain behavioral and physiological traits, even between anglers of similar skill levels. Selection pressures based on angler technique and/or skill level should be investigated in further detail to discern the influence of angler behavior on the phenotypes of captured fish. These angler differences pose a unique challenge by increasing the

complexity to achieve effective fisheries management that can be applied to all types and skill levels of anglers.

### 3.5 Tables

Table 3.1. Results of intra-class correlation (ICC) analysis to define repeatability of 5 behaviors generated during laboratory assays with largemouth bass. Confidence intervals and p-values (with  $p < 0.05$  are in bold text) show the strength in the repeatability for each behavior. All measured behaviors, except for freeze time, were repeatable.

Behavior	Repeatability	Confidence interval	p
Swim activity pre-predator (s)	0.245	$0.068 < \text{ICC} < 0.407$	<b>0.004</b>
Time spent in center pre-predator (s)	0.472	$0.319 < \text{ICC} < 0.601$	<b>&lt;0.001</b>
Swim activity post-predator (s)	0.395	$0.232 < \text{ICC} < 0.537$	<b>&lt;0.001</b>
Time spent in center post-predator (s)	0.487	$0.337 < \text{ICC} < 0.613$	<b>&lt;0.001</b>
Freeze time (s)	0.167	$-0.013 < \text{ICC} < 0.337$	0.035

Table 3.2. Principal component analysis (PCA), factor loadings and variance for repeatable behaviors generated from laboratory assays with largemouth bass. The behaviors measured, as well as ICC analyses to quantify repeatability, are given in Table 3.1. Factor loadings above 0.4 are shown, as behaviors above this threshold can be used to explain relationships between factors within a PC.

Factor	PC1 loadings
Swim activity pre-predator (s)	-0.511
Time spent in center pre-predator (s)	0.585
Time spent in center post-predator (s)	0.538
Eigenvalue	2.28
% Variance explained	57.01

Table 3.3. Summary of a two-way ANOVA testing for effects of angler, pond, and their interaction on the PC scores of captured fish. The behaviors used to generate PC scores were repeatable across two behavioral assays, and results of repeatability analyses are show in Table 3.1. Results of PC analyses are shown in Table 3.2. Angler was the only predictor variable with a significant influence on the PC scores of captured largemouth bass ( $p < 0.05$  in bold text).

Predictor variable	F-value	p
<b>Angler</b>	3.36	<b>0.041</b>
Pond	0.29	0.751
Angler $\times$ pond	0.31	0.873

Table 3.4. Summary of linear mixed effects model relating the PC score of captured largemouth bass across angling sessions and pond zones (fixed effects), with pond and angler included in the model as random effects. None of the fixed effects influenced the PC score of captured largemouth bass. Data are visualized in Figure 3.2.  $R^2_m$  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 random factors.

Variable	Parameter estimate	Standard error	df	t-value	p	$r^2_m$	$r^2_c$
Angling session	-0.319	1.017	69.750	0.693	0.544	0.035	0.148
Structure zone	-1.067	1.115	69.920	0.957	0.342		
No structure zone	-0.597	1.101	69.110	0.542	0.589		
Angling session $\times$ structure zone	0.405	0.567	69.550	0.714	0.478		
Angling session $\times$ open zone	0.484	0.575	68.870	0.840	0.404		
Random effects	Variance	Standard deviation					
Pond (intercept)	0.042	0.204					
Angler (intercept)	0.283	0.532					
Residual	2.450	1.565					

Table 3.5. Summary of multinomial regression explaining which zone in the pond largemouth bass were captured in across 5 angling sessions in three replicate ponds. Principal component score, pond, and angler were the predictor variables in the multinomial regression, and the dependent variable was the pond zone where fish were captured (either the no structure zone, the structure zone, or both). A diagram of the pond is shown in Figure 3.1, the measured behaviors to generate PC scores are shown in Table 3.1, and the results of PC analyses are given in Table 3.2. Angler was the only predictor variable with a significant influence on the pond zones where largemouth bass were captured ( $p < 0.05$  in bold text).

Variable	LR $\chi^2$	Degrees of freedom	p
PC score	2.752	2	0.253
Pond	1.779	4	0.776
Angler	13.492	4	<b>0.009</b>



### 3.6 Figures

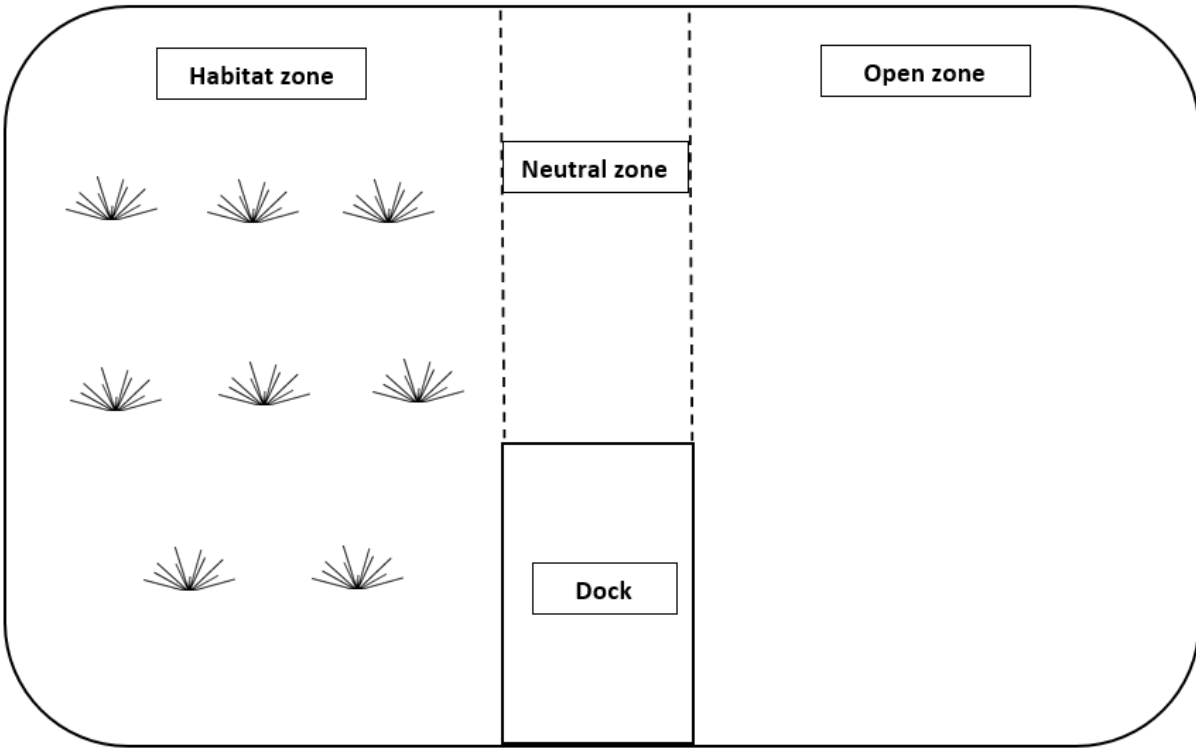


Figure 3.1. Overhead view of the experimental pond design. Each of the 3 replicate ponds had 1) a structure zone, equipped with 8 porcupine attractors for fish to utilize as habitat, 2) a no structure zone, devoid of structures and vegetation due to benthic liners, providing no habitat for stocked largemouth bass and 3) a neutral zone separating the structure zone and the no structure zone, which extended from a dock. The neutral zone did not have porcupine attractors or benthic liners.

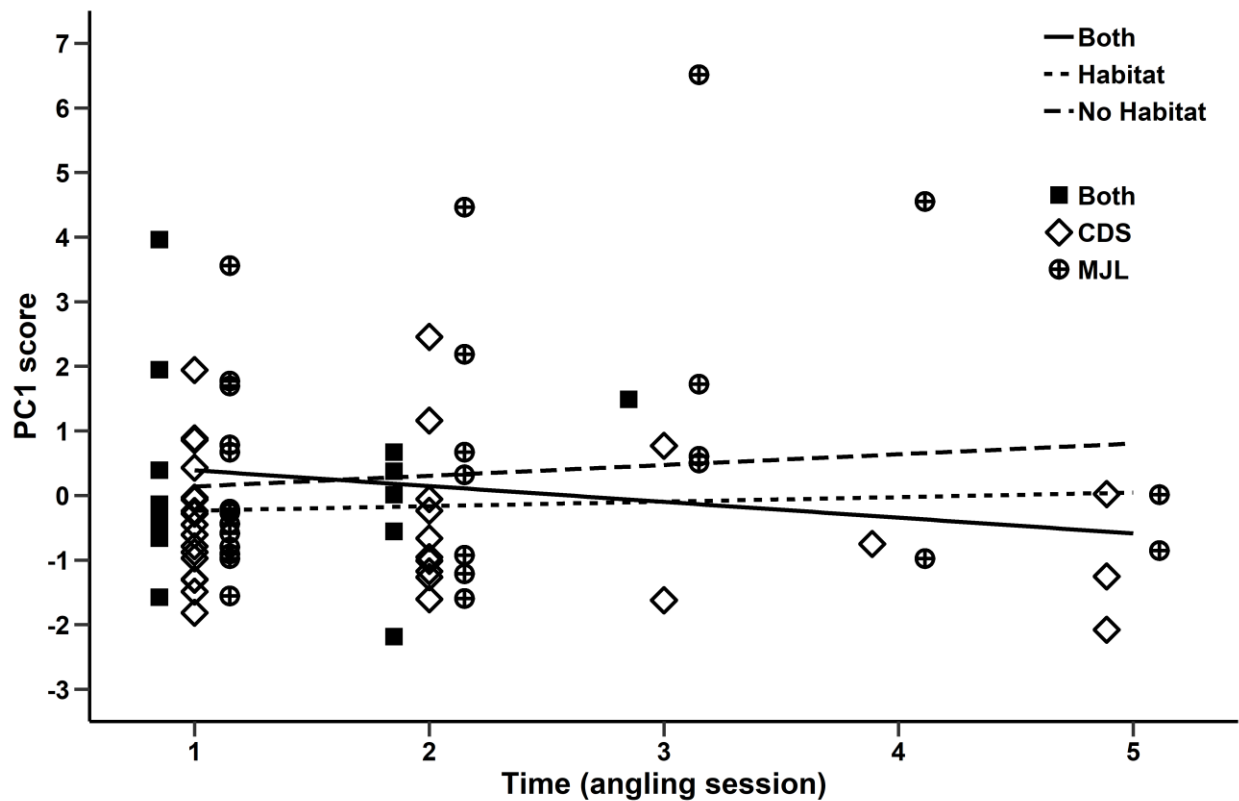


Figure 3.2. Differences in PC scores of largemouth bass captured across 5 different angling sessions in three replicate ponds. The short-dashed line shows the change in PC score across angling sessions for largemouth bass captured exclusively in the structure zone, the long-dashed line shows the change in PC score for largemouth bass captured in the no structure zone, and the solid line shows the change in PC score over angling sessions for largemouth bass captured in both pond zones. Symbol shape represents fish captured by CDS (open diamond), MJL (circle with plus sign) or both anglers (filled circle). Results of statistical analyses are shown in Table 3.4.

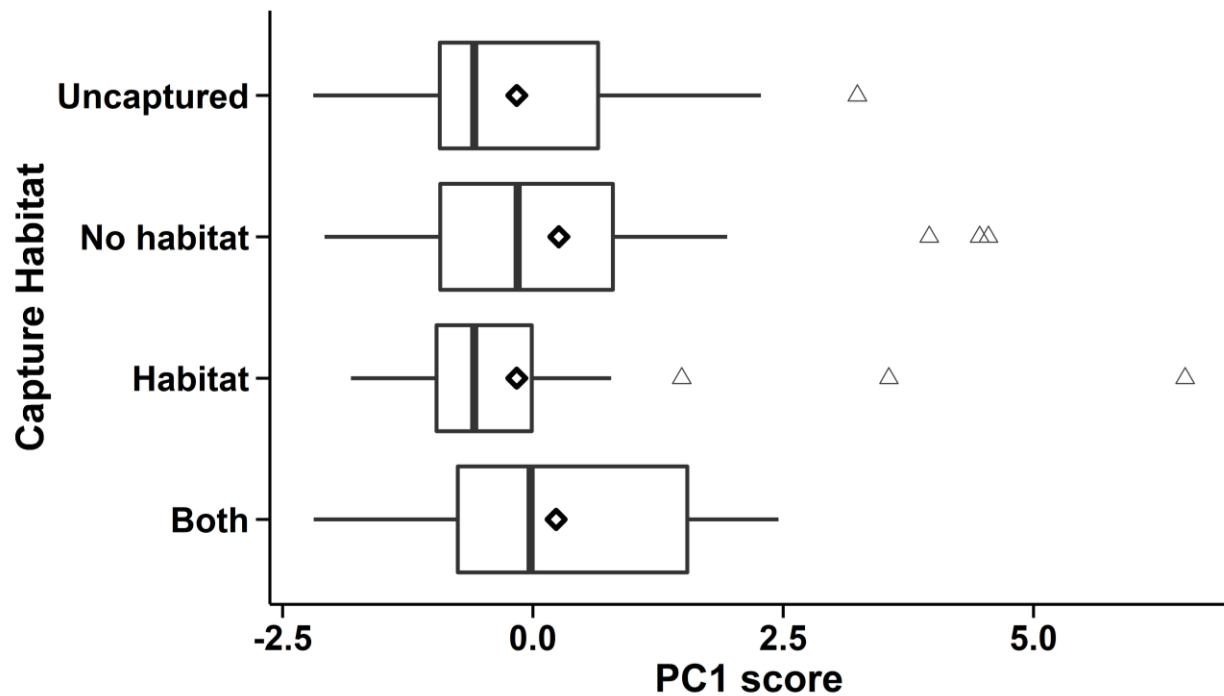


Figure 3.3. Distribution of PC scores for largemouth bass captured in pond zones that received supplemental habitat (habitat), received no supplemental habitat (no habitat), were captured in both structure and no structure zones (both), or were uncaptured. Angling occurred in three replicate ponds by two experienced anglers across five angling sessions. The thick vertical lines inside the boxes show the median, the diamonds represent the mean, the whiskers represent the upper and lower quartiles  $\pm$  the interquartile range, and the open triangles represent outliers with values less than the first quartile minus 1.5 times the interquartile range or greater than the third quartile plus 1.5 times the interquartile range. Behavioral metrics that make up the PC score were repeatable, and are shown in Table 3.1.



## **CHAPTER 4: FOOD DEPRIVED FISH ARE INACTIVE AND STRESSED, BUT DO NOT ALTER LURE INSPECTIONS**

### **4.1 Introduction**

Coping styles are suites of correlated behavioral and physiological traits that can explain how individual animals, including fish, react to stressful situations (Overli et al. 2007). 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 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 (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). Because behavioral and physiological traits are closely linked within coping styles, consistent relationships have been shown between activity behaviors and cortisol responsiveness (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). 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 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 two 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 (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 (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 in 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 fish coping styles 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 would be expected to increase activity rates (Beukema 1968), thereby increasing the probability of encountering fishing lures, and increasing the likelihood of capture. However, if fish experience increased stress responsiveness due to low food availability, then cortisol concentrations may increase, leading 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 with 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 (Philipp et al. 2009; Laugen et al. 2014; Mittelbach et al. 2014; Hessenauer et al. 2015, 2016). 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 were either fed or food deprived for 2-weeks. Then, behavior assays and cortisol responsiveness measurements were repeated. 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.

## **4.2 Methods**

### *4.2.1 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 =  $280 \pm 18.0$  mm; mass =  $284 \pm 70.3$  g; relative weight (a ratio of mass to length through the use of a species' standard weight; (Henson 1991)) =  $89.3 \pm 8.74$ ). Fish were evenly divided and held in 8 circular 1,135 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 (Tooele, Utah). Four days after arrival, fish were implanted with passive integrated transponder (PIT) tags (10 mm [length] × 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.

#### 4.2.2 Behavior Assays

Fish were moved from outdoor tanks to indoor isolation tanks to acclimate overnight for 15 - 23 hours 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 two 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 ± 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 two 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 (Wilson et al. 1993; Naslund and Johnsson 2016; Tucker et al. 2018). 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 10 additional 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.

#### *4.2.3 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 one 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 (Romero and Reed 2005; Lawrence et al. 2018). 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.

#### *4.2.4 Feeding Treatments*

Following the initial behavior assay and baseline cortisol responsiveness, fish were held in one of two circular 1,135 L or one of two rectangular 180 × 65 cm, 379 L indoor holding tanks

and randomly divided into two treatments (four 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  $9.4 \pm 0.4 \text{ mg/L}$ , 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 ( $TL_{\text{food deprived}} = 278 \pm 13 \text{ mm}$ ,  $TL_{\text{fed}} = 282 \pm 22 \text{ mm}$ ,  $mass_{\text{food deprived}} = 270 \pm 36 \text{ g}$ ,  $mass_{\text{fed}} = 298 \pm 93 \text{ g}$ ,  $relative\ weight_{\text{food deprived}} = 88 \pm 6$ ,  $relative\ weight_{\text{fed}} = 91 \pm 11$ ; t-tests,  $t = -0.61, -1.14, \text{ and } -0.99$ , respectively,  $p > 0.05$  for all three 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, cortisol responsiveness, and fishing lure inspections. 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). All other aspects of the behavior assay were identical to those described above.

#### *4.2.5 Statistical Analysis*

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 with eigenvalues  $> 1$  and factors with loadings  $> 0.50$  after varimax rotation were included as primary factors for associated PCs (Hair 1998; Ho 2006; Budaev 2010). 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 two 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, two 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 three 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 two separate PCAs (one pre-treatment and one 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 was included as a random effect. Lastly, two additional generalized linear mixed effects models with Poisson error distributions were carried out, one pre-treatment and one 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 ID was included as a random effect. All models described above initially included relative weight, water temperature, and behavior arena number as fixed effects, but these terms were subsequently removed as they did not have significant effects on any of the models (Engqvist 2005; Crawley 2013). 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), ‘lsmeans 2.30-0’ (Lenth 2016), ‘ggplot2 2.2.1’ (Wickham 2009), and ‘gridExtra 2.3’ (Auguie 2017) packages. The significance level for all tests was set at  $\alpha = 0.05$ .

### **4.3 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.3). 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 4.1). 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 either fed or food deprived for 2-weeks resulted in two principle components, with PC1 and

PC2 accounting for 46% and 31% of the variance, respectively (Table 4.2). This second PCA separated activity behaviors performed pre- and post- lure introduction, leading them to be grouped ‘pre-lure 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 4.3). 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 4.4). In addition, maximum cortisol concentration was significantly correlated with both baseline cortisol and cortisol responsiveness pre- and post-treatment (Table 4.4), 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 4.5; Figure 4.1). Both maximum cortisol concentrations and cortisol responsiveness were not different between treatment groups either pre- or post-treatment (Table 4.5; Figure 4.1). 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 4.6; Figure 4.2). 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 4.6).

Neither PC scores nor cortisol metrics had significant effects on the number of times fish visited lures prior to the feeding/food deprivation treatments (Table 4.7). However, baseline cortisol concentrations significantly influenced lure visits after largemouth bass in both the fed and food deprived treatments, whereby fish with low baseline cortisol made more visits to the fishing lure than fish with high baseline cortisol (Table 4.7; Figure 4.3).

#### **4.4 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 (Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010). Therefore, to survive, fish need to be able to adjust to environments with low food availability, often through physiological and behavioral changes (Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010). 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 (Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010). Twelve weeks of food deprivation, for example, caused Atlantic cod (*Gadus morhua*) to demonstrate decreased sustained swimming activity (Martínez et al. 2002). 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 (Mendez and Wieser 1993; Gingerich et al. 2010; Jobling 2011). 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 a parental care period, 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 after air exposure and handling during blood draws, may be lower than in the absence of these chronic environmental stressors (Wendelaar Bonga 1997; Abrahams 2011). 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 two stressors combine, the overall stress response may be negated through antagonism instead of enhanced through synergism (Folt et al. 1999). 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 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. More importantly, largemouth bass with high cortisol responsiveness (i.e., reactive fish) 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. Because results from the current study did not demonstrate a link between cortisol responsiveness and lure inspections, it is likely that cortisol responsiveness does 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 of 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 (*Solea senegalensis*) 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 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. 2014). 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 of 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 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 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 (Mendez and Wieser 1993; Jobling 2011; Killen et al. 2011). 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 (Navarro and Gutierrez 1995; Wang et al. 2006; McCue 2010; Tucker et al. 2018). Because activity did not influence lure inspections either pre- or post- feeding treatment, however, 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 or other potential environmental stressors to better define behavioral plasticity in various ecological settings. 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 styles refer 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, 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 (Sih and Bell 2008). Activity behaviors and cortisol metrics of largemouth bass did not combine to exhibit distinct coping styles.

Results from this study can inform fisheries management in four important ways. First, results reinforce the idea that there are many steps leading to capture, and lure inspection behaviors are different from lure-striking behaviors (Lennox et al. 2017). Because this study focused on lure inspection, which may not be directly correlated with lure-striking behavior, it is possible that cortisol responsiveness and activity do not influence this lure inspection step

leading to capture. Behavioral and physiological traits may only be applicable to specific steps leading to whether fish are vulnerable to capture by hook-and-line, so future studies can investigate links between other traits to discern predictors that may determine encounter, inspection, lure-striking, and ingestion behaviors leading to capture. Second, environmental stressors, such as food deprivation, may have little influence on lure inspection behaviors, so the likelihood of artificial selection of behavioral and physiological traits through recreational angling are expected to be similar across these environmental contexts. Therefore, if largemouth bass are food deprived in exploited populations, managers would not expect to see enhanced evidence of selective fishing exploitation on activity and cortisol responsiveness traits. However, other environmental constraints must also be further investigated. Third, food deprivation can decrease activity rates and increase baseline cortisol concentrations of largemouth bass, which may have negative ecological impacts on foraging and angling vulnerability. For example, in species that actively swim in search of prey, food deprivation may lead to further energetic stress whereby fish reduce swimming activities to save energy at the cost of decreased foraging success (Mendez and Wieser 1993; Jobling 2011). A decrease in angler satisfaction may also occur in environments where prey is scarce for the target fish, whereby some species may decrease their activity rates and be less likely to encounter fishing lures, leading to a decreased likelihood of capture. Lastly, because coping styles can predispose fish to angling capture, it is important for fisheries managers to understand coping styles and how they relate to angling vulnerability in order to minimize effects of fisheries-induced evolution in fish populations. But, if behavior and stress responsiveness traits are uncoupled in certain species, including largemouth bass, these traits must be investigated independently to predict hook-and-line vulnerability. Relationships determined from this study can be used to



help fisheries managers further protect largemouth bass from potential negative consequences inflicted through angling exploitation.

## 4.5 Tables

Table 4.1. 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)	<b>0.545</b>
Post-lure distance moved (cm)	<b>0.536</b>
Eigenvalue	2.71
% variance explained	67.76

Table 4.2. 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 loaded positively on PC1, while activity and distance moved post-lure introduction loaded positively on PC2. Therefore, PC1 can be used to describe pre-lure introduction behaviors and PC2 can be used to describe post-lure introduction behaviors.

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

Table 4.3. 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 ( $p < 0.05$ ) 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	<b>0.94</b>	0.11
Post-treatment lure visits	0.74	-	0.06	<b>0.99</b>
Pre-treatment time spent in proximity to lure (s)	<b>&lt; 0.01</b>	0.76	-	0.10
Post-treatment time spent in proximity to lure (s)	0.53	<b>&lt; 0.01</b>	0.60	-

Table 4.4. Results from a 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 4.1), but both PC1 and PC2 scores are shown for post-treatment behaviors, as the second PCA determined 2 principal components (Table 4.2). 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 ( $p < 0.05$ ) are in bold.

Pre-treatment					
	Baseline cortisol	Maximum cortisol	Cortisol responsiveness	PC1 score	
Baseline cortisol	-	<b>0.37</b>	0.10	0.14	
Maximum cortisol	<b>0.04</b>	-	<b>0.92</b>	-0.18	
Cortisol responsiveness	0.61	<b>&lt;0.01</b>	-	-0.13	
PC1 score	0.46	0.97	0.75	-	
Post-treatment					
	Baseline cortisol	Maximum cortisol	Cortisol responsiveness	PC1 score	PC2 score
Baseline cortisol	-	<b>0.57</b>	0.13	0.02	-0.09
Maximum cortisol	<b>&lt;0.01</b>	-	<b>0.82</b>	-0.01	0.11
Cortisol responsiveness	0.50	<b>&lt;0.01</b>	-	0.03	0.22
PC1 score	0.91	0.95	0.85	-	0.04
PC2 score	0.65	0.54	0.23	0.84	-

Table 4.5. 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 random factors. Significant variables within each model ( $p < 0.05$ ) are in bold.

Factors	coefficient	SEM	df	t	p	$r^2_m$	$r^2_c$
Baseline cortisol							
Intercept	51996.69	6367.50	57.81	8.17	<0.001	0.20	0.25
<b>Treatment</b>	-18887.47	9153.35	57.83	-2.06	<b>0.043</b>		
<b>Time</b>	-27331.69	8888.82	29.42	-3.08	<b>0.005</b>		
Treatment $\times$ time	13563.84	12570.69	29.42	1.08	0.289		
Maximum cortisol							
Intercept	187213.81	19801.39	47.58	9.46	<0.001	0.10	0.53
Treatment	-51368.76	28362.91	48.46	-1.81	0.076		
Time	-19523.53	20800.85	28.60	-0.94	0.356		
Treatment $\times$ time	6057.79	29416.85	28.60	0.21	0.838		
Cortisol responsiveness							
Intercept	135217.12	18951.47	48.69	7.14	<0.01	0.05	0.48
Treatment	-32239.17	27155.57	49.52	-1.19	0.241		
Time	6747.00	20362.52	29.07	0.33	0.743		
Treatment $\times$ time	-6687.02	28796.95	29.07	-0.23	0.818		

Table 4.6. Summary of mixed effects models explaining activity (time spent swimming or distance moved during behavior assays) and the number of lure (novel object) inspections performed by largemouth bass that were food deprived or fed for 2 weeks. Independent variables in the models include treatment (fed vs. food deprived), time (prior to feeding/food deprivation or after 2 weeks of feeding/food deprivation), and their interaction. Fish ID was used as a random effect in both models as the same fish were tested before and after the 2 week feeding/food deprivation period. 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.  $r^2_m$  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 ( $p < 0.05$ ) are in bold.

Factors	coefficient	SEM	df	t/z	p	$r^2_m$	$r^2_c$
Activity							
Intercept	193.19	28.63	58.00	6.75	<0.001	0.12	0.12
Treatment	18.08	41.16	58.00	0.44	0.662		
<b>Time</b>	106.81	41.16	58.00	2.60	<b>0.012</b>		
Treatment $\times$ time	-50.45	58.20	58.00	-0.87	0.390		
Lure visits							
Intercept	-0.83	0.43	-	-1.90	0.057	0.09	0.09
Treatment	0.41	0.54	-	0.76	0.447		
Time	0.21	0.41	-	0.50	0.617		
Treatment $\times$ time	0.42	0.53	-	0.80	0.426		

Table 4.7. 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 4.2. Independent variables in the models include treatment (fed or food deprived), PC scores (see Tables 4.1 and 4.2), baseline cortisol, and cortisol responsiveness (Table 4.4). Fish ID was used as a random effect in both models.  $r^2_m$  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 ( $p < 0.05$ ) are in bold.

Factors	coefficient	SEM	z	p	$r^2_m$	$r^2_c$
Pre-treatment						
Intercept	-0.66	0.93	-0.71	0.480	0.14	0.14
Treatment	1.06	0.70	1.52	0.130		
PC1	0.24	0.20	1.19	0.236		
Baseline cortisol	<0.01	<0.01	-0.44	0.660		
Cortisol responsiveness	<0.01	<0.01	-0.34	0.731		
Post-treatment						
Intercept	-0.92	0.95	0.97	0.332	0.63	0.63
Treatment	0.37	0.69	0.55	0.586		
PC1	<0.01	0.28	0.03	0.974		
PC2	0.01	0.30	-0.05	0.964		
<b>Baseline cortisol</b>	<0.01	<0.01	-2.24	<b>0.025</b>		
Cortisol responsiveness	<0.01	<0.01	-0.19	0.852		



## 4.6 Figures

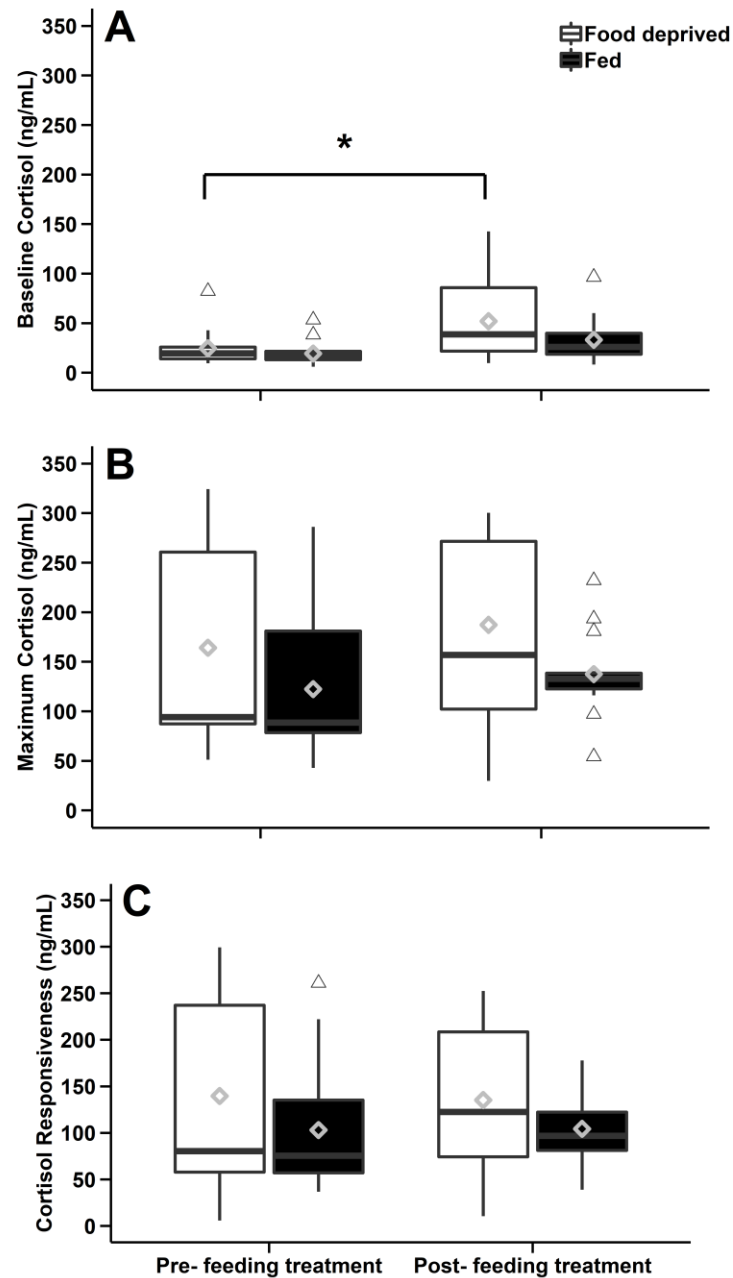


Figure 4.1. Boxplots showing A) baseline cortisol, B) maximum cortisol, and C) cortisol responsiveness values both pre- and post-feeding treatment. White boxplots represent largemouth bass that were food deprived for 2-weeks, while black boxplots represent largemouth bass that were fed for 2-weeks. The 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 ( $p=0.04$ ), and analyses are in Table 4.5.

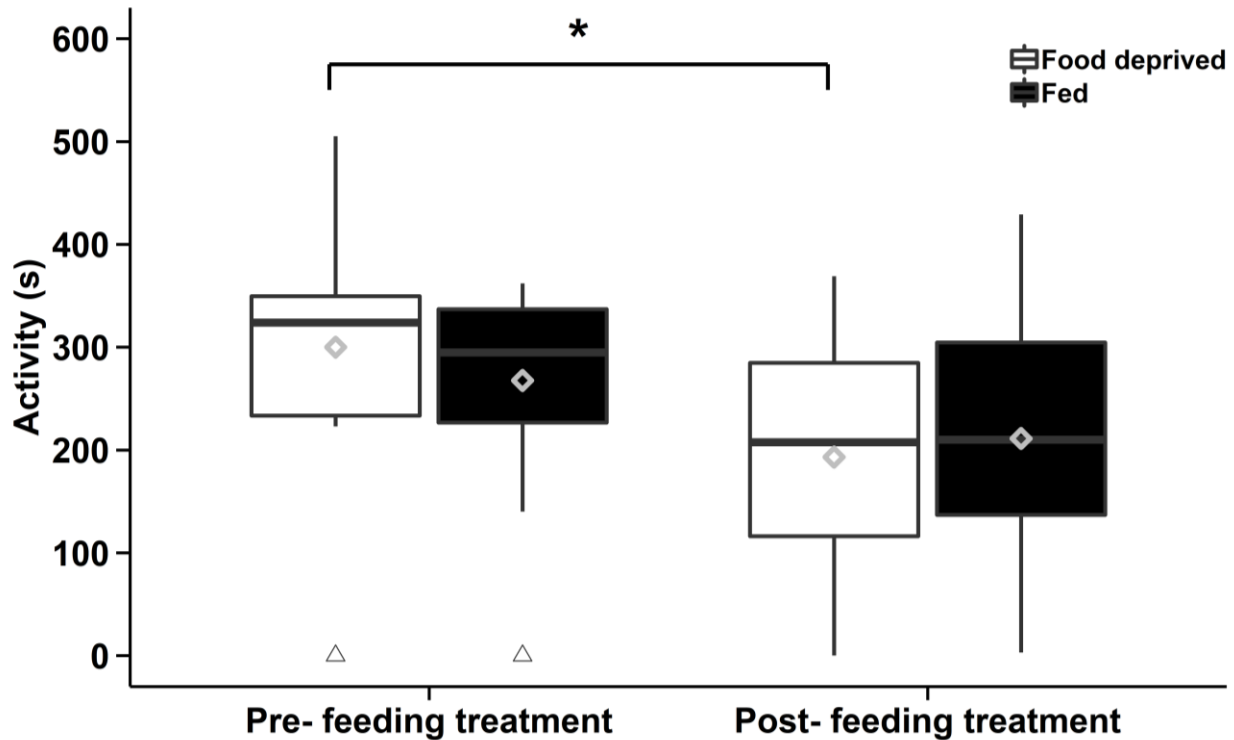


Figure 4.2. Boxplots showing pre-lure introduction activity (which was correlated with post-lure introduction activity) in a behavior arena (time spent swimming, which was correlated with distance moved) for largemouth bass that had been fed or food deprived for 2 weeks. White boxplots represent largemouth 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 ( $p = 0.04$ ). Statistical tests are shown in Table 4.6.

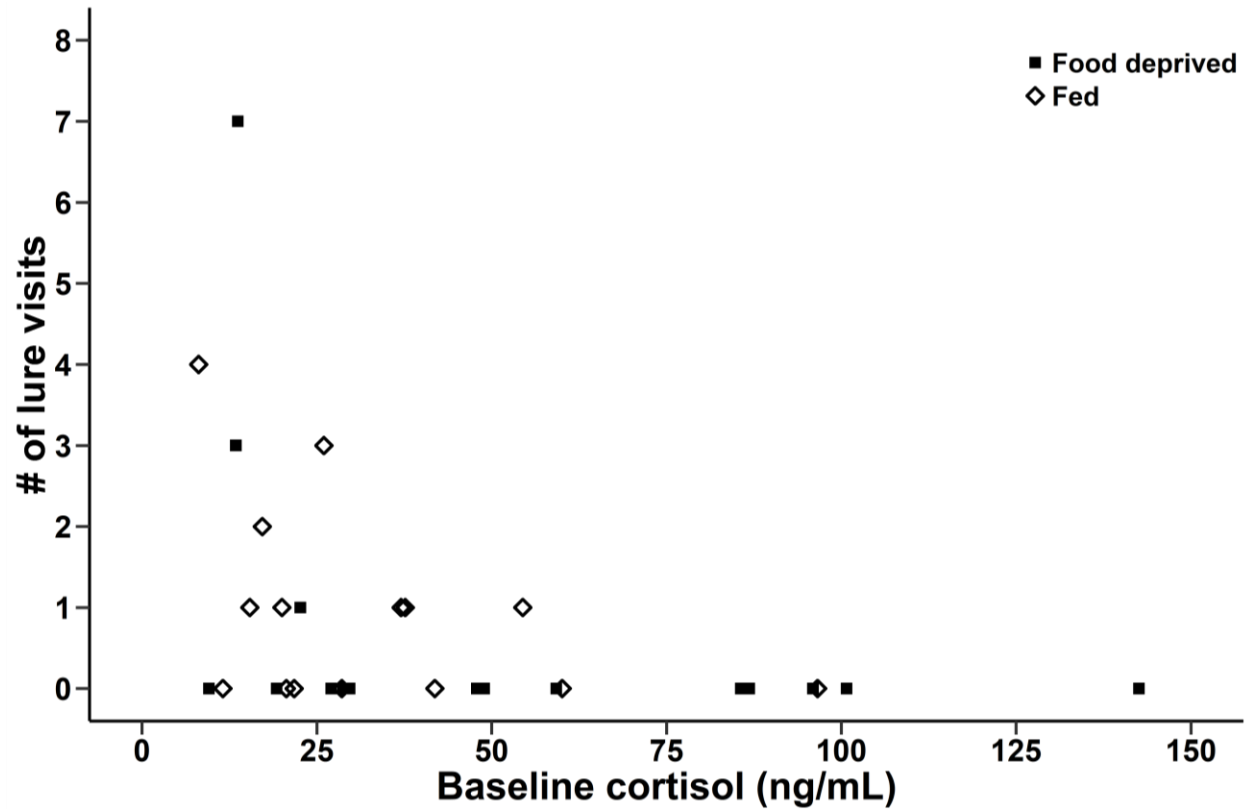


Figure 4.3. Point graph showing the relationship between baseline cortisol concentration (ng/mL) and number of times largemouth bass visited the presented fishing lure (novel object inspection, which was correlated to time spent in proximity to the lure) in a behavior arena post-treatment (after 2 weeks of feeding or food deprivation;  $p = 0.03$ ). 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.

## **CHAPTER 5: SUMMARY AND BROADER CONTEXT**

As fisheries-induced evolution is expected to occur due to the disproportionate capture and harvest of largemouth bass with specific traits, behavioral and physiological drivers that predispose individuals to recreational angling capture must be understood. Fisheries-induced evolution has the potential to artificially select for fish of specific behavioral phenotypes and stress responsiveness, with the potential to minimize genetic and phenotypic diversity within exploited bass populations. Individual angling vulnerability can also be modulated by changes in environmental conditions where angling occurs, including differences in food and habitat availability. However, angling vulnerability has traditionally been measured in static environments, which are not representative of the dynamic natural systems where recreational fishing occurs. Thus, my thesis research sought to fill knowledge gaps about the interactions between environmental conditions, behavioral phenotypes, and physiological traits to determine angling vulnerability in largemouth bass. This was completed by answering three questions in separate, but complementary studies: 1) do food availability, exploratory behaviors, and boldness behaviors interact to determine angling vulnerability?; 2) do largemouth bass activity and boldness behaviors combine with habitat preferences to determine angling vulnerability?; and 3) does food deprivation interact with activity behaviors and stress responsiveness to determine novel object (lure) inspection?

The first thesis chapter was the first study to quantify the interaction between food availability and behavioral phenotypes to predict angling vulnerability in largemouth bass. We found that the interaction between food availability and behavioral phenotypes of largemouth bass did not predict capture, whereby individuals with different behavioral phenotypes were equally vulnerable to capture across food availability contexts. Largemouth bass capture

vulnerability was similar across behavioral phenotypes as well. However, regardless of behavioral phenotype, largemouth bass in the experimental pond with no minnows present were more vulnerable to capture than were bass in the experimental pond stocked with minnows. This chapter provides evidence that largemouth bass of all behavioral phenotypes have increased vulnerability when food availability is low.

The second thesis chapter used a novel approach and combined behavioral phenotypes and habitat preferences of largemouth bass to determine angling vulnerability. Behavioral phenotype and habitat preference did not interact to determine angling vulnerability of largemouth bass, meaning specific behavioral phenotypes were not preferentially captured in one habitat type over the other. The behavioral phenotypes of captured and uncaptured largemouth bass were similar, as were the catch rates where artificial structure was present and absent. This chapter provides evidence that the presence or absence of artificial porcupine attractors does not alter capture vulnerability of largemouth bass, even of differing behavioral phenotypes.

The third thesis chapter was the first study to use fishing lures to quantify the combined effects of food deprivation, activity behaviors and cortisol responsiveness on novel object inspections of largemouth bass. Largemouth bass lure visits were not predicted by activity behaviors, cortisol responsiveness, or by feeding treatments. However, post-feeding/ food deprivation, largemouth bass with low baseline cortisol concentrations were more likely to inspect fishing lures across feeding treatments. Additionally, food deprived largemouth bass had reduced activity rates and increased baseline cortisol concentrations when compared to their fed conspecifics. Overall, baseline cortisol concentration was the only factor to predict lure inspection post-feeding/ food deprivation across treatments, providing evidence that activity and food deprivation may not be drivers of novel object inspection in largemouth bass.

Collectively, results from these three data chapters provide knowledge about angling vulnerability across varying environmental contexts. The general pattern across the chapters is that behavioral phenotype, including activity, exploration, and boldness, do not predict lure inspections or capture vulnerability of largemouth bass within the environmental contexts considered, including food availability and habitat types. Additionally, individual traits, including behavioral phenotype and cortisol responsiveness, do not interact with measured environmental contexts to determine lure inspections or capture vulnerability. However, angling vulnerability increased for largemouth bass of all behavioral phenotypes when food availability was low, and baseline cortisol concentrations for largemouth bass predicted lure inspections post-feeding treatment for fed and food deprived bass. Results indicate that environmental contexts measured here, including food availability and the presence or absence of additional artificial structure, may not have large influences on angling vulnerability of largemouth bass. In addition, the lack of relationships between behavioral and physiological traits on lure inspections and capture illustrate that largemouth bass angling vulnerability may not be trait selective based on activity, exploration, boldness, or cortisol responsiveness. Thus, it is possible that largemouth bass populations experiencing recreational exploitation may only be minimally affected by fisheries-induced evolution within the measured contexts.

## REFERENCES

- Abrahams M V. (2011) Behavioral responses to the environment | A survival guide for fishes: How to obtain food while avoiding being food. Elsevier Inc.
- Ahrens RNM, Walters CJ, Christensen V (2012) Foraging arena theory. *Fish Fish* 13:41–59. doi: 10.1111/j.1467-2979.2011.00432.x
- Alós J, Palmer M, Rosselló R, Arlinghaus R (2016) Fast and behavior-selective exploitation of a marine fish targeted by anglers. *Sci Rep* 6:. doi: 10.1038/srep38093
- Anderson RO, Heman ML (1969) Angling as a factor influencing catchability of largemouth bass. *Trans Am Fish Soc* 98:317–320
- Arlinghaus R, Alós J, Pieterek T, Klefoth T (2017a) Determinants of angling catch of northern pike (*Esox lucius*) as revealed by a controlled whole-lake catch-and-release angling experiment—The role of abiotic and biotic factors, spatial encounters and lure type. *Fish Res* 186:648–657. doi: 10.1016/j.fishres.2016.09.009
- Arlinghaus R, Laskowski KL, Alós J, et al (2017b) Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. *Fish Fish* 18:360–373. doi: 10.1111/faf.12176
- Arnold TW (2010) Uninformative parameters and model selection using Akaike's Information Criterion. *J Wildl Manage* 74:1175–1178. doi: 10.1111/j.1937-2817.2010.tb01236.x
- Asakawa A, Inui A, Kaga T, et al (2001) A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology* 74:143–147. doi: 10.1159/000054680
- Askey PJ, Richards SA, Post JR, Parkinson EA (2006) Linking angling catch rates and fish

- learning under catch-and-release regulations. *North Am J Fish Manag* 26:1020–1029. doi: 10.1577/M06-035.1
- Auguie B (2017) gridExtra: Miscellaneous Functions for “Grid” Graphics
- Ayala FJ, Campbell CA (1974) Frequency-dependent selection. *Annu Rev Ecol Syst* 5:115–138. doi: 10.1016/B978-0-12-374984-0.00558-1
- Baayen RH, Shafaei-Bajestan E (2019) languageR: Analyzing linguistic data: A practical introduction to statistics
- Barton K (2017) MuMIn: Multi-Model Inference
- Basic D, Winberg S, Schjolden J, et al (2012) Context-dependent responses to novelty in Rainbow trout (*Oncorhynchus mykiss*), selected for high and low post-stress cortisol responsiveness. *Physiol Behav* 105:1175–1181. doi: 10.1016/j.physbeh.2011.12.021
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using {lme4}. *J Softw* 67:1–48
- Baumann JR, Oakley NC, McRae BJ (2016) Evaluating the effectiveness of artificial fish habitat designs in turbid reservoirs using sonar imagery. *North Am J Fish Manag* 36:1437–1444. doi: 10.1080/02755947.2016.1227401
- Bell AM (2007) Future directions in behavioural syndromes research. *Proc R Soc B Biol Sci* 274:755–761. doi: 10.1098/rspb.2006.0199
- Bell AM, Hankison SJ, Laskowski KL (2009) The repeatability of behaviour: a meta-analysis. *Anim Behav* 77:771–783. doi: 10.1016/j.anbehav.2008.12.022
- Bell AM, Stamps JA (2004) Development of behavioural differences between individuals and



- populations of sticklebacks, *Gasterosteus aculeatus*. *Anim Behav* 68:1339–1348. doi: 10.1016/j.anbehav.2004.05.007
- Benus RF, Den Daas S, Koolhaas JM, Van Oortmerssen GA (1990) Routine formation and flexibility in social and non-social behaviour of aggressive and non-aggressive male mice. *Behaviour* 112:176–193
- Beukema AJJ (1968) Predation by the three-spined stickleback ( *Gasterosteus aculeatus* L .): The influence of hunger and experience. *Behaviour* 31:1–126
- Binder TR, Nannini MA, Wahl DH, et al (2012) Largemouth bass selected for differential vulnerability to angling exhibit similar routine locomotory activity in experimental ponds. *Trans Am Fish Soc* 141:1252–1259. doi: 10.1080/00028487.2012.688919
- Biro PA, Beckmann C, Stamps JA (2010) Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proc R Soc B* 277:71–77
- Biro PA, Post JR (2008a) Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. *Proc Natl Acad Sci* 105:2919–2922. doi: 10.1073/pnas.0708159105
- Biro PA, Post JR (2008b) Rapid depletion of genotypes with fast growth and bold personality traits from harvested fish populations. *Proc Natl Acad Sci U S A* 105:2919–2922. doi: 10.1073/pnas.0708159105
- Biro PA, Sampson P (2015) Fishing directly selects on growth rate via behaviour: implications of growth- selection that is independent of size. *Proc R Soc B* 282:. doi: 10.1098/rspb.2014.2283

- Bolding B, Bonar S, Divens M (2004) Use of artificial structure to enhance angler benefits in lakes, ponds, and reservoirs: A literature review. *Rev Fish Sci* 12:75–96. doi: 10.1080/10641260490273050
- Bolker BM, Brooks ME, Clark CJ, et al (2008) Generalized linear mixed models : a practical guide for ecology and evolution. *Trends Ecol Evol* 24:127–135. doi: 10.1016/j.tree.2008.10.008
- Budaev S V. (2010) Using principal components and factor analysis in animal behaviour research: Caveats and guidelines. *Ethology* 116:472–480. doi: 10.1111/j.1439-0310.2010.01758.x
- Chivers DP, Zhao X, Ferrari MCO (2007) Linking morphological and behavioural defences: Prey fish detect the morphology of conspecifics in the odour signature of their predators. *Ethology* 113:733–739. doi: 10.1111/j.1439-0310.2006.01385.x
- Coltman DW, O'Donoghue P, Jorgenson JT, et al (2003) Undesirable evolutionary consequences of trophy hunting. *Nature* 426:655–658. doi: 10.1038/nature02187.1.
- Conover DO, Munch SB, Arnott SA (2009) Reversal of evolutionary downsizing caused by selective harvest of large fish. *Proc R Soc B* 276:2015–2020. doi: 10.1098/rspb.2009.0003
- Conrad JL, Weinersmith KL, Brodin T, et al (2011) Behavioural syndromes in fishes: A review with implications for ecology and fisheries management. *J Fish Biol* 78:395–435. doi: 10.1111/j.1095-8649.2010.02874.x
- Cook K V., O'Connor CM, Gilmour KM, Cooke SJ (2011) The glucocorticoid stress response is repeatable between years in a wild teleost fish. *J Comp Physiol A Neuroethol Sensory,*

- Neural, Behav Physiol 197:11189–1196. doi: 10.1007/s00359-011-0680-3
- Cooke SJ, Cowx IG (2004) The role of recreational fishing in global fish crises. *Bioscience* 54:857–859
- Cooke SJ, Steinmetz J, Degner JF, et al (2003) Metabolic fright responses of different-sized largemouth bass (*Micropterus salmoides*) to two avian predators show variations in nonlethal energetic costs. *Can J Zool* 81:699–709. doi: 10.1139/z03-044
- Cooke SJ, Suski CD, Ostrand KG, et al (2007) Physiological and behavioral consequences of long-term artificial selection for vulnerability to recreational angling in a teleost fish. *Physiol Biochem Zool* 80:480–490. doi: 10.1086/520618
- Crawley MJ (2013) *The R Book*, 1st edn. John Wiley & Sons, Ltd., West Sussex, United Kingdom
- Demers E, York B, McKinley RS, et al (1996) Activity patterns of largemouth and smallmouth bass determined with electromyogram biotelemetry. *Trans Am Fish Soc* 125:434–439
- Dingemanse NJ, Barber I, Wright J, Brommer JE (2012) Quantitative genetics of behavioural reaction norms: Genetic correlations between personality and behavioural plasticity vary across stickleback populations. *J Evol Biol* 25:485–496. doi: 10.1111/j.1420-9101.2011.02439.x
- Dingemanse NJ, Wolf M (2010) Recent models for adaptive personality differences : a review. *Philos Trans R Soc B Biol Sci* 365:3947–3958. doi: 10.1098/rstb.2010.0221
- Dotson JR, Allen MS, Kerns JA, Poudel WF (2013) Utility of restrictive harvest regulations for trophy largemouth bass management. *North Am J Fish Manag* 33:499–507. doi:

10.1080/02755947.2013.769921

Dragulescu AA, Arendt C (2018) *xlsx: Read, Write, Format Excel 2007 and Excel*

97/2000/XP/2003 Files

Dunmall KM, Cooke SJ, Schreer JF, McKinley RS (2001) The effect of scented lures on the hooking injury and mortality of smallmouth bass caught by novice and experienced anglers.

North Am J Fish Manag 21:242–248

Engqvist L (2005) The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Anim Behav* 70:967–971. doi:

10.1016/j.anbehav.2005.01.016

Erisman BE, Allen LG, Claisse JT, et al (2011) The illusion of plenty : hyperstability masks collapses in two recreational fisheries that target fish spawning aggregations. *Can J Fish Aquat Sci* 68:1705–1716. doi: 10.1139/F2011-090

Fedele AD (2017) Influences of catch-and-release angling on fish avoidance behavior.

University of Nebraska

Fernö A, Huse I (1983) The effect of experience on the behaviour of cod (*Gadus morhua* L.)

Towards a baited hook. *Fish Res* 2:19–28. doi: 10.1016/0165-7836(83)90100-5

Festa-Bianchet M (2016) When does selective hunting select, how can we tell, and what should we do about it? *Mamm Rev* 47:76–81. doi: 10.1111/mam.12078

Folt CL, Chen CY, Moore M V, Burnaford J (1999) Synergism and antagonism among multiple stressors. *Limnol Oceanogr* 44:864–877

Fox J, Weisberg S (2011) *An (R) companion to applied regression*, Second edition

- Fraser NHC, Metcalfe NB, Thorpe JE (1993) Temperature-dependent switch between diurnal and nocturnal foraging in salmon. *Proc R Soc London B* 252:135–139. doi: 10.1098/rspb.1993.0057
- Fu S-J, Zeng L-Q, Li X-M, et al (2009) The behavioural, digestive and metabolic characteristics of fishes with different foraging strategies. *J Exp Biol* 212:2296–2303. doi: 10.1242/jeb.027102
- Gallagher AJ, Creel S, Wilson RP, Cooke SJ (2017) Energy landscapes and the landscape of fear. *Trends Ecol Evol* 32:88–96. doi: 10.1016/j.tree.2016.10.010
- Gamer M, Lemon J, Singh IFP (2012) {irr: Various coefficients of interrater reliability and agreement}
- Gingerich AJ, Philipp DP, Suski CD (2010) Effects of nutritional status on metabolic rate, exercise and recovery in a freshwater fish. *J Comp Physiol B* 180:371–384. doi: 10.1007/s00360-009-0419-4
- Godin J-GJ, Crossman SL (1994) Hunger-dependent predator inspection and foraging behaviours in the threespine stickleback (*Gasterosteus aculeatus*) under predation risk. *Behav Ecol Sociobiol* 34:359–366. doi: 10.1007/BF00197006
- Gutowsky LFG, Sullivan BG, Wilson ADM, Cooke SJ (2017) Synergistic and interactive effects of angler behaviour, gear type, and fish behaviour on hooking depth in passively angled fish. *Fish Res* 186:612–618. doi: 10.1016/j.fishres.2016.05.026
- Hair JF (1998) *Multivariate Data Analysis with Readings*, 5th edn. Prentice Hall, Upper Saddle River, NJ

- Hanson KC, Cooke SJ (2009) Nutritional condition and physiology of paternal care in two congeneric species of black bass (*Micropterus* spp.) relative to stage of offspring development. *J Comp Physiol B* 179:253–266. doi: 10.1007/s00360-008-0309-1
- Härkönen L, Hyvärinen P, Niemelä PT, Vainikka A (2016) Behavioural variation in Eurasian perch populations with respect to relative catchability. *Acta Ethol* 19:21–31. doi: 10.1007/s10211-015-0219-7
- Härkönen L, Hyvärinen P, Paappanen J, et al (2014) Explorative behavior increases vulnerability to angling in hatchery-reared brown trout (*Salmo trutta*). *Can J Fish Aquat Sci* 71:1900–1909. doi: 10.1139/cjfas-2014-0221
- Harrell Jr. FE (2019) Hmisc: Harrell miscellaneous
- Hart APJB, Webster MM, Ward AJW (2009) Individual boldness affects interspecific interactions in sticklebacks. *Behav Ecol Sociobiol* 63:511–520
- Heermann L, Emmrich M, Heynen M, et al (2013) Explaining recreational angling catch rates of Eurasian perch, *Perca fluviatilis*: The role of natural and fishing-related environmental factors. *Fish Manag Ecol* 20:187–200. doi: 10.1111/fme.12000
- Heino M, Baulier L, Boukal DS, et al (2013) Can fisheries-induced evolution shift reference points for fisheries management? *ICES J Mar Sci* 70:707–721. doi: 10.1093/icesjms/fst077
- Heino M, Pauli BD, Dieckmann U (2015) Fisheries-induced evolution. *Annu Rev Ecol Evol Syst* 46:461–480
- Henson JC (1991) Quantitative description and development of a species-specific growth form for largemouth bass, with application to the relative weight index. Texas A&M University,

College Station

- Hessenauer JM, Vokoun J, Davis J, et al (2016) Loss of naivety to angling at different rates in fished and unfished populations of largemouth bass. *Trans Am Fish Soc* 145:1068–1076. doi: 10.1080/00028487.2016.1194894
- Hessenauer JM, Vokoun JC, Suski CD, et al (2015) Differences in the metabolic rates of exploited and unexploited fish populations: A signature of recreational fisheries induced evolution? *PLoS One* 10:. doi: 10.1371/journal.pone.0128336
- Ho R (2006) *Handbook of univariate and multivariate data analysis and interpretation with SPSS*. Chapman and Hall/ CRC
- Hollins J, Thambithurai D, Köeck B, et al (2018) A physiological perspective on fisheries-induced evolution. *Evol Appl* 1–16. doi: 10.1111/eva.12597
- Huntingford FA, Wright PJ (1993) The development of adaptive variation in predator avoidance in freshwater fishes. *Mar Behav Physiol* 23:45–61. doi: 10.1080/10236249309378856
- Jackson DA, Peres-Neto PR, Olden JD (2001) What controls who is where in freshwater fish communities – the roles of biotic, abiotic, and spatial factors. *Can J Fish Aquat Sci* 58:157–170. doi: 10.1139/cjfas-58-1-157
- Jenjan H, Mesquita F, Huntingford F, Adams C (2013) Respiratory function of common carp with different stress coping styles: a hidden cost of personality traits? *Anim Behav* 85:1245–1249
- Jobling M (2011) Bionenergetics in aquaculture settings. *Encycl. Fish Physiol.* 1664–1674
- Killen SS (2011) Energetics of foraging decisions and prey handling. *Encycl. Fish Physiol.*

- Killen SS, Adriaenssens B, Marras S, et al (2016) Context dependency of trait repeatability and its relevance for management and conservation of fish populations. *Conserv Physiol* 4:1–19. doi: 10.1093/conphys/cow007
- Killen SS, Marras S, McKenzie DJ (2011) Fuel, fasting, fear: Routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass. *J Anim Ecol* 80:1024–1033. doi: 10.1111/j.1365-2656.2011.01844.x
- Killen SS, Marras S, Metcalfe NB, et al (2013) Environmental stressors alter relationships between physiology and behaviour. *Trends Ecol Evol* 28:651–658. doi: 10.1016/j.tree.2013.05.005
- Killen SS, Marras S, Ryan MR, et al (2012) A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Funct Ecol* 26:134–143. doi: 10.1111/j.1365-2435.2011.01920.x
- Klefoth T, Skov C, Kuparinen A, Arlinghaus R (2017) Toward a mechanistic understanding of vulnerability to hook-and-line fishing: Boldness as the basic target of angling-induced selection. *Evol Appl* 1–13. doi: 10.1111/eva.12504
- Koolhaas JM (2008) Coping style and immunity in animals: Making sense of individual variation. *Brain Behav Immun* 22:662–667. doi: 10.1016/j.bbi.2007.11.006
- Koolhaas JM, de Boer SF, Coppens CM, Buwalda B (2010) Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Front Neuroendocrinol* 31:307–321. doi: 10.1016/j.yfrne.2010.04.001



- Kortet R, Vainikka A, Janhunen M, et al (2014) Behavioral variation shows heritability in juvenile brown trout *Salmo trutta*. *Behav Ecol Sociobiol* 68:927–934. doi: 10.1007/s00265-014-1705-z
- Krause J (1994) The influence of food competition and predation risk on size-assortative shoaling in juvenile chub ( *Leuciscus cephalus* ). *Ethology* 96:105–116
- Krause J, Loader SP, McDermott J, Ruxton GD (1998) Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proc R Soc B Biol Sci* 265:2373–2379. doi: 10.1098/rspb.1998.0586
- Kuparinen A, Merilä J (2007) Detecting and managing fisheries-induced evolution. *Trends Ecol Evol* 22:652–659. doi: 10.1016/j.tree.2007.08.011
- Kuznetsova A, Brockhoff PB, Christensen RHB (2017) {lmerTest} package: Tests in linear mixed effects models. *J Stat Softw* 82:1–26
- Laird NM, Ware JH (1982) Random-effects models for longitudinal data. *Biometrics* 38:963–974
- Lander JP (2018) coefplot: Plots coefficients from fitted models
- Laugen AT, Engelhard GH, Whitlock R, et al (2014) Evolutionary impact assessment: Accounting for evolutionary consequences of fishing in an ecosystem approach to fisheries management. *Fish Fish* 15:65–96. doi: 10.1111/faf.12007
- Lawrence MJ, Jain-Schlaepfer S, Zolderdo AJ, et al (2018) Are 3 minutes good enough for obtaining baseline physiological samples from teleost fish? *Can J Zool* 96:774–786
- Lennox RJ, Alos J, Arlinghaus R, et al (2017) What makes fish vulnerable to capture by hooks?

A conceptual framework and a review of key determinants. *Fish Fish* 1–25

Lennox RJ, Diserud OH, Cooke SJ, et al (2016) Influence of gear switching on recapture of Atlantic salmon (*Salmo salar*) in catch-and-release fisheries. *Ecol Freshw Fish* 25:422–428. doi: 10.1111/eff.12223

Lenth R V. (2016) Least-squares means: The R package lsmeans. *J Stat Softw* 69:1–33

Lima SL (1998) Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. *Adv Study Behav* 27:215–290. doi: 10.1016/S0065-3454(08)60366-6

Lindstrom MJ, Bates DM (1990) Nonlinear mixed effects models for repeated measures data. *Biometrics* 46:673–687

Louison MJ, Adhikari S, Stein JA, Suski CD (2017) Hormonal responsiveness to stress is negatively associated with vulnerability to angling capture in fish. *J Exp Biol* 200:2529–2535. doi: 10.1242/jeb.150730

Louison MJ, Hage VM, Stein JA, Suski CD (2019) Quick learning , quick capture : largemouth bass that rapidly learn an association task are more likely to be captured by recreational anglers. *Behav Ecol Sociobiol* 73:23

Martínez M, Guderley H, Nelson JA, et al (2002) Once a fast cod, always a fast cod: Maintenance of performance hierarchies despite changing food availability in cod (*Gadus morhua*). *Physiol Biochem Zool* 75:90–100

Matthias BG, Allen MS, Ahrens RNM, et al (2014) Hide and seek: Interplay of fish and anglers influences spatial fisheries management. *Fisheries* 39:261–269. doi:

10.1080/03632415.2014.903836

- Mazué GPF, Dechaume-Moncharmont FX, Godin JGJ (2015) Boldness-exploration behavioral syndrome: Interfamily variability and repeatability of personality traits in the young of the convict cichlid (*Amatitlania siquia*). *Behav Ecol* 26:900–908. doi: 10.1093/beheco/arv030
- McCue MD (2010) Starvation physiology: Reviewing the different strategies animals use to survive a common challenge. *Comp Biochem Physiol Part A* 156:1–18. doi: 10.1016/j.cbpa.2010.01.002
- Menard S (2002) Applied logistic regression analysis, 2nd edn. Sage Publications, Inc., Thousand Oaks
- Mendez G, Wieser W (1993) Metabolic responses to food deprivation and refeeding in juveniles of *Rutilus rutilus* (Teleostei : Cyprinidae). *Environ Biol Fishes* 36:73–81
- Midway SR, Hasler CT, Wagner T, Suski CD (2017) Predation of freshwater fish in environments with elevated carbon dioxide. *Mar Freshw Res A-H*. doi: 10.1071/MF16156
- Mittelbach GG, Ballew NG, Kjelson MK (2014) Fish behavioral types and their ecological consequences. *Can J Fish Aquat Sci* 71:927–944. doi: 10.1139/cjfas-2013-0558
- Mogensen S, Post JR, Sullivan MG (2014) Vulnerability to harvest by anglers differs across climate, productivity, and diversity clines. *Can J Fish Aquat Sci* 71:416–426. doi: 10.1139/cjfas-2013-0336
- Monk CT, Arlinghaus R (2018) Eurasian perch, *Perca fluviatilis*, spatial behaviour determines vulnerability independent of angler skill in a whole-lake reality mining experiment. *Can J Fish Aquat Sci* 75:417–428. doi: 10.1139/cjfas-2017-0029

- Monk CT, Arlinghaus R (2017) Encountering a bait is necessary but insufficient to explain individual variability in vulnerability to angling in two freshwater benthivorous fish in the wild. *PLoS One* 12:1–25. doi: 10.1371/journal.pone.0173989
- Murphy BR, Willis DR, Springer TA (1991) The relative weight index in fisheries management: Status and needs. *Fisheries* 16:30–38
- Nannini MA, Wahl DH, Philipp DP, Cooke SJ (2011) The influence of selection for vulnerability to angling on foraging ecology in largemouth bass *Micropterus salmoides*. *J Fish Biol* 79:1017–1028. doi: 10.1111/j.1095-8649.2011.03079.x
- Naslund J, Johnsson JI (2016) State-dependent behavior and alternative behavioral strategies in brown trout (*Salmo trutta* L.) fry. *Behav Ecol Sociobiol* 70:2111–2125. doi: 10.1007/s00265-016-2215-y
- Navarro I, Gutierrez J (1995) Fasting and starvation. Elsevier Science B.V., Barcelona
- Oksanen J, Blanchet FG, Friendly M, et al (2018) vegan: Community ecology package
- Olsen EM, Heino M, Lilly GR, et al (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* 428:932–935. doi: 10.1038/nature02453.1.
- Overli O, Sorensen C, Pulman KGT, et al (2007) Evolutionary background for stress-coping styles: Relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neurosci Biobehav Rev* 31:396–412
- Pauli BD, Sih A (2017) Behavioural responses to human-induced change: Why fishing should not be ignored. *Evol Appl* 10:231–240. doi: 10.1111/eva.12456
- Pauli BD, Wiech M, Heino M, Utne-Palm AC (2015) Opposite selection on behavioural types by

- active and passive fishing gears in a simulated guppy *Poecilia reticulata* fishery. *J Fish Biol* 86:1030–1045. doi: 10.1111/jfb.12620
- Pettersson LB, Brönmark C (1993) Trading off safety against food: State dependent habitat choice and foraging in crucian carp. *Oecologia* 95:353–357
- Philipp DP, Cooke SJ, Claussen JE, et al (2009) Selection for vulnerability to angling in largemouth bass. *Trans Am Fish Soc* 138:189–199. doi: 10.1577/T06-243.1
- Pinheiro J, Bates D, DebRoy S, Sarkar D (2017) {nlme}: Linear and nonlinear mixed effects models
- Post JR, Sullivan M, Cox S, et al (2002) Canada’s recreational fisheries: The invisible collapse? *Fisheries* 27:6–17. doi: 10.1577/1548-8446(2002)027<0006:CRF>2.0.CO;2
- Reale D, Garant D, Humphries MM, et al (2010) Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos Trans R Soc B Biol Sci* 365:4051–4063. doi: 10.1098/rstb.2010.0208
- Réale D, Reader SM, Sol D, et al (2007) Integrating animal temperament within ecology and evolution. *Biol Rev* 82:291–318. doi: 10.1111/j.1469-185X.2007.00010.x
- Redpath TD, Cooke SJ, Suski CD, et al (2010) The metabolic and biochemical basis of vulnerability to recreational angling after three generations of angling-induced selection in a teleost fish. *Can J Fish Aquat Sci* 67:1983–1992. doi: 10.1139/F10-120
- Revelle W (2018) psych: Procedures for personality and psychological research
- Reznick DN, Ghalambor CK (2005) Can commercial fishing cause evolution? Answers from guppies (*Poecilia reticulata*). *Can J Fish Aquat Sci* 62:791–801. doi: 10.1139/f05-079

- Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations : What empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112–113:183–198. doi: 10.1023/A
- Ricklefs RE, Wikelski M (2002) The physiology / life- history nexus. *Trends Ecol Evol* 17:462–468
- Ringler NH (1983) Variation in foraging tactics of fishes. In: David L.G. Noakes et al. (ed) *Predators and prey in fishes*, 2nd edn. Dr W. Junk Publishers, pp 159–171
- Rogers KB, Bergersen EP (1999) Utility of synthetic structures for concentrating adult northern pike and largemouth bass. *North Am J Fish Manag* 19:1054–1065
- Romero LM, Reed JM (2005) Collecting baseline corticosterone samples in the field: Is under 3 min good enough? *Comp Biochem Physiol - A Mol Integr Physiol* 140:73–79. doi: 10.1016/j.cbpb.2004.11.004
- Roni P (2019) Does river restoration increase fish abundance and survival or concentrate fish ? The effects of project scale, location, and fish life history. *Fisheries* 44:7–19. doi: 10.1002/fsh.10180
- Ruiz-Gomez MDL, Kittilsen S, Höglund E, et al (2008) Behavioral plasticity in rainbow trout (*Oncorhynchus mykiss*) with divergent coping styles : When doves become hawks. *Horm Behav* 54:534–538. doi: 10.1016/j.yhbeh.2008.05.005
- Sass GG, Gaeta JW, Allen MS, et al (2018) Effects of catch-and-release angling on a largemouth bass (*Micropterus salmoides*) population in a north temperate lake, 2001–2005. *Fish Res* 204:95–102. doi: 10.1016/j.fishres.2018.02.012

- Shrout PE, Fleiss JL (1979) Intraclass correlations : Uses in assessing rater reliability. Psychol Bull 86:420–428
- Sih A (2013) Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Anim Behav 85:1077–1088. doi: 10.1016/j.anbehav.2013.02.017
- Sih A, Bell AM (2008) Insights for behavioral ecology from behavioral syndromes. In: Advances in the Study of Behavior. pp 227–281
- Silva PIM, Martins CIM, Engrola S, et al (2010) Individual differences in cortisol levels and behaviour of Senegalese sole (*Solea senegalensis*) juveniles: Evidence for coping styles. Appl Anim Behav Sci 124:75–81. doi: 10.1016/j.applanim.2010.01.008
- Sink TD, Lochmann RT, Fecteau KA (2008) Validation, use, and disadvantages of enzyme-linked immunosorbent assay kits for detection of cortisol in channel catfish, largemouth bass, red pacu, and golden shiners. Fish Physiol Biochem 34:95–101. doi: 10.1007/s10695-007-9150-9
- Sloman KA (2011) Behavioral responses to the environment | Anthropogenic influences on fish behavior. Encycl. Fish Physiol. 783–789
- Smokorowski KE, Pratt TC (2007) Effect of a change in physical structure and cover on fish and fish habitat in freshwater ecosystems – a review and meta-analysis. Environ Rev 15:15–41. doi: 10.1139/A06-007
- Stein JA, Shultz AD, Cooke SJ, et al (2012) The influence of hook size, type, and location on hook retention and survival of angled bonefish (*Albula vulpes* ). Fish Res 113:147–152. doi:

10.1016/j.fishres.2011.11.001

Stoner AW (2004) Effects of environmental variables on fish feeding ecology : Implications for the performance of baited fishing gear and stock assessment. *J Fish Biol* 65:1445–1471. doi: 10.1111/j.1095-8649.2004.00593.x

Suski CD, Philipp DP (2004) Factors affecting the vulnerability to angling of nesting male largemouth and smallmouth bass. *Trans Am Fish Soc* 133:1100–1106. doi: 10.1577/T03-079.1

Sutter DAH, Suski CD, Philipp DP, et al (2012) Recreational fishing selectively captures individuals with the highest fitness potential. *Proc Natl Acad Sci* 109:20960–20965. doi: 10.1073/pnas.1212536109

Symonds MRE, Moussalli A (2011) A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike’s information criterion. *Behav Ecol Sociobiol* 65:13–21. doi: 10.1007/s00265-010-1037-6

Taylor MK, Cook K V., Hasler CT, et al (2012) Behaviour and physiology of mountain whitefish (*Prosopium williamsoni*) relative to short-term changes in river flow. *Ecol Freshw Fish* 21:609–616. doi: 10.1111/j.1600-0633.2012.00582.x

Thomson JS, Watts PC, Pottinger TG, Sneddon LU (2012) Plasticity of boldness in rainbow trout, *Oncorhynchus mykiss*: Do hunger and predation influence risk-taking behaviour? *Horm Behav* 61:750–757. doi: 10.1016/j.yhbeh.2012.03.014

Tix JA, Hasler CT, Sullivan C, et al (2017) Elevated carbon dioxide has limited acute effects on *Lepomis macrochirus* behaviour. *J Fish Biol* 90:751–772. doi: 10.1111/jfb.13188



- Toms CN, Echevarria DJ, Jouandot DJ (2010) A methodological review of personality-related studies in fish : Focus on the shy-bold axis of behavior. *Int J Comp Psychol* 23:1–25
- Tucker EK, Hasler CT, Suski CD, et al (2018) Glucocorticoid and behavioral variation in relation to carbon dioxide avoidance across two experiments in freshwater teleost fishes. *Biol Invasions* 9:. doi: 10.1007/s10530-018-1842-9
- Uusi-Heikkilä S, Whiteley AR, Kuparinen A, et al (2015) The evolutionary legacy of size-selective harvesting extends from genes to populations. *Evol Appl* 8:597–620. doi: 10.1111/eva.12268
- Vainikka A, Tammela I, Hyvärinen P (2016) Does boldness explain vulnerability to angling in Eurasian perch *Perca fluviatilis*? *Curr Zool* 62:109–115. doi: 10.1093/cz/zow003
- VanDeValk AJ, Forney JL, Jackson JR, et al (2005) Angler catch rates and catchability of walleyes in Oneida Lake, New York. *North Am J Fish Manag* 25:1441–1447. doi: 10.1577/M04-171.1
- Venables WN, Ripley BD (2002) *Modern applied statistics with S*, fourth. Springer, New York
- Volkoff H (2011) Hormonal control of reproduction and growth-control of appetite in fish. *Encycl. Fish Physiol.* 1509–1514
- Wang GD, Zhai W, Yang HC, et al (2013) The genomics of selection in dogs and the parallel evolution between dogs and humans. *Nat Commun* 4:1860–1869. doi: 10.1038/ncomms2814
- Wang T, Hung CCY, Randall DJ (2006) The comparative physiology of food deprivation: From feast to famine. *Annu Rev Physiol* 68:223–251. doi:

10.1146/annurev.physiol.68.040104.105739

Wegener MG, Schramm HL, Neal JW, Gerard PD (2018) Effect of fishing effort on catch rate and catchability of largemouth bass in small impoundments. *Fish Manag Ecol* 25:66–76.

doi: 10.1111/fme.12268

Weimer EJ, Brown ML, Blackwell BG (2014) Quantifying differences in habitat use between anglers and large bluegills. *Prairie Nat* 46:4–10

Wendelaar Bonga SE (1997) The stress response in fish. *Physiol Rev* 77:591–625

Werner EE, Gilliam JF, Hall DJ, Mittelbach GG (1983) An experimental test of the effects of predation risk on habitat use in fish. *Ecology* 64:1540–1548

Wickham H (2009) *ggplot2: Elegant graphics for data analysis*

Wickham H (2017) *tidyverse: Easily install and load the “Tidyverse”*

Wickham H, Hester J, Francois R (2018) *readr: Read rectangular text data*

Wilson ADM, Binder TR, McGrath KP, et al (2011a) Capture technique and fish personality: angling targets timid bluegill sunfish, *Lepomis macrochirus*. *Can J Fish Aquat Sci* 68:749–757. doi: 10.1139/f2011-019

Wilson ADM, Binder TR, McGrath KP, et al (2011b) Capture technique and fish personality: angling targets timid bluegill sunfish, *Lepomis macrochirus*. *Can J Fish Aquat Sci* 68:749–757. doi: 10.1139/f2011-019

Wilson ADM, Brownscombe JW, Sullivan B, et al (2015) Does angling technique selectively target fishes based on their behavioural type? *PLoS One* 10:1–14. doi: 10.1371/journal.pone.0135848

- Wilson DS, Coleman K, Clark AB, Biederman L (1993) Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): An ecological study of a psychological trait. *J Comp Psychol* 107:250–260. doi: 10.1037//0735-7036.107.3.250
- Young RG, Hayes JW (2004) Angling pressure and trout catchability: Behavioral observations of brown trout in two New Zealand backcountry rivers. *North Am J Fish Manag* 24:1203–1213. doi: 10.1577/M03-177.1
- Zhang Z (2016) Residuals and regression diagnostics: focusing on logistic regression. *Ann Transl Med* 4:195. doi: 10.21037/atm.2016.03.36
- Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. *Methods Ecol Evol* 1:3–14. doi: 10.1111/j.2041-210X.2009.00001.x

## APPENDIX A: SUPPLEMENTARY TABLES AND FIGURES

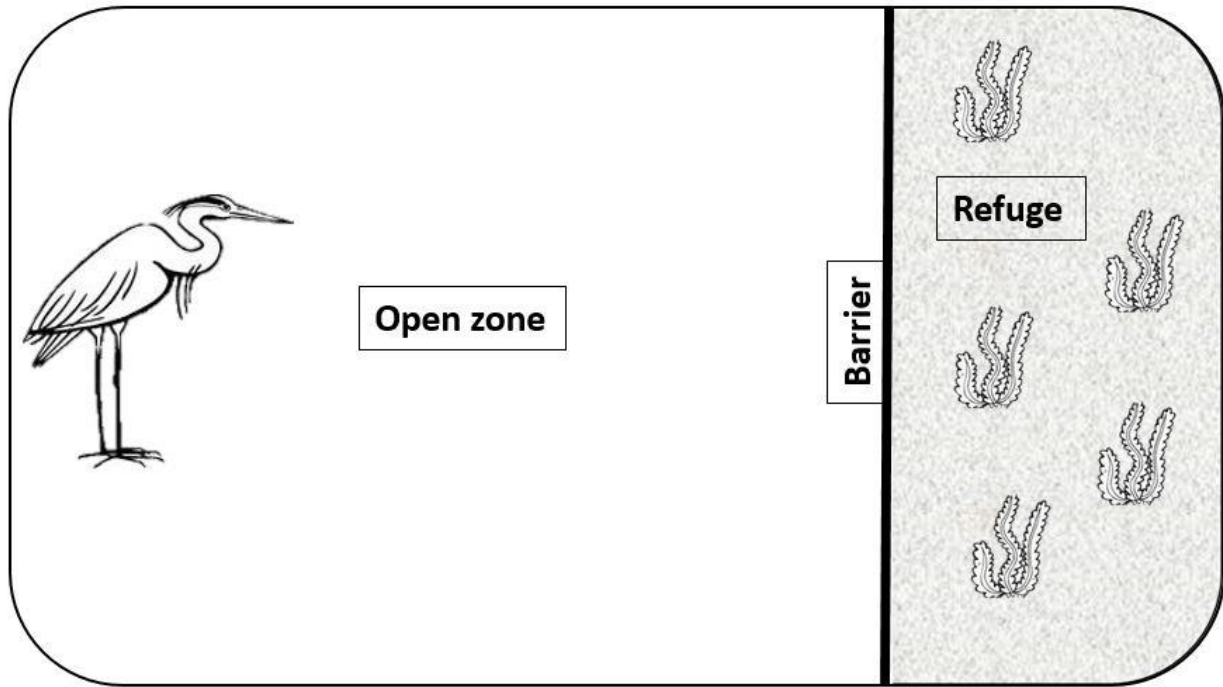


Figure A.1. Overhead view of the chapter 2 behavioral arena, with the refuge on the right, separated from the open zone on the left, with a removable barrier. The heron model simulation was conducted at the far left of the open zone, opposite the refuge.

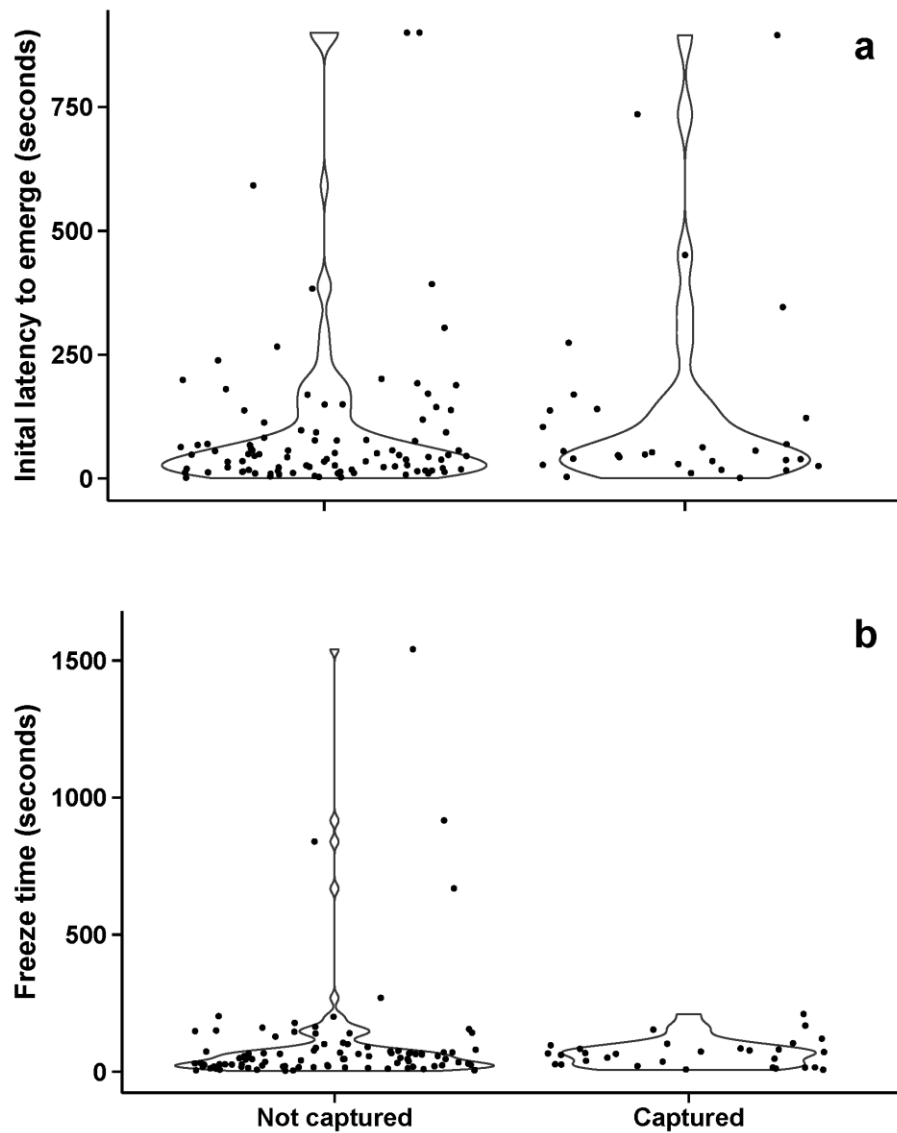


Figure A.2. Violin plots with jitter plots overlaid comparing behavioral scores between chapter 2 captured and uncaptured largemouth bass. The points represent individual times to perform the initial latency to emerge behaviors (a) and freeze behaviors (b). The overlaid shapes show the frequency of individuals to perform these behaviors at their associated times, with thin lines representing few individuals with associated scores and wide shapes representing many fish with the indicated behavioral scores.

Table A.1. Summary of generalized linear model to explain changes in largemouth bass catch rates across consecutive angling sessions across all experimental ponds in chapter 3. Catch rates in angling sessions two through five were compared to angling session one. Angling sessions that had significantly different catch rates compared to angling session one ( $p < 0.05$ ) are in bold text.

Variable	Parameter estimate	Standard error	z-value	p
Angling session 2	-0.360	0.238	-1.513	0.130
<b>Angling session 3</b>	-1.459	0.351	-4.155	<b>&lt;0.001</b>
<b>Angling session 4</b>	-1.682	0.385	-4.368	<b>&lt;0.001</b>
<b>Angling session 5</b>	-0.871	0.281	-3.102	<b>0.002</b>

Table A.2. Summary of generalized linear mixed effects model to explain number of times individual fish were captured in chapter 3, with PC score, and pond zone as fixed effects and fish ID, angler, and pond as random effects.  $R^2_m$  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.

Variable	Parameter estimate	Standard error	Z-value	<i>p</i>	$r^2_m$	$r^2_c$
Intercept	0.793	0.180	4.398	<0.001	0.958	0.958
PC score	0.009	0.062	0.142	0.887		
<b>Structure zone</b>	-0.615	0.245	-2.512	<b>0.012</b>		
<b>No structure zone</b>	-0.623	0.242	-2.575	<b>0.010</b>		
Uncaptured	-0.343	<0.001	0.000	1.000		
Random effects	Variance	Standard deviation				
Fish ID	<0.001	<0.001				
Angler	<0.001	<0.001				
Pond	<0.001	<0.001				

Table A.3. Summary statistics of scores from behavior assays, combined for pre- and post-feeding treatment behaviors of largemouth bass in chapter 4. Time spent swimming and distance moved were measured separately both pre- and post- lure introduction.

Behavior	Mean $\pm$ SD	Median	Range
Time spent swimming pre-lure (s)	243 $\pm$ 120	244	0-505
Time spent swimming post-lure (s)	344 $\pm$ 204	326	0-841
Distance moved pre-lure (cm)	4,682 $\pm$ 5,784	3,225	0-35,203
Distance moved post-lure (cm)	7,592 $\pm$ 13,190	3,504	0-83,971
Number of lure visits	1.05 $\pm$ 1.70	0	0-7
Time spent in proximity of lure (s)	5.29 $\pm$ 29.9	0	0-236