

RESEARCH ARTICLE

Hormonal responsiveness to stress is negatively associated with vulnerability to angling capture in fish

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ABSTRACT

Differences in behavior and physiology amongst individuals often alter relative fitness levels in the environment. However, the ideal behavioral/physiological phenotype in a given environment may be altered by human activity, leading to an evolutionary response in the affected population. One example of this process can be found in fisheries (including recreational freshwater fisheries), where selective capture and harvest of individuals with certain phenotypes can drive evolutionary change. While some life history traits and behavioral tendencies influencing capture likelihood have been studied, the physiological mechanisms driving this vulnerability remain poorly understood. To address this, we assessed how two major physiological characteristics (hormonal responsiveness to stress and metabolic phenotype) and one behavioral characteristic (boldness) impact the likelihood of an individual being captured by anglers. Largemouth bass, *Micropterus salmoides*, derived from a population artificially selected for differential angling vulnerability were assessed for boldness and for stress responsiveness (as indicated by plasma cortisol levels) following an air-exposure challenge. Largemouth bass were then stocked into a pond where experimental angling trials took place, and a subset of captured and uncaptured fish were afterwards assessed for metabolic phenotype. The results showed that stress responsiveness was the primary driver of angling vulnerability, with individuals that experienced lower rises in cortisol following the air-exposure challenge more likely to be captured. Neither boldness nor metabolic phenotype influenced capture probability. The results from this study indicate that fisheries-induced selective pressure may act on physiology, potentially altering stress responsiveness and its associated behaviors in populations exploited by recreational anglers.

KEY WORDS: Fisheries-induced evolution, Largemouth bass, Stress-coping style, Behavioral syndromes, Metabolic rate

INTRODUCTION

A large body of research has documented the alteration of exploited fish populations via selective capture of particular phenotypes, a phenomenon known as fisheries-induced evolution (FIE) (Devine et al., 2012; Jorgensen et al., 2007). The majority of these studies have focused on the alteration of life-history traits (Kuparinen and Hutchings, 2012), including decreases in growth rate, lower total reproductive output and reduced age at maturity in populations of

marine species that have been subjected to commercial harvest (Devine et al., 2012; Kuparinen and Merilä, 2007). Similar processes may also be occurring in freshwater fisheries primarily targeted by recreational hook-and-line anglers (Kendall and Quinn, 2011; Nussle et al., 2009), which may reduce the overall fitness of individuals in the population (Sutter et al., 2012). Whether in freshwater or marine ecosystems, FIE has the potential to greatly alter the ecology of the affected populations.

While alterations in growth rate, reproductive rate and the timing of maturation have been identified as outcomes of FIE in exploited populations, it has been posited that inter-individual differences in behavior are the proximate mechanisms responsible for FIE, specifically those behaviors that predispose individuals to capture by commercial or recreational gear (Biro and Post, 2008; Uusi-Heikkilä et al., 2008). Consistent behavioral differences among individuals, which are alternatively referred to as ‘behavioral syndromes’, ‘personalities’ or ‘stress-coping styles’, have been thoroughly studied in a host of animal taxa (Sih et al., 2004). These differences among individuals are typically parsed into behavioral axes, including boldness, aggression and activity levels (Réale et al., 2007), and the physiological mechanisms that underpin many of these behavioral differences have also been defined. For instance, studies of stress-coping styles have examined the relationship between levels of boldness and neuroendocrine responsiveness to stress, with ‘proactive’ individuals being marked by bolder behavior and a less pronounced hypothalamic-pituitary–adrenal (hypothalamic-pituitary–interrenal in teleost fish and amphibians) axis response to stress, as measured by cortisol concentrations in the blood (Koolhaas et al., 1999; Overli et al., 2005). Levels of boldness and/or aggression have also been linked with metabolic rate and aerobic capacity (Careau et al., 2008; Metcalfe et al., 1995), under the presumption that individuals with intrinsically high metabolic demands will need to behave more boldly and/or more aggressively to acquire and defend sufficient resources to satisfy higher energetic requirements (Biro and Stamps, 2010; Stamps, 2007).

For FIE to influence the physiology or behavior of recreationally targeted species, the traits in question must be linked to a propensity to strike a fishing lure. At present, links between physiological traits and angling vulnerability within individuals have been relatively unexplored, although some studies have made comparisons among lines artificially selected for differing angling vulnerability (Cooke et al., 2007; Redpath et al., 2010). Direct causal links between behavioral type and angling have, however, been studied more frequently. For example, previous work has shown that bolder and more active individuals may be more likely to be caught on hook-and-line gear (Härkönen et al., 2014; Klefoth et al., 2013), while individuals with higher growth rates have been found to be more vulnerable to commercial netting (Biro and Post, 2008). It is important to note that, while previous work has independently examined the correlation of boldness (Wilson et al., 2011) as well as metabolic rate (Redpath et al., 2010) with angling vulnerability, no

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studies have taken an integrative approach to simultaneously examine the relative influence of behavior, metabolic rate and hormones in driving the likelihood of capture. Defining these relationships is of critical importance in determining which characteristics may be under selective pressure, and what types of evolutionary alterations we can expect to see in populations exploited by recreational anglers.

The goal of the present study was to define the physiological and behavioral mechanisms responsible for driving the vulnerability of fish to recreational angling capture. To accomplish this goal, we utilized a population of largemouth bass, *Micropterus salmoides* Lacépède, artificially selected to vary in their vulnerability to angling (Philipp et al., 2009). Several studies have examined these selected lines for differences in their metabolic characteristics/growth (Cooke et al., 2007; Redpath et al., 2009, 2010), reproductive output (Sutter et al., 2012), foraging ecology (Nannini et al., 2011) and daily activity (Binder et al., 2012). However, none of these studies have attempted to link characteristics to angling vulnerability on the individual level, instead focusing on between-line comparisons. These approaches, while highly useful, ignore the fact that angling vulnerability and its associated characteristics may still vary within each line. In this study, we therefore looked to quantify factors driving vulnerability to angling in individuals, rather than comparing lines to draw inferences. In addition, hormonal/physiological characteristics may possibly play a major role in determining whether an individual is vulnerable to capture (Biro and Sampson, 2015; Cooke et al., 2009), but have been understudied with respect to angling vulnerability in fish. We predicted that individuals with lower neuroendocrine responsiveness to stress (as indicated by plasma cortisol levels), greater levels of boldness and higher metabolic rates (i.e. proactive copers) would be more likely to be captured by hook-and-line angling. This hypothesis was formulated because of the greater rates of exploration, aggression and feeding motivation in individuals with these characteristics in prior studies (Killen et al., 2014; Koolhaas et al., 1999; Stamps, 2007). The results from this study will enhance our knowledge of the factors (especially physiological factors) driving angling vulnerability, which has been unexplored to date.

MATERIALS AND METHODS

Study animals

This study made use of a population of largemouth bass that has been the subject of an artificial selection experiment to produce lines of fish that differ in their vulnerability to angling (Philipp et al., 2009). Beginning in 1976 and ending in 1980, a population of wild largemouth bass from Ridge Lake near Charleston, IL, USA, was angled to divide the population into individuals that were captured numerous times (hereafter high vulnerability bass, HVB) and those not vulnerable to capture (hereafter low vulnerability bass, LVB). Both HVB and LVB were subsequently removed from the lake, given fin clips to identify assignment to HVB and LVB lines, and returned to the Illinois Natural History Survey's (INHS) aquatic research facility in Champaign, IL, USA, where they were held in a set of identical, earthen-bottom ponds. HVB and LVB fish were allowed to breed within their respective lines, and offspring were similarly marked with a fin clip to identify HVB and LVB individuals. Angling to further select LVB and HVB based on catchability continued for an additional three generations. Following the third generation of selection, the selection regime was halted (no additional angling). However, within-line breeding was continued to produce additional generations of pure HVB and

LVB, as well as reciprocal hybrid lines (H×L and L×H). The current study utilized the 6th generation of largemouth bass (spawned in 2012) derived from this selection experiment, and was conducted in 2015.

All largemouth bass used in the current study were held in one of several 0.12 ha ponds at the INHS aquatic research facility from the time they were spawned until the outset of experiments. Ponds at this facility were maintained in an identical fashion, with similar vegetative cover, fish density and abundance of forage (fathead minnows, *Pimphales promelas*), such that the effects of differential experience and habitat availability on behavior and physiology were minimized (Brydges and Braithwaite, 2009). Prior to experimental trials, ponds containing fish were drained, and each fish was checked for a fin clip to identify lineage then implanted with a passive integrated transponder (PIT) tag (Biomark Inc., Boise, ID, USA) for individual identification. Fish were then moved to one of eight circular 1135 l holding tanks connected to an adjacent pond with a flow-through system providing a continuous supply of fresh water where they were held for a period of 5 days to recover from handling. While being held, fish were fed fathead minnows *ad libitum*. All procedures described were approved by the University of Illinois Institutional Care and Use Committee (IACUC), protocol no. 14230.

Boldness and stress responsiveness

A total of 113 largemouth bass ($N=23$ HVB, 26 LVB, 37 H×L, 27 L×H; mean total length 230.9 mm, range 211–255 mm; mean total mass 159.8 g, range 118–223 g) were assessed for boldness and stress responsiveness. No difference in size was present across lines (analysis of variance, ANOVA on mass, d.f.=3, $F=0.967$, $P=0.41$). Prior to boldness assessment trials, each fish was PIT-identified and isolated overnight indoors in a 27 l opaque plastic holding tank provided with a continual supply of re-circulating water. Temperature for all indoor tanks was maintained at 23°C by a TK 500 Heater-Chiller (Teco, Revenna, Italy), with dissolved oxygen levels maintained above 8.0 mg l⁻¹ by an air stone connected to an air compressor.

At the outset of the experiment, largemouth bass were first assessed for boldness. The arena to test boldness consisted of a 180×65 cm opaque rectangular tank filled to a depth of 35 cm and transected into four equally sized zones. The first 'refuge' zone was separated from the three remaining zones (the 'open area') by an opaque Plexiglas divider. The refuge was covered with naturally colored gravel and artificial aquarium plants, while the open area contained no substrate or vegetative cover. Prior to the start of each trial, a focal fish was quickly netted from its individual holding tank and placed into the refuge zone of the arena. The fish was allowed to acclimate in the refuge for a period of 10 min before the divider was raised via a pulley system from behind a blind, allowing the fish to swim about in the arena for 30 min. Each trial was videotaped from above using a GoPro™ 3 camera, and three measures of boldness were quantified – the latency for the fish's entire body to cross the line separating the refuge zone from the rest of the arena, the amount of time spent by fish in the open area, and the total number of zone lines crossed by the fish. Each fish was assessed only once for boldness. While repeatability of behavior is necessary to identify that behavior as consistent and intrinsic to an individual (Bell et al., 2009), previous work has shown that fish behavior with open field tests is repeatable (Kortet et al., 2014; Mazue et al., 2015; Webster et al., 2009). In addition, we were concerned that repeatedly testing each fish would result in a loss of novelty of the environment on the second test (i.e. habituation), which can alter what behaviors are

actually being assessed compared with the first test (Dingemans et al., 2012; Réale et al., 2007).

After the boldness trial, the fish was returned to its individual holding tank, where it was again held overnight. The following day, two blood samples were taken from each fish to quantify cortisol concentration before and after a stressor, following procedures previously used for largemouth bass (Cook et al., 2011). In each case, the fish in question was quickly removed from its individual holding tank and a blood sample (baseline) was acquired within 2 min via caudal puncture using a 23-gauge heparinized needle. The fish was then given an air-exposure challenge in a container lined with wet towels for a period of 3 min, and was subsequently held in a 68 l tank filled with fresh pond water for a period of 25 min to allow cortisol levels to peak. Following this period, a second, post-stress blood sample was acquired in the same way as the baseline sample. All blood samples were immediately centrifuged at 6000 rpm for 2 min to extract plasma, which was temporarily stored in liquid nitrogen prior to transport to the laboratory, where samples were permanently stored at -80°C . Cortisol was quantified using an enzyme-linked immunosorbent assay (ELISA, kit no. ADI-901-071, Enzo Life Sciences[®], Farmingdale, NY, USA) previously validated for use in largemouth bass (Sink et al., 2008). Stress responsiveness was defined as the difference between post-stress and baseline cortisol concentrations for each individual. The air-exposure challenge and sample collection was conducted only once per fish, as recent work in largemouth bass has shown stress responsiveness to be repeatable (Cook et al., 2011), and we wanted to avoid excess stress resulting from multiple handling events. Following blood collection, all fish were stocked into a single 0.12 ha pond containing abundant fathead minnows and held for a period of 1 month before angling trials commenced.

Angling trials

Angling trials began in July of 2015 and consisted of 10, 2 h angling sessions performed over the course of 7 days. Each session took place at one of three times as determined by random selection: morning (06:00–08:00 h), midday (12:00–14:00 h) or evening (18:00–20:00 h). All angling was performed by a pair of experienced anglers. Medium-action rods spooled with 2.7 kg Berkley Trilene[®] monofilament line, commonly used by anglers targeting largemouth bass, were used for all three lure presentations, which included a 7.6 cm watermelon-colored plastic worm (Gary Yamamoto Custom Baits[™]) rigged ‘wacky’ style on a size 2 Eagle Claw[®] J-hook, a Strike King[®] 1 g white single-bladed spinnerbait tipped with a 0.95 cm white curly tail grub, and a single size 2 J-hook baited with a live nightcrawler *Lumbricus terrestris* suspended from a slip-bobber. We chose to use multiple lure types as fish with different behavior types may have preferences for striking certain types of lures (Wilson et al., 2015), and the three lure types used here allowed for a range of presentation speeds (the spinnerbait was retrieved quickly, the plastic worm was retrieved slowly, and the live nightcrawler was static) to maximize catch rates. The pond was divided into six equal sections (60 m \times 20 m), and the two anglers simultaneously fished a different section during each of three, 40 min periods within a 2 h angling session. Following each period, the anglers moved to a new section and switched lures as determined by random selection. Thus, during each 2 h session (which contained three periods), each section of the pond was fished once, and each angler used each of the three lure types. Upon capture, each largemouth bass was identified via PIT tag before being quickly released back into the pond. No bleeding or other injury was observed in any captured fish. At the conclusion of the

angling trials, the pond was drained and 88 of the original 113 fish were recovered. Unrecovered fish, which had presumably died ($N=25$), did not differ from the collected fish in boldness, cortisol levels or mass (Student’s *t*-test, $P>0.3$ for all tests). Because none of the unrecovered fish had been captured previously, we also assumed that those fish had died prior to the start of angling trials. For consistency, only the 88 recovered fish (19 HVB, 23 LVB, 26 H \times L, 20 L \times H) were considered in subsequent analyses.

Metabolic rate assessment

Because of the length of time that would be needed to process all 88 fish, metabolic rate assessment was performed only on a subsample of 40 fish: 20 that had been captured one or more times, and 20 that had not been captured. Specifically, sampling all 88 fish would have required sampling to continue well into October, when cooling weather conditions and decreasing photoperiod could potentially influence our results (Evans, 1984). Fish mass did not differ across experimental groups (ANOVA, $P>0.05$). All metabolic assessments took place 6 weeks following the completion of angling (fish were held in the meantime in a 0.12 ha pond stocked with fathead minnows), and were performed using static, intermittent-flow respirometry (Loligo Systems[™], Tjele, Denmark) following the methods of Redpath et al. (2010) with a few modifications (5.26 l respirometry chambers were used, and measurement cycles were lengthened to a 20 min ‘flush’, 2 min ‘wait’ and 10 min measurement phase). During measurements, all chambers were submerged in a 500 l square tank. Oxygen saturation in the tank was maintained near 100% by a pair of air stones, and kept at 23°C using heater-chillers. Measurements of oxygen saturation in the chambers were taken every 5 s during the measurement phase by a fiber-optic dissolved oxygen probe (calibrated regularly during the study) that allowed for the calculation of oxygen consumption (\dot{M}_{O_2} , in mg O_2 consumed kg^{-1} fish h^{-1}).

Each fish was loaded into a chamber in the afternoon and held overnight to collect data to determine standard metabolic rate (SMR), which was calculated as the mean of the five lowest \dot{M}_{O_2} values (Nelson and Chabot, 2011). The following morning, fish were removed from their chambers and temporarily placed in a 550 l tank, where they were exercised to exhaustion by manual chasing with a net for 5 min (Suski et al., 2007) before being returned to the respirometry chambers for an additional four measurement cycles. The highest individual \dot{M}_{O_2} value from these measurements was taken as the fish’s maximum metabolic rate (MMR) and aerobic scope (AS) was defined as the difference between MMR and SMR for each fish (Redpath et al., 2010). All equipment (chambers, pumps and tubing) was sterilized between trials with a 10% bleach solution, and final \dot{M}_{O_2} values were corrected to account for background metabolic activity (Rodgers et al., 2016).

Statistical analysis

To simplify measurement of boldness, principal components analysis (PCA) was performed based on the correlation matrix derived from the three individual boldness metrics (latency to emerge, time spent in the open, number of zones crossed). Principal components with eigenvalues over 1 were extracted using varimax rotation on the maximum likelihood solution (Kaiser, 1960). To determine the effect of boldness, baseline cortisol, stress responsiveness, fish mass and/or selected line on whether a fish was captured, we ran logistic regression models on all possible combinations of predictors (including models that included predictors independently, as well as full and null models).

Table 1. Factor loadings and variance explained following principal components analysis on boldness metrics for largemouth bass

Factor	PC1 loadings	PC2 loadings
Time spent in open (s)	0.881	0.256
Number of zone lines crossed	0.871	0.307
Latency to emerge from refuge (s)	−0.761	0.649
Eigenvalue	2.11	0.58
% Variance explained	70.45	19.35

Largemouth bass ($N=88$) were assessed for latency to emerge from a refuge in a novel arena, the number of zones crossed and the amount of time spent away from the refuge within a 30 min trial.

Logistic regression models were then compared using Akaike's information criterion, adjusted for small sample sizes (AIC_C), with top models selected based on ΔAIC_C values at or lower than 2 (Arnold, 2010).

To assess whether capture was size selective, fish mass was compared between captured and uncaptured fish using t -tests. All other metrics measured (baseline cortisol, stress responsiveness and boldness for the full set of 88 fish, and SMR, MMR and AS for the subset of 40 fish assessed with respirometry) were compared across captured and uncaptured fish using t -tests. Normality of data was assessed via examination of residual quantile–quantile plots, and homogeneity of variances was assessed by visual examination of fitted residuals (Anscombe and Tukey, 1963). All analyses were performed using R version 3.2.1 (R Core Team, Vienna, Austria) and the level of significance (α) was set at 0.05.

RESULTS

Ten angling sessions resulted in a total of 92 fish captures, with 38 captures occurring in the first angling session. Of the lures used, the plastic worm proved to be the most effective (57 captures), followed by the spinnerbait (28 captures) and the live nightcrawler (7 captures). Thirty largemouth bass, out of the total population of 88, were not captured. Fifty-eight of the 88 recovered largemouth bass were captured at least once, with 25 of these being captured multiple times. Fish captured once did not differ from those captured multiple times in boldness, stress responsiveness or metabolic rate (ANOVA, $P>0.44$ in all cases).

PCA revealed that the three boldness metrics were highly correlated. Only a single component was extracted (PC1, hereafter referred to as the 'boldness score') with an eigenvalue greater than 1; this single component explained over 70% of the variation in boldness behavior (Table 1). Individuals with high boldness scores tended to leave the refuge sooner, spend more time exploring open

zones and cross more lines relative to fish with lower boldness scores.

Stress responsiveness (i.e. the difference between post-stress and baseline cortisol concentration) alone was the top model explaining whether a fish was captured by anglers, and stress responsiveness was featured as a predictor variable in all of the top 10 models (Table 2). No additional models had a ΔAIC_C value lower than 2 (Table 2). However, models that contained stress responsiveness along with boldness score and baseline cortisol concentration had ΔAIC_C values of 2.02 and 2.04, respectively, suggesting some possible role for these factors in influencing vulnerability to angling (Table 2). Selected line did not factor in any of the top seven models, indicating that a fish's line in this experiment did not influence whether it was captured (Table 2).

Stress responsiveness differed significantly between captured and uncaptured fish (Table 3). Baseline cortisol concentration across all fish was 20.47 ng ml^{-1} and did not differ between captured and uncaptured fish, and instead the difference in stress responsiveness was driven by post-stress cortisol concentrations that were 48% higher in uncaptured fish (Fig. 1). Angling was not size selective, indicated by the fact that size was not included in any of the top logistic regression models, and also by the fact that mass did not differ statistically between captured and uncaptured fish (Table 3). Captured and uncaptured fish did not differ in any other metric assessed, including boldness, SMR, MMR and AS (Table 3, Fig. 1B,C).

DISCUSSION

Data from the current study indicate that neuroendocrine stress responsiveness was the strongest driver of angling vulnerability in largemouth bass when compared against other behavioral or physiological parameters. More specifically, largemouth bass that were captured by anglers showed a lower rise in plasma cortisol levels following an air-exposure challenge compared with largemouth bass that were not captured. Cortisol is the primary stress hormone in fish, which rises in response to stressors to mobilize energy reserves for use in responding to an external challenge (Bonga, 1997). High stress responsiveness, as defined by relatively large rises in cortisol following a stressor, is associated with the 'reactive' stress-coping style in many studies of animal behavior (Overli et al., 2005). This high responsiveness has been linked to shy and less aggressive behavior (Archard et al., 2012), as well as increased flexibility and learning capacity in dealing with environmental change (de Lourdes Ruiz-Gomez et al., 2011). In the case of largemouth bass in the current study, highly responsive individuals were less likely to be

Table 2. Top 10 binary logistic models

Model	AIC_C	ΔAIC_C	$-2 \log$ likelihood	W_i
Stress responsiveness	109.68	0	105.54	0.46
Stress responsiveness+boldness	111.70	2.02	105.41	0.17
Stress responsiveness+baseline cortisol	111.72	2.04	105.43	0.16
Stress responsiveness+fish mass	111.78	2.10	105.49	0.16
Stress responsiveness+fish mass+boldness	113.70	4.02	105.22	0.06
Stress responsiveness+baseline cortisol+boldness	113.75	4.07	105.27	0.06
Stress responsiveness+fish mass+baseline cortisol	113.85	4.17	105.37	0.06
Stress responsiveness+line	114.35	4.67	108.06	0.04
Stress responsiveness+fish mass+baseline cortisol+boldness	115.81	6.13	105.08	0.02
Stress responsiveness+fish mass+line	116.33	6.65	107.85	0.02

Comparison of models assessing the effect of baseline cortisol concentration, stress responsiveness (post-stress cortisol concentration minus baseline concentration), boldness, fish mass and selected line on whether a largemouth bass was captured during angling trials ($N=88$). Comparisons were made using Akaike's information criterion, adjusted for small sample sizes (AIC_C).

$-2 \log$ likelihood scores and model likelihoods (W_i) are also given.

Table 3. Statistical output of Student's *t*-tests

Variable	<i>t</i>	<i>P</i>	d.f.
Baseline cortisol	−0.80	0.42	87
Stress responsiveness	2.80	0.006	87
Boldness score	−0.36	0.71	87
SMR	−1.00	0.32	38
MMR	−0.52	0.60	38
AS	−0.22	0.81	37
Fish mass	−0.56	0.57	87

Comparison of baseline cortisol, stress responsiveness, boldness score, standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS) and fish mass between captured and uncaptured largemouth bass following assessment and experimental angling trials.

Variables that differed significantly between captured and uncaptured fish are given in bold.

captured, which may be linked in part to shyer and less aggressive behavioral tendencies. Reduced vulnerability may also be related to a tendency to freeze and/or not respond aggressively to the sudden appearance of a fishing lure nearby, as freezing behavior has also been shown to be linked with high stress responsiveness (Koolhaas et al., 1999). Regardless of what behavior is being affected by underlying physiology, it appears that stress responsiveness is negatively associated with angling vulnerability.

Interestingly, boldness had little influence on vulnerability to capture, with no difference in boldness score found between captured and non-captured fish. Boldness is typically defined as a propensity to take risks, whether in exploring novel environments or continuing to be active in the face of threats (Bell and Sih, 2007). Previous work has sought to link this risk-taking behavior to angling vulnerability, and results have been inconsistent. Some studies indicate that bolder and more exploratory individuals are more vulnerable to capture by anglers (Härkönen et al., 2014, 2016; Klefoth et al., 2013); other work has found bold individuals to be less vulnerable to angling (Wilson et al., 2011); and still other work has found no connection between boldness and angling vulnerability (Kekäläinen et al., 2014; Vainikka et al., 2016). Inconsistent findings linking boldness to angling vulnerability may be due to differences in methodology across studies (Beckmann and Biro, 2013), or may indicate that a relationship between boldness and vulnerability is context dependent and may fluctuate depending on factors such as the species in question or the time of year

(for instance, during the spawning season, vulnerability to angling may depend to a greater degree on factors related to aggressiveness; see Sutter et al., 2012). If the latter is the case, then studies of how behavioral and physiological characteristics affect angling vulnerability should take this into account so that these context-driven patterns may be better understood.

In contrast to our predictions, we found that metabolic phenotype (SMR, MMR and AS) was not an important predictor of angling vulnerability. This finding was contrary to our initial predictions, which were based on a number of previous studies documenting that high metabolic rates may increase the likelihood of a fish being captured, albeit via different gear types (Biro and Post, 2008). Other work has also indicated that angling pressure may lead to a reduction in metabolic rate in exploited populations, likely as a result of the selective capture of individuals with higher metabolism (Hessenaueer et al., 2015). Alterations to metabolic phenotype via the selective capture and removal of individuals with high metabolic rates could potentially have fitness-related outcomes for exploited populations, as metabolism is closely linked to growth rate and overall productivity as well as the likelihood of mortality (Biro and Stamps, 2008; Myles-Gonzalez et al., 2015). Not all experiments have indicated that this metabolic alteration will occur as a result of FIE in exploited populations. For instance, an artificial selection experiment performed on zebrafish, *Danio rerio*, resulted in no alteration in metabolic rate associated with simulated size-selective capture (Uusi-Heikkilä et al., 2015). The context in which angling occurs may be an important factor in determining the evolutionary changes imparted by the selective capture of certain individuals in a population, as some angling techniques may preferentially capture individuals with higher metabolic rates while other techniques do not. In a similar vein to our conclusions regarding the role of boldness in driving angling vulnerability, a compelling future avenue of research resides in the need to determine how different contexts (variable temperatures, seasons, capture techniques and/or targeted species) may lead to the capture-driven selection for or against different physiological traits.

Our results showed no role of selected line in driving vulnerability to angling. The lines of fish used in the current study were generated based on their response to recreational angling over three generations (Philipp et al., 2009), but selection pressures had stopped for three generations prior to use in this study. The

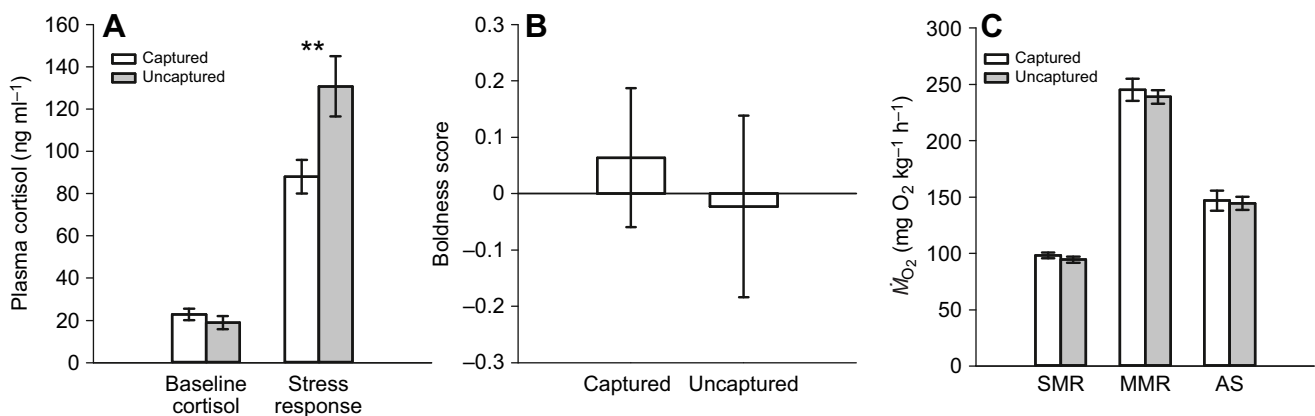


Fig. 1. Comparison of cortisol levels, boldness and metabolic variables between captured and uncaptured largemouth bass. (A) Baseline and post-stress cortisol. (B) Boldness score. (C) Metabolic variables: SMR, standard metabolic rate; MMR, maximum metabolic rate; AS, aerobic scope. For each fish, a baseline and post-stress plasma sample were taken; similarly, for metabolic rate, a single measurement of SMR and MMR was taken. Significant differences between captured and uncaptured fish for a given metric are denoted by asterisks. All bars are shown as means \pm s.e.m. For cortisol concentrations and boldness score, $N=58$ captured and $N=30$ uncaptured fish; for metabolic rate, $N=20$ captured and $N=20$ uncaptured fish.

selection regime had previously resulted in numerous differences between HVB and LVB as determined by studies of the 3rd to 5th generations, with HVB showing higher rates of recovery from exercise (Cooke et al., 2007), higher MMR and AS (Redpath et al., 2010), higher gonadosomatic indices (Redpath et al., 2009), greater aggression and angling vulnerability while nesting (Sutter et al., 2012) and lower rates of prey rejection (Nannini et al., 2011). The failure to detect a line effect with respect to capture likelihood in the present study is likely the result of differences in our classification of fish as vulnerable relative to the criteria used to establish the selected lines. In the present study, a vulnerable individual was defined as being captured once within a 10 day angling experiment, whereas HVB in the original selection regime were defined as being captured three or more times over an entire summer (Philipp et al., 2009). Also, the present study utilized different lure types and presentation styles compared with previous work, a difference that may have allowed for the capture of fish with a wider range of behavioral and physiological traits compared with those captured by single lure types, thus muting the line effect of catchability (Wilson et al., 2015). It is also possible that differences in angling vulnerability have become less distinct between HVB and LVB over the past three unselected generations because of a relaxation of the selection regime. However, a reversal of the aforementioned differences after only three generations of no angling would be quite rapid based on work that suggests human-induced evolutionary changes should take longer to reverse than induce (Conover et al., 2009). In summary, the lack of differences between the lines in catchability may be simply due to how the word ‘catchability’ is defined, with different characteristics being associated with the propensity to be caught via different lure types.

The results from the current study provide a number of new insights into the role of physiological characteristics driving capture vulnerability in recreational fisheries, and some of the potential long-term consequences of harvest by recreational anglers. It has been demonstrated previously that stress responsiveness and its behavioral and physiological correlates may be linked to fitness and overall productivity (Biro and Stamps, 2008). Because stress responsiveness is a heritable trait (Overli et al., 2005), the potential exists for this trait to be under selective pressure in recreationally angled populations through selective harvest and/or angling-induced mortality. While this pattern would hold significance in these populations, the actual degree of negative consequence of this is not entirely clear. On the one hand, populations that have experienced harvest and/or mortality due to angling may experience a selective alteration of physiological and behavioral traits, with selection leaving behind fish that respond reactively (for instance by freezing) to threats such as predators, territorial intrusions and attempts at predation of their nests. This, of course, could have cascading effects on the overall fitness of the population, especially in environments where greater fitness would otherwise be imparted by the maintenance of more proactive characteristics (Sutter et al., 2012). On the other hand, selection favoring reactive individuals could actually impart some fitness benefits in the form of increased behavioral flexibility in changing environments (Grootuis and Carere, 2005), which is a characteristic often found in individuals with higher levels of stress responsiveness (de Lourdes Ruiz-Gomez et al., 2011; Ruiz-Gomez et al., 2008). This ability of an individual to adjust its behavior in the face of changing environments may be especially important in the face of the rapid environmental change brought on by human activity (Sih, 2013). Regardless of the outcome, it is likely that fisheries selection on physiology will have an impact on

fitness levels in exploited populations, which will need to be closely examined and monitored in the years to come.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.J.L., S.A., J.A.S., C.D.S.; Methodology: M.J.L., S.A., C.D.S.; Software: S.A., C.D.S.; Formal analysis: M.J.L.; Writing - original draft: M.J.L.; Writing - review & editing: M.J.L., J.A.S., C.D.S.; Supervision: J.A.S., C.D.S.; Project administration: J.A.S., C.D.S.; Funding acquisition: J.A.S.

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