Capture technique and fish personality: angling targets timid bluegill sunfish, *Lepomis macrochirus*

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Abstract: Size-selective harvesting associated with commercial and recreational fishing practices has been shown to alter life history traits through a phenomenon known as fishing-induced evolution. This phenomenon may be a result of selection pathways targeting life-history traits directly or indirectly through correlations with behavioral traits. Here, we report on the relationship between individual differences in behavior and capture technique (beach seining versus angling) in wild-caught juvenile bluegill sunfish (*Lepomis macrochirus*). Both fish caught by using a seine net (seined) and fish caught by using a lure (angled) were individually tested under standardized laboratory conditions for their boldness, water-column use, and general activity. Observed inter-individual differences in boldness were strongly correlated with method of capture in the wild. Fish caught by angling were more timid and had fewer ectoparasites than fish caught using a seine net. However, this relationship did not carry over to an experiment in a large outdoor pool with seine-caught, individually tagged wild fish, where bolder individuals were more likely to be angled in open water away from refuges than more timid individuals, based on their previously assessed boldness scores. Our study is both novel and important, as it describes the relationship between capture technique and boldness in a natural population and underscores the potential risk of sampling biases associated with method of animal capture for behavioral, population, and conservation biologists.

Résumé : On a démontré que les captures sélectives en fonction de la taille associées aux pratiques de pêche commerciale et sportive changent les traits du cycle biologique par un phénomène connu sous le nom d'évolution induite par la pêche. Ce phénomène peut être dû à des voies de sélection qui ciblent les traits du cycle biologique directement ou indirectement par des corrélations avec les traits comportementaux. Nous traitons ici de la relation entre les différences individuelles de comportement et les techniques de capture (senne de plage et pêche à la ligne) chez des jeunes crapets arlequins (*Lepomis macrochirus*) capturés en nature. Nous avons vérifié individuellement chez des crapets pris à la senne et à la ligne la présence de hardiesse, l'utilisation de la colonne d'eau et l'activité générale dans des conditions standard de laboratoire. Les différences de hardiesse observées entre les individus sont en forte corrélation avec la méthode de capture en nature. Les poissons pris à la ligne sont plus timides et portent moins d'ectoparasites que les individus capturés à la senne. Cependant, cette relation ne s'est pas maintenue lors d'une expérience dans une grande piscine extérieure avec des poissons capturés à la senne et marqués individuellement; les individus plus hardis (d'après leur résultat dans les tests antérieurs de hardiesse) avaient plus de chance d'être pris à la ligne en eau libre loin des refuges que les individus plus timides. Notre étude est à la fois originale et importante puisqu'elle décrit la relation entre la technique de capture et la hardiesse dans une population naturelle; elle souligne aussi le risque potentiel d'erreurs systématiques associées à la méthode de capture des animaux dans les études biologiques de comportement, de démographie et de conservation.

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Introduction

Animal "personality" is commonly defined as individual differences in behavior that are consistent across time and (or) situations, and can incorporate a broad range of behavioral axes (e.g., boldness, sociability, aggressiveness) (Réale et al. 2007). Interest in the underlying proximate and evolutionary bases of animal personality has been steadily increasing in recent years (e.g., Réale et al. 2007; Smith and Blumstein

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2008; van Oers et al. 2008). While some studies have examined a variety of natural selection pressures shaping such behavioral traits (e.g., predation, environmental heterogeneity; see Dingemanse and Réale 2005), others have highlighted the potential impact of anthropogenic factors on the evolution of both morphological and behavioral traits (McDougall et al. 2006; Allendorf and Hard 2009). For example, size-selective harvesting associated with commercial and recreational fishing practices is thought to alter life history traits (e.g., earlier sexual maturation, reduced adult body size) through a phenomenon known as fishing-induced evolution (FIE) (Heino and Godo 2002; Kuparinen and Merila 2007; Uusi-Heikkila et al. 2008). However, life-history changes associated with fishing practices may be a result of selection pathways targeting life-history traits directly (e.g., decreased intrinsic growth) or indirectly through correlations with behavioral traits (Conover and Munch 2002; Walsh et al. 2006; Arlinghaus et al. 2009). Indeed, the efficacy of certain animal capture methods does appear to be dependent more on the behavior of the targeted animals than on their morphological traits per se (see Biro and Post 2008). For example, individuals that are more active, exploratory, and likely to take risks may be disproportionately susceptible to capture and, as a consequence, this may result in a variety of deleterious side-effects (e.g., rapid depletion of certain behavioral phenotypes and genotypes from a population) that may have important implications for species management and conservation (McDougall et al. 2006; Biro and Post 2008; Allendorf and Hard 2009). In addition, this phenomenon may have further implications for the manner by which animals are collected for behavioral research, as different capture methods may unintentionally target specific behavioral phenotypes (e.g., personality types), resulting in potential sampling biases (Biro and Dingemanse 2009).

One axis of personality of considerable interest to behavioral biologists is that of boldness. Boldness may be defined as the tendency of an individual to take risks (Wilson et al. 1994). The ecological and evolutionary importance of boldness is evident in both its taxonomic expression and the broad range of other ecologically relevant behaviors it is associated with. Individual differences along the boldness axis have been documented in a variety of taxa, including aquatic animals such as fishes (e.g., Ward et al. 2004; Webster et al. 2009; Wilson and Godin 2009) and cephalopods (e.g., Sinn et al. 2006, 2008). Boldness is also associated with numerous ecologically relevant behaviors such as general activity and space use (Wilson and McLaughlin 2007), intermittent locomotion (Wilson and Godin 2010), sociability (Ward et al. 2004; van Oers et al. 2005; Webster et al. 2007), antipredator behavior (Wilson and Godin 2009; Jones and Godin 2010), invasiveness (Rehage and Sih 2004), dispersal (Fraser et al. 2001), mate selection (Godin and Dugatkin 1996), foraging (Farwell and McLaughlin 2009), and exploration (Wilson and Stevens 2005; Wilson and Godin 2009). Furthermore, boldness is also correlated with fitness-related traits (Smith and Blumstein 2008; Wilson et al. 2010), can be heritable (Dingemanse et al. 2002; Brown et al. 2007), and subject to natural (Réale and Festa-Bianchet 2003) and “unnatural” (Biro and Post 2008) selection.

Here, we used both field and laboratory techniques with juvenile bluegill sunfish (*Lepomis macrochirus*) to ascertain whether recreational angling differentially targets timid or bold individuals and, additionally, to compare different capture practices for the potential presence of a sampling bias. Sunfish are particularly amenable to this type of study as they are easily captured using a variety of methods (e.g., line angling, seine net), are abundant in many freshwater systems (Scott and Crossman 1998; Spotte 2007), and are known to exhibit individual-level differences in boldness (Wilson et al. 1993; Wilson and Godin 2009, 2010). Furthermore, bluegill sunfish exhibit dramatic changes in morphological and behavioral life-history traits in response to harvesting exploitation by recreational angling (Coble 1988; Jennings et al. 1997; Beard and Essington 2000). Since bluegill sunfish are a popular sport fish across North America (Spotte 2007), any potential capture biases associated with individual differences in personality traits may have significant evolutionary and ecological consequences for the affected populations, as well as the quality of the fishery (Beard and Essington 2000; Biro and Post 2008), and may influence management strategies (Ashley et al. 2003).

Firstly, to quantitatively assess the relationship between angling and boldness in bluegill sunfish, we captured juveniles from a large northern temperate lake (using both line angling and seine net techniques) and exposed them to a standardized behavioral test quantifying refuge emergence time (a measure of boldness), general activity, and water column use following the methodology of Wilson and Godin (2009). Should recreational angling target either bold or timid individuals differentially, we predicted that fish captured via angling in the wild would be bolder on average than those individuals caught haphazardly with a beach seine net. Secondly, to characterize the relationship between boldness and susceptibility to angling, all seined individuals were placed in a large outdoor pool (following behavioral testing), wherein we attempted to recapture these individuals using a standardized angling protocol. We predicted that the individuals that were scored as being bolder during laboratory trials would be more likely to be caught via angling than more timid individuals, as per our previous prediction for field-captured fish. Lastly, since bolder individuals tend to spend more time in the riskier areas of the water column (e.g., near surface, open water; Wilson and Godin 2009, 2010), we also predicted that, on average, bolder individuals would be more likely to be caught in the open portion of the pool, than near a provided refuge, when compared with more timid fish. Understanding the relationship between recreational angling and fish boldness should contribute to our understanding of the potential ecological and evolutionary consequences of such harvest practices on natural populations, and also offer further insight into the potential for sampling biases in behavioral research based on method of animal capture.

Materials and methods

Field collections

Between 10 June and 20 July 2009, we used two different collection techniques to capture 230 juvenile (year 1+) bluegill sunfish (total length (TL), 98–148 mm; mass, 14.1–58.5 g) from the shallow littoral zone of several small bays located >600 m apart in Lake Opinicon, Ontario, Canada (44°34′N, 76°21′W). One of these collection techniques involved the use of a 20 m beach seine (5 mm mesh size) to capture a subset of the above individuals (N = 120, 98–148 mm TL, 14.9–51.8 g). Seining involved making numerous attempts of short duration (2–3 min for net movement, 5–7 min for fish collection from the net). Such brief seining attempts were used to minimize physical injury and stress among captured fish. Following each attempt, all captured fish of the appropriate size were immediately placed inside a cooler with fresh lake water (see below).

The second collection technique involved a standardized 10 s angling protocol by which we captured the remaining portion of individuals used in the study (N = 110, 99–148 mm TL, 14.1–58.5 g) over several collection trips. This short angling duration (i.e., 10 s) is common for sunfish (Cooke et al. 2003). These individuals were captured (angled) from the same bays as those sampled with the beach seine. Only small, barbless hooks baited with a 1 cm piece of earthworm (*Lumbricus* sp.) were used to capture fish. Moreover, individuals had to be caught, have the hook removed, and be placed inside a cooler within 10 s to be included in our study. Any individuals caught that did not follow this protocol, or individuals that were injured (e.g., any evidence of bleeding) as a result of angling practices, were excluded from further study. This protocol insured that any physical trauma and stress associated with capture would be minimized.

Following capture (via both methods), the fish were transported in coolers containing lake water (mean temperature = 23.4 °C) to our laboratory facilities at the Queen's University Biological Station, located on the shoreline of Lake Opinicon (boat transit time < 30 min). Between 16 and 20 (mean = 18) individuals were captured during each collection period, twice per week. Seining and angling did not occur on the same day.

Experimental holding conditions and experimental apparatus

On arrival at the laboratory, fish captured either by angling or seining were placed in one of two large holding tanks (171 L, 61 cm × 67 cm × 42 cm, L × W × H). Each holding tank was aerated and continually provided with fresh water from the lake via a flow-through system. The tanks were exposed to ambient sunlight through several windows in the laboratory. All fish were held overnight prior to experimental testing the following day. Since angled and seined fish were, respectively, caught on consecutive days (not concurrently), the particular holding tank assigned to each group was randomly assigned each week to reduce the likelihood of any holding tank bias. Each holding tank was equipped with six plastic aquarium plants of various sizes (height range = 20–60 cm) to provide cover and minimize agonistic interactions between fish. The density of sunfish (<20 on average) held in each holding tank falls within the range of densities observed in free-ranging juvenile bluegill sunfish in the shallow littoral zone of Lake Opinicon and similar nearby lakes in eastern Ontario (A.D.M. Wilson, personal observation). Fish were fed according to a standardized protocol such that they were provided commercial flake food (TetraMin) ad libitum following Experiment 1 and prior to being moved to an outdoor pool for Experiment 2. Fish were not fed on the day of their capture to avoid confounding variables associated with any differences in feeding caused by stress during capture and experimental holding.

During behavioral testing, each fish was placed in one of two identical glass aquaria (82 L, 92 cm × 30 cm × 30 cm). Each aquarium contained lake water (23 ± 1 °C; replaced daily) and was exposed to overhead fluorescent lighting. Each aquarium was divided into thirds, both horizontally and vertically, with lines drawn on the front panel. In doing so, each aquarium was divided into nine distinct zones of equal size to facilitate the observation of fish water column use. A refuge area, consisting of three plastic aquarium plants for cover, was located on one side of the test aquarium and was separated from the remainder of the aquarium by an opaque white plastic partition equipped with a sliding door. This gated partition was located 25 cm from the left side of the aquarium (see Fig. 1 in Wilson and Godin 2009). Aquaria were covered externally with brown cardboard at both ends and the back wall to prevent external disturbance and interaction between subjects.

Both aquaria were placed behind an observation blind to minimize external disturbance and to facilitate the quantification of behavioral parameters. Behaviors were quantified using the behavioral analysis software JWatcher (version 1.0) (Blumstein and Daniel 2007).

Behavioral experiments

Experiment 1: latency to exit a refuge and explore a novel environment

Approximately 24 h after capture (by either seining or angling), each focal fish was gently caught using an aquarium dip-net and placed within the refuge portion of an experimental aquarium (see above). The focal fish was then given 15 min to acclimatize to the refuge prior to the onset of behavioral testing. Following this period, the sliding door of the test aquarium was lifted manually via a remote pulley system operated from behind the blind, thereby allowing the focal fish to exit the refuge area and swim freely throughout the remainder of the aquarium (hereafter the "arena"). Each fish was given 20 min to exit the refuge and explore the arena. On exiting the refuge, several behavioral measures were quantified, namely latency to exit the refuge, water column use, and total time spent active in the arena (see Day 1 protocol in Wilson and Godin 2009). Because we were primarily interested in measures of risk-taking, only behaviors exhibited in the open arena (and not in the refuge) were quantified. Any individual that did not exit the refuge within the 20 min pre-trial period was assigned a latency-to-exit score of 20 min.

At the completion of behavioral trials, focal fish that were caught initially via seining were anesthetized using tricaine methanesulfonate (MS-222, 1:10 000 v/v dilution) buffered with sodium bicarbonate, and individually tagged on their dorsum with visible elastomer implant tags (Northwest Marine Technology, Inc.) following a standardized protocol (see Wilson and Godin 2009). Following the tagging procedure, these fish were returned to their respective holding tank for recuperation until the onset of Experiment 2 the next day.

Only seined fish were held over for further behavioral tests; those individuals caught initially via angling were euthanized using a lethal dose of buffered MS-222. We then recorded the standard length (SL) and wet mass (M) of these individuals and calculated their body condition as [M (g)·SL^{-3} (cm)] × 100 (Bolger and Connolly 1989). In addition, we counted external parasites (e.g., black spot, *Uvulifer ambloplitis*) on the body of each fish, as parasite load can affect behavior in fishes (Barber et al. 2000), and has been shown to be associated with boldness in sunfishes (Wilson et al. 1993; Barber et al. 2000). Further, we made additional morphological measurements (e.g., total length, girth) on each fish, and excluded for the study any fish that was found to be reproductively mature (N = 2).

Experiment 2: boldness and susceptibility of seined fish to angling practices

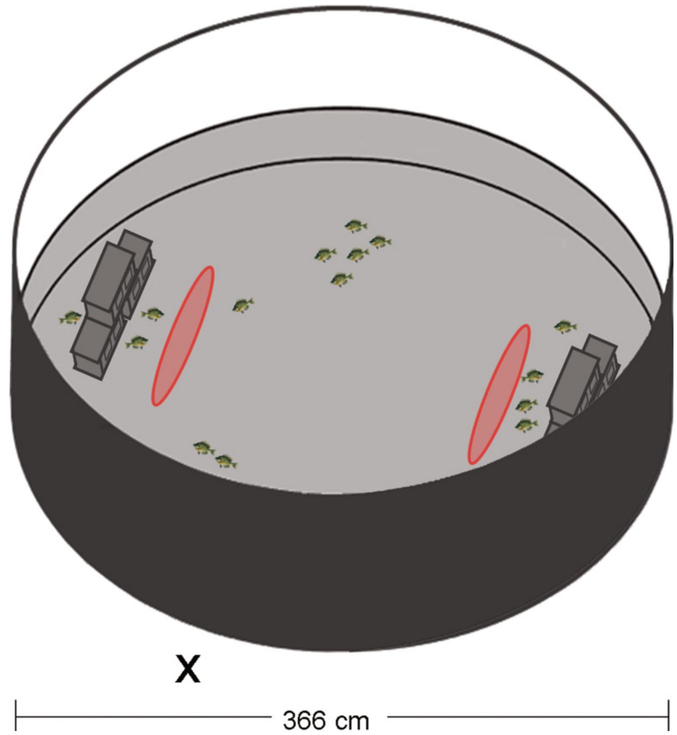
Approximately 16 h after being tested for boldness and tagged, a group of 15 fish (which had been previously seined from the lake) was transported from the indoor holding facilities to a large outdoor fibreglass pool tank (Fig. 1) to assess the susceptibility of seined fish to a recreational angling protocol related to that used in the field. Following their addition to the outdoor tank, the test fish were given an additional 24 h to acclimatize to the tank before being subjected to a standardized angling protocol (see below). A total of eight such replicate groups of fish were similarly tested. This experiment aimed to characterize the relationship between fish boldness level and susceptibility to being captured by angling in a novel experimental environment.

The outdoor pool consisted of a large, grey, fibreglass circular tank (366 cm diameter) and was exposed to natural sunlight. The oxygen level and temperature (23 ± 1 °C) were consistently maintained via a continuous inflow of fresh water from the lake into the tank and surface outflow. Water level was maintained at 46 cm, and the base of the tank was covered uniformly with natural rubble (~5 cm deep). Additionally, two refuges were added, each consisting of three concrete cinderblocks (each block 40 cm × 20 cm × 20 cm) stacked in the shape of a pyramid. The refuges faced each other from opposite sides of the tank (~200 cm apart) and were located 30 cm from the tank wall (Fig. 1).

The angling protocol used in this experiment mimicked the one used in the field as closely as possible. The observer (ADMW) was positioned centrally, such that he was perpendicular to the direction of the two refuges and equidistant to each of them. During three distinct time periods (beginning at 0900, 1300, and 1800 h) over the course of any day of the experiment, attempts were made to capture fish using a standardized angling technique. This technique was slightly modified from the angling protocol used in the field, but utilized the same equipment (i.e., hook, bait choice). Each fishing attempt lasted 20 min and involved alternating the dangling of the baited hook 30 cm from the front of each refuge for approximately 1 min. Though the exact position of the hook varied each time, the distance from refuge remained relatively constant (see Fig. 1).

When caught, individuals were identified, placed in coolers containing lake water, and brought back to the laboratory for measurement and determination of reproductive status (immature juvenile vs. mature adult, as above). After the last fishing period (1820 h), any individuals that remained uncaught in the experimental pool were netted out and similarly processed. The time individuals were caught and their position of approach to the hook prior to being caught (i.e., open area vs. within refuge) were recorded for each fish. On the rare instance where a fish bit the hook but escaped capture (N = 3), an attempt was made to visually identify it based on its tag and where possible (N = 2) the fish was counted as being caught during that collection period.

Fig. 1. Schematic representation of the experimental pool tank used in Experiment 2 to quantify the susceptibility of seined fish to angling practices based on their individual boldness score obtained from Experiment 1. The shaded ovals represent the areas wherein the baited hook was alternately positioned on each side of the tank during experimental trials. The stacked cinderblocks provided refuge for the fish. The symbol X denotes the position of the angler-observer.



Experiment 3: stress assessment of the beach seining and angling protocols

To ascertain the role, if any, capture method might have played in influencing the behavior of focal fish, we measured plasma cortisol levels (ng·mL^{-1}) in a number of seined (N = 21, 110–142 mm TL) and angled (N = 30; 100–141 mm TL) individuals collected using the exact same capture and holding protocols as described above. These individuals represent those fish from which sufficient amounts of plasma were collected within 30 s to allow the quantification of plasma cortisol. Each holding tank contained 20 individuals that were caught using either angling or seining techniques, on two separate occasions, so as to mirror the conditions used for fish holding prior to Experiments 1 and 2.

Plasma cortisol is a common stress hormone in fishes and is commonly used as an indicator of overall stress during laboratory and field experimentation in fishes (Barton 2002). Following a 24 h acclimatization period in laboratory holding tanks (as experienced by fish in Experiments 1 and 2), we collected blood plasma samples from each of the fish to ascertain their level of stress, which we assume would be representative of that of fish just prior to behavioral testing in Experiments 1 and 2. It is important to note that the objective of this experiment was to ascertain whether our two methods of capture per se (and not our behavioral testing) are associated with different levels of stress in juvenile sunfish. Consequently, fish used in Experiments 1 and 2 were purposely not sampled for plasma cortisol following their initial holding period in the laboratory, as this procedure is invasive and would have confounded the subsequent behavioral trials that the fish were exposed to in both the laboratory aquaria (Experiment 1) and outdoor pool tank (Experiment 2).

We collected blood plasma samples from anesthetized fish (buffered MS-222, 1:10 000 *v*/*v* dilution) via caudal puncture using a 1 mL heparinised syringe with a 25 g needle (Becton-Dickson, New Jersey) and immediately stored the samples in liquid nitrogen for subsequent analysis. We measured plasma cortisol levels using a commercial kit (ImmunoChem Cortisol 125I RIA kit, MP Biomedicals, Orangeburg, New York) and a Cobra Auto-Gammer (Hewlett-Packard, Palo Alto, California), following the methods outlined by Gamperl et al. (1994).

Data analyses

First, we compared recorded individual behavioral measures across the two experimental groups (i.e., seined vs. angled) using the nonparametric Mann–Whitney *U* test. These comparisons were made to assess behavioral differences between experimental groups based on capture method (Experiment 1; seined vs. angled fish) and within an experimental group (Experiment 2; seined fish) based on fish behavior exhibited in the laboratory.

Second, to test for any relationship between behavioral and morphological parameters, individual traits that were strongly correlated and best represented by a single value were collapsed into a first principal component score (PC1) using principal components analysis (PCA) (Table 1). This procedure was only performed on water column use, as general activity, latency-to-exit-a-refuge, body size, and body condition comprise independent, but at times correlated, behavioral variables. We then obtained across-context correlations between the PC1 score for water column use and other traits using the nonparametric Spearman's rank correlation test. To minimize Type 1 errors in our statistical analyses, the alpha level (α = 0.05) was adjusted to be more conservative using the sequential Bonferroni correction (Rice 1989).

Lastly, we used an analysis of covariance (ANCOVA) to compare the plasma cortisol concentrations of fish captured by seining with those captured by angling. Total body length was used as a covariate.

Results

Experiment 1: latency to exit a refuge and explore a novel environment

In general, fish caught by seining were bolder, having shorter latencies to emerge from refuge ($Z_1 = -4.44$, $P < 0.0001$), and had more ectoparasites ($Z_1 = -2.77$, $P = 0.006$) than fish caught via angling (Figs. 2*a* and 2*b*). However, they did not differ in general activity ($Z_1 = 0.84$, $P = 0.401$), water column use (upper zone: $Z_1 = -1.36$, $P = 0.174$; lower zone: $Z_1 = 1.32$, $P = 0.189$), total body length ($Z_1 = -1.95$, $P = 0.051$), or body condition ($Z_1 = -0.528$, $P = 0.604$) (Figs. 2*c*, 2*d*, 2*e*, and 2*f*, respectively).

Spearman rank correlation tests between latency-to-exit-a-refuge (refuge emergence) and time spent active, total body length, body condition, and the PC1 score for water column use showed consistent relationships within and between experimental groups. For all fish combined, refuge emergence was strongly negatively correlated with both time spent active ($r_s = -0.31$, $N = 134$, $P = 0.0003$) and water column use ($r_s = -0.29$, $N = 134$, $P = 0.0006$). Refuge emergence was also negatively correlated with total body length ($r_s = -0.21$, $N = 228$, $P = 0.002$), but not with body condition ($r_s = 0.003$, $N = 228$, $P = 0.964$). The direction and relative strength of the above relationships were similar for both angled and seined groups, with the exception that refuge emergence and total body length were significantly negatively correlated with each other for the angled group ($r_s = -0.30$, $N = 109$, $P = 0.002$), but not for the seined group ($r_s = -0.07$, $N = 119$, $P = 0.450$).

Experiment 2: boldness and susceptibility of seined fish to angling practices

On average, 40% of fish were caught by angling in the pool across the replicates (mean = 6 fish per replicate, range = 2–10). There was no relationship between their behavior (e.g., refuge emergence, water column use, general activity) in Experiment 1 and whether or not individuals were caught or the order in which they were caught when angling in the outdoor pool (range $Z_1 = -0.91$–0.77, all $P > 0.05$). However, when considering the location (open water vs. refuge) where individuals were located just prior to approaching the baited hook and being caught, fish that were in open water tended to be bolder (shorter latencies to exit the refuge, Experiment 1) than individuals that were in the refuge ($Z_1 = -3.62$, $P = 0.0003$). Similarly, individuals that spent more time swimming in the bottom portion of the open arena in the laboratory (Experiment 1) were also more likely to be hiding in refuge prior to being caught in the outdoor pool (Experiment 2) ($Z_1 = -2.11$, $P = 0.035$). There was no other relationship evident between morphological or behavioral variables and location prior to capture (range $Z_1 = -1.61$ to 1.12, all $P > 0.05$).

Experiment 3: stress assessment of beach seining and angling protocols

When controlling for fish body length (as a covariate), there was no significant difference in plasma cortisol levels between seined (mean ± standard deviation, SD; 92 ± 108 ng·mL^{-1}) and angled (167 ± 133 ng·mL^{-1}) fish (ANCOVA; $F_{[1,44]} = 2.11$, $P = 0.153$). However, larger individuals tended to exhibit higher cortisol levels than smaller individuals after capture (ANCOVA; $F_{[1,44]} = 4.52$, $P = 0.039$), irrespective of capture method.

Discussion

Our study addresses three areas of current interest in behavioral ecology, namely, animal personality, sampling biases associated with field capture techniques, and the potential impact of animal capture methods on different behavioral types

Table 1. Principal component analysis loadings of behavioral variables used to generate a principal component score (PC1) for water column use in juvenile bluegill sunfish.

Trait	Behavioral variables	Loadings for PC1	Variation explained (%)
Water column use	Time spent in upper zone	0.7071	71.9
	Time spent in lower zone	–0.7071	

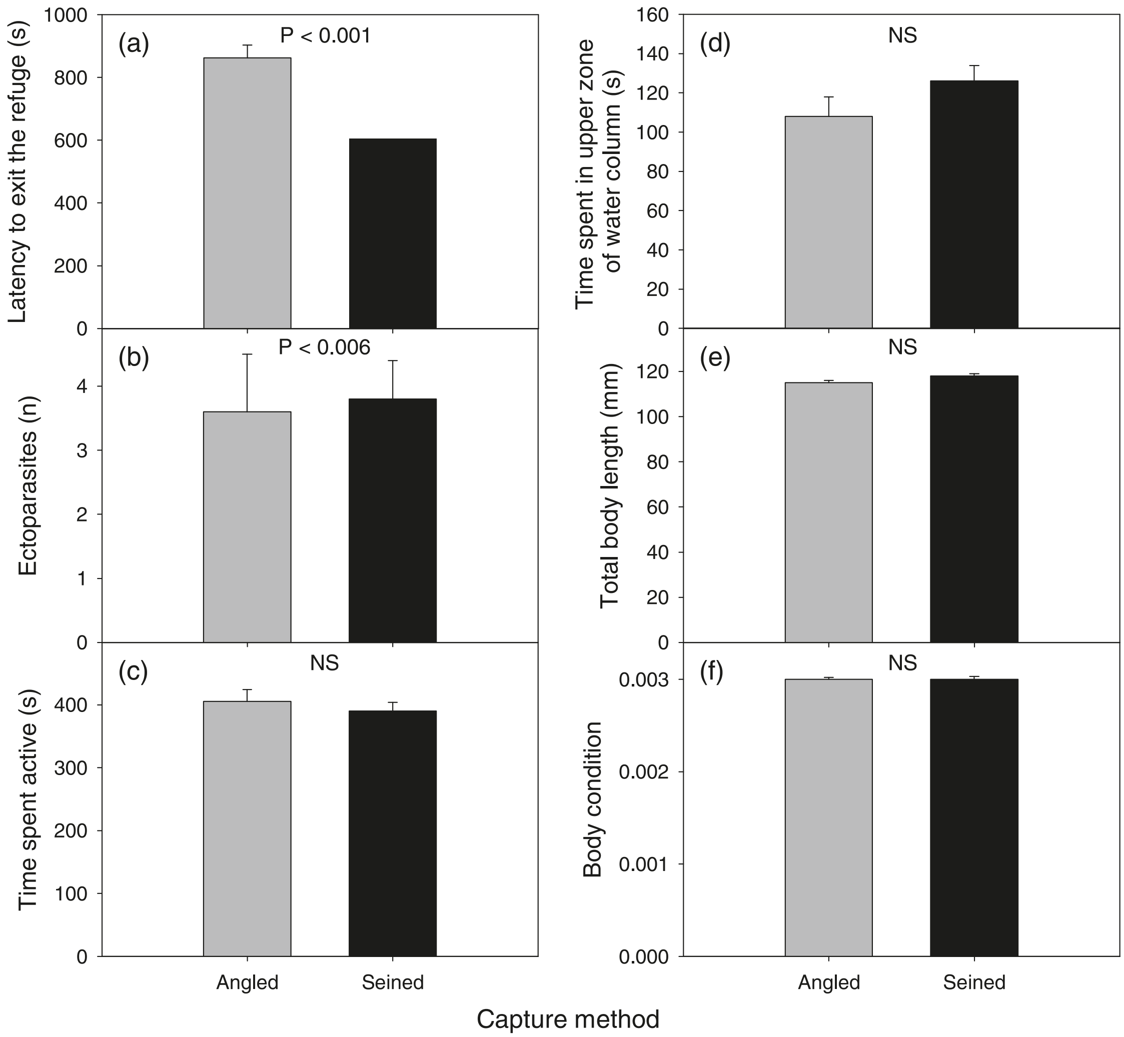


Fig. 2. Mean (±SE) for (*a*) refuge emergence, (*b*) ectoparasites, (*c*) general activity, (*d*) time spent in upper zone of the water column, (*e*) total body length, and (*f*) body condition in juvenile bluegill sunfish caught by either angling or beach seining capture techniques. Gray bars represent individuals caught via angling; black bars represent individuals caught via seining. *P*-values shown were obtained using the Mann–Whitney *U* test. NS denotes a nonsignificant difference between the means being compared.

(i.e., fishing-induced evolution). We sought to ascertain the nature of the relationships between these variables, if any, using the bluegill sunfish. We found that juvenile sunfish exhibited significant inter-individual differences in boldness (refuge emergence) and that these differences were strongly correlated with capture method in the wild. However, water column use and general activity did not differ between seined and angled fish. Interestingly, although the data supported our prediction that recreational angling may target individuals based on their behavioral type (i.e., bold/timid), the direction of this relationship was opposite to that initially expected. Rather than being more bold, sunfish that were caught in the lake using a standard angling technique were, on average, more timid and had fewer ectoparasites than individuals caught with a beach seine. However, this relationship between boldness and angling did not carry over to our experiment in an outdoor pool. Bolder individuals were, however, more likely to be angled when located in open water away from refuges than more timid individuals, based on their previously assessed boldness score. Although a small number of studies have been conducted on captive populations and (or) in artificial ponds/lakes (e.g., Wilson et al. 1993; Biro and

Post 2008), the current study is the first to our knowledge to have characterized a relationship between capture technique and individual boldness in a wild population of fish.

The current study is important for behavioral, population, and conservation biologists, as the relationship between fish personality traits (e.g., boldness) and their susceptibility to recreational and commercial harvesting practices may result in anthropogenic selection pressures affecting the phenotypic composition of natural fish populations. At the population level, Biro and Post (2008) recently demonstrated that certain fast-growing phenotypes of rainbow trout (*Oncorhynchus mykiss*) that differ behaviorally from slow-growing phenotypes may be up to three times more vulnerable to commercial fishing practices and, as such, be susceptible to overexploitation and fishing-induced evolution (Uusi-Heikkila et al. 2008). Should this risk of overexploitation exist for bluegill sunfish, either through recreational or commercial fishing practices, it may have important ecological consequences for the affected populations (e.g., decreased intrinsic population growth rate) as well as the quality of the fishery (Beard and Essington 2000). Indeed, both commercial and recreational fisheries operate for bluegill sunfish in many freshwater systems across Ontario, including Lake Opinicon, emphasizing the possibility for such exploitation to occur. In addition, should different capture techniques target individuals differentially based on behavioral type, behavioral researchers may be introducing a heretofore largely unrecognized sampling bias into their experiments (Biro and Dingemanse 2009).

Our finding that angling targets timid rather than bold sunfish is both interesting and novel, as it suggests a trend opposite in direction to our initial prediction and what little information is currently available on the relationship between personality traits and capture technique in fishes (Wilson et al. 1993; Biro and Post 2008). However, in the current study, this relationship appears to make sense ecologically. As with normal angling practices for sport fish such as sunfish, we frequented locations in or near areas of dense cover (e.g., rocky crevices, submergent vegetation) as well as open areas when fishing in Lake Opinicon. However, whilst fish were caught via angling from all locations, slightly more individuals (15%) were caught near refuge than in open areas, in spite of equal time spent fishing in open and refuge areas. Individuals caught in or near refuges may be more timid than individuals caught in open water, as juvenile fish commonly use such cover for predator avoidance (Godin 1997). Juvenile sunfish in particular are known to exhibit high site fidelity for these types of locations prior to reaching larger body sizes (McCairns and Fox 2004; Wilson and Godin 2009). In contrast, a large beach seine captures individuals from open areas as well as those in refuge, which is why it is a recommended technique for avoiding sampling biases associated with personality traits (Wilson et al. 1993; Biro and Dingemanse 2009). This assertion is also supported by our outdoor pool experiment (Experiment 2), which revealed that bolder fish (in terms of refuge emergence) tended to be located in the open water, whereas more timid fish tended to be located near the refuges prior to capture . Since our measure of boldness had to do with individual tendency to exit a refuge, the finding that timid individuals were more likely to be caught when angling near refuge may not be so counterintuitive. As such, this finding has important implications for the manner by which species such as sunfish are caught for behavioral research, as well as for understanding the potential impact of intensive angling on the behavioral and phenotypic composition of fish populations.

The observed relationships between boldness (latency-to-exit-a-refuge), water column use, and general activity were consistent and did not differ between the two capture groups, although the magnitude of these relationships did vary between seined and angled fish. Bolder individuals tended to spend more time near the water surface and were more active in general than more timid individuals. These relationships are consistent with other recent studies of boldness in juvenile bluegill sunfish in another similar, but unconnected, southern Ontario lake (Wilson and Godin 2009, 2010). While it has been suggested that correlations between particular behavioral traits should, and likely do, vary between locales (Dingemanse et al. 2007), evidence also suggests that certain selective agents (e.g., predation) can shape behavioral correlations in a predictable manner (Bell 2005). The consistency observed between the results of the current study and others (Wilson and Godin 2009, 2010) on juvenile bluegill sunfish may be a result of similar predation pressures within each study locale, similar environmental factors because of their close geographical proximity, or some combination of these and other factors. This finding may provide important insights into the nature and geographic distribution of sunfish boldness traits and how they have evolved in Ontario lakes.

Although we cannot eliminate the possibility that environmental or experimental factors may have influenced our findings with respect to the relationship between capture technique and boldness, the results do not appear to be related to different stress levels associated with angling or seining. We found no significant difference in plasma cortisol level between the two capture-method groups when controlling for body size (Experiment 3). This result is not unexpected, as even when exposed to severe stress associated with fisheries capture, physiological recovery in fish is generally quite rapid, with most physiological parameters returning to baseline values within 4 to 6 h of capture (Barton 2002; Cooke and Suski 2005). Similarly, while we did have a slight over-representation (i.e., 15%) of timid fish caught near refuge versus open water when angling in the wild, it is not likely large enough to solely explain the observed difference in boldness behavior between angled and seined fish, but, more likely reflects accurately the distribution of these juvenile fish in the wild. Moreover, because the fish in the pool experiment demonstrated the same, though nonsignificant, trend when comparing fish caught in the open versus refuge, a sampling bias based on habitat is unlikely.

We recognize certain limitations to our study, which prompt the cautious interpretation of some of the results. First, the observed differences in ectoparasite load between angled and seined fish are difficult to interpret. This finding may reflect either differences in habitat use or foraging strategy among individual fish, as susceptibility to parasitic infection can depend on habitat occupancy and diet (e.g., McCairns and Fox 2004) or a statistical artefact (resulting from large standard errors). Consequently, the ecological implications of this particular result should be considered cautiously. Second, we were unable to accurately quantify how individual interactions between conspecifics in the pool could have potentially influenced their behavior and thereby the likelihood of their differential capture by angling in Experiment 2. For example, if certain individuals were more aggressive or territorial than others, then the less aggressive and socially subordinate individuals in the pool may have behaved more cautiously and been less willing to feed on the angling lure than otherwise. Lastly, our results may have been dependent on the type of lure used. The use of our simple lure may not have elicited the same type of behavioral response in sunfish as other more complicated fishing lures (that vary in colour, design, size, and recovery technique) may have; but we did not test different types of lures in the current study. Owing to these limitations, the implications of our findings for predatory fish species or for individuals of different developmental stages (i.e., adult vs. juvenile) remain subjects in need of further investigation. This is particularly true since juvenile bluegill sunfish are primarily prey fish for larger piscivorous fishes (e.g., largemouth bass, *Micropterus salmoides*; northern pike, *Esox lucius*; Scott and Crossman 1998). As such, sunfish behavior and the angling protocols used to catch them may differ considerably from the angling techniques commonly used on predatory species. Given these differences, a similar study using alternative techniques on larger, predatory species may still demonstrate the directional relationship between capture technique and boldness that we initially predicted and as such, should be further investigated.

Nonetheless, our study describes, for the first time, a relationship between boldness and recreational fish angling practices, and strongly suggests that a potential sampling bias can be associated with fish-capture technique. Our findings should therefore be of particular interest to behavioral, population, and conservation biologists.

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