THE ECOLOGICAL CONSEQUENCES OF FORAGING BOLDNESS IN JUVENILE BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*)

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Zoology – Doctor of Philosopy
Ecology, Evolutionary Biology, and Behavior – Dual Major

2015

ABSTRACT

THE ECOLOGICAL CONSEQUENCES OF FORAGING BOLDNESS IN JUVENILE BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*)

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Biodiversity is manifest at all levels of ecological organization, from individuals to populations to communities, and elucidating the factors that generate and maintain this biodiversity is a central goal of ecology. Recently, there has been a surge of interest amongst behavioral ecologists to understand the degree to which individuals within a population differ in their behavioral types (often referred to as “animal personalities”) and how this inter-individual behavioral variation is maintained. To date, most of the work on animal personalities has been conducted in the controlled environment of the laboratory.

For my dissertation research, I studied the consequences of foraging boldness in juvenile bluegill sunfish (*Lepomis macrochirus*) using a combination of laboratory behavioral assays and experiments conducted in semi-natural ponds. Juvenile bluegill are a model system because they respond to tradeoffs in energy gain and predation risk, creating an ideal system to investigate consequences of individual differences in risk taking behaviors. A conflict between taking risks to acquire more energy and using refuge to avoid predators creates an ideal system to explore the consequences of inter-individual variation in foraging boldness.

Chapter 1 reviews the evidence for behavioral variation in fish and focuses on the factors that can select for and maintain inter-individual variation in behavioral types within a population.

In Chapter 2, I examined the behavioral structure, repeatability, and effect of social context on foraging boldness of juvenile bluegill under predation threat. I found that conspecific behavior and group composition affected the repeatability and foraging success of focal fish.

Such group dynamics may increase the variability in observed behaviors, but it is important to recognize that individual behavioral types are likely a complex combination of responses to physiological and social cues along with genetic predisposition and lifetime learning.

In Chapter 3, I determined the effects of boldness on growth, survival, and physiological investment into injury repair in a set of experiments in which juvenile bluegill were behaviorally assayed in the laboratory, individually marked, and then transferred to an experimental pond that mimics the environment of a natural lake. Bluegill that exhibited increased foraging under predation threat in the laboratory generally had higher growth rates in the field, but the magnitude of this effect was modified by ecological factors such as conspecific density and predator abundance. Surprisingly, there were no differences in survival based on boldness. However, shyer fish exhibited higher levels of fin regeneration, an indication that they invested more energy into injury repair.

In Chapter 4, I examined the diets and associated habitat use of individual bluegill sampled from one of the pond experiments. I found that bolder individuals made greater use of the risky open-water habitat in the field and benefited by increasing their foraging gain. Additionally, bolder individuals showed higher use of pelagic habitats among fish that were at early stages of an ontogenetic niche shift.

In Chapter 5, I highlighted the use of Passive Integrated Transponder (PIT) antenna systems as a tool for quantifying individual differences in habitat use, activity, and movement behaviors of aquatic organisms in field settings.

I dedicate this dissertation to my dad, Ken Kjelvik,
who fostered my ecological curiosity by teaching me the ways of the natural world
and instilling a deep respect for the intricacies of nature.

ACKNOWLEDGMENTS

First, I thank Gary Mittelbach for agreeing to be my academic advisor and delving back into the world of bluegill foraging ecology that he explored for his own dissertation. His ability to frame results from my research within established patterns led to interesting connections that I find to be the most rewarding aspects of my dissertation. In addition, he encouraged my desire to maintain connections with fisheries management by organizing and co-authoring a review paper exploring the implications of individual level behavioral variation on common management practices.

My graduate advisory committee, Mary Bremigan, Tom Getty, and Brian Roth provided great feedback throughout the years and on the final dissertation. They all show great sincerity in the mentoring of graduate students, and thus provided constructive critiques and feedback in a positive and helpful manner. I would also like to thank Kay Gross and Louise Mead for advocating for graduate students at KBS and in BEACON, respectively. Among several other things, Kay Gross provided research money, teaching assistantship positions, and undergraduates mentoring opportunities that greatly shaped my graduate experience and is integral to what makes KBS a nationally recognized ecological research site. I am grateful that I was introduced to Louise Mead as a science education mentor. Her enthusiasm, creativity, and encouragement led to the continued support of Data Nuggets through various funding sources. Now a nationally recognized program with a solid research team of its own, I owe much to her support at the beginning of the project.

I would like to thank funding sources for supporting my research, teaching, and mentoring experiences throughout the years. In particular, BEACON, MSU College of Natural

Sciences, EEBB, the Graduate School, KBS Lauff, KBS URA and REU programs, KBS LTER, and Sigma Xi. Additionally, parts of this dissertation were funded by NSF DDIG-1210438.

I would like to thank the many great undergraduates I have had the opportunity to work with during the summer months at KBS; Laurel Lindemann, Dustin Rhodes, Emily Fisher, Ben Staton, Kacie Jonasen, Susanna LaGory, Genevieve Pugesek, Becca Blundell, Zack Davis and Emily Gauci. I am proud to see the growth and development of each mentee as they follow their dreams and pursue diverse career paths. They offered fresh and motivating perspectives on various aspects of science. I am thankful I got to get to know them and hope I impacted their lives a fraction of the amount they affected mine.

I am also thankful for the time my dissertation writing buddies, Tomomi Suwa and Christine Sprunger, dedicated to checking in daily during our final stretch. It has been an honor to share the ups and downs of our final semesters together and I can't wait to see what is in store for us all as we move on.

I would also like to thank others who played substantial supportive roles in my life. My two friends, Greg Triemstra and Larry Bushing, always went above and beyond to help me with my research and to make sure we had fun along the way. And finally, I thank Derek Ogle, who encouraged me to pursue independent research and graduate school during my final years at Northland College. His passion, enthusiasm, and support both in and out of academic settings has served as a model to strive for, and has undoubtedly shaped my own academic values.

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CHAPTER ONE

FISH BEHAVIORAL TYPES AND THEIR ECOLOGICAL CONSEQUENCES

Abstract

Fish have proven to be model organisms for the study of animal personalities, and a rich literature documents consistent interindividual behavioral differences in a variety of species. However, relatively few studies have examined the ecological consequences of such consistent interindividual differences in behaviors in fish or other organisms, especially under field conditions. In this review and perspective, we discuss the factors that may lead to the formation and maintenance of behavioral types in fish populations. We then examine what is known about the effects of personality variation on individual growth and survival, breeding behaviors and reproductive success, habitat use, diet, and ontogenetic niche shifts, migration and dispersal, as well as potential consequences for species interactions and ecosystem functioning. We focus as much as possible on studies conducted under natural or seminatural conditions, as such field studies are most relevant to elucidating the ecological consequences of behavioral variation. Finally, we discuss the potential importance of consistent individual differences in behaviors to fisheries management and conservation, specifically examining consequences for recreational and commercial fishing, hatchery rearing, and stock enhancement.

Introduction

Fish, like many other vertebrates, show consistent individual differences in behavior despite maintaining a high degree of behavioral plasticity. These consistent interindividual differences in behavior have been variously termed animal personalities (Dall et al. 2004), behavioral profiles (Groothuis and Trillmich 2011), temperaments (Réale et al. 2007), coping styles (Koolhaas et al. 1999), or behavioral syndromes (Sih et al. 2004*a*, 2004*b*). Much of the large and rapidly expanding literature on animal personalities involves studies with fish (Stamps 2007), and it was early work by Huntingford (1976, 1982) with sticklebacks, and Ehlinger and Wilson (1988) and Wilson et al. (1993, 1994) with sunfish, that provided some of the first evidence that individuals may exhibit consistent differences in behavioral traits within a population (e.g., individuals may be relatively shy or bold, aggressive or timid). Wilson et al. (1993) suggested that such consistent behavioral differences between individuals represented more than random variation around an adaptive mean. Rather, "individual differences are interpreted not as the raw material on which natural selection acts but as the end product of natural selection" (Wilson et al. 1993: page 255). That is, variation in behavioral traits may be maintained within a population because such variation represents different adaptive solutions to a complex environment (e.g., Wolf and McNamara 2012).

Although studies of animal personalities initially struggled to gain traction against the idea that variation in phenotype is expected within a population and therefore does not require a special explanation (Wilson 1998), subsequent years have validated the early insights of Huntingford, Wilson, and their colleagues. Today the study of consistent individual differences in behavior enjoys a vigorous growth, as evidenced by the publication of a number of recent reviews (e.g., Sih et al. 2004*a*, 2004*b*; Réale et al. 2007; Sih and Bell 2008; Stamps and Groothuis 2010; Wolf and Weissing 2012), including three reviews devoted specifically to fish (Toms et al. 2010; Budaev and Brown 2011; Conrad et al. 2011). As Wolf and McNamara (2012) note, three key features associated with personalities have been observed in a variety of species: (*i*) variation, individuals differ in their behaviors; (*ii*) consistency, individual differences in behaviors are stable over time; and (*iii*) correlations, certain behavioral traits (e.g., boldness, aggression, and exploration) tend to be correlated among individuals.

Although consistent individual differences in behavior are now well documented in fish and other organisms, for the most part these studies have been conducted in the laboratory under simplified and highly controlled conditions. Studies of behavioral trait variation under natural or seminatural conditions are still quite rare (e.g., Fraser et al. 2001; Biro et al. 2007; Adriaenssens and Johnsson 2011*a*). While laboratory studies have opened the door to the rich array of behavioral diversity found in nature, a key question before us is what are the ecological consequences of consistent individual differences in behavior to organisms in the wild (Dingemanse and Réale 2005; Archard and Braithwaite 2010; Bolnick et al. 2011; Adriaenssens and Johnsson 2011*a*; Sih et al. 2012; Wolf and Weissing 2012). These ecological consequences include potential effects on an individual's survival and reproductive success, population dynamics (through influences on species' vital rates, e.g., growth, fecundity, and survival), community structure and species diversity (through influences on species interactions), and on the conservation and management of natural resources (Figure 1). To understand these consequences of animal personalities, we need to study organisms in the complex environments found in nature (Stamps and Groothuis 2010).

Our goal in this paper is to review what is known about the ecological consequences of behavioral trait variation in fishes at the individual, population, and community levels, including the consequences of personality for the conservation and management of fishes. More than 20 years ago, Wilson et al. (1993) noted that the ecological consequences of such consistent individual differences had not been studied in a natural population of any species. Despite the explosion of research into animal personalities since the paper of Wilson et al, the ecological and evolutionary consequences of consistent interindividual differences in behavioral traits in natural populations are only now coming into focus (Bolnick et al. 2011; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012). We begin our review with a short description of terminology and measurement issues in the study of animal personalities, followed by a discussion of the proximate and ultimate factors that may lead to consistent interindividual differences in behavior. We then examine what is known about the ecological consequences of behavioral types in fishes, including effects at the individual, population, and community levels. We focus as much as possible on studies conducted under natural or seminatural conditions, as such field studies are most relevant to elucidating the ecological consequences of behavioral variation. Finally, we discuss the potential importance of consistent individual differences in behavior to fisheries management and conservation, specifically examining consequences for recreational and commercial fishing, hatchery rearing, and stock enhancement.

Terminology and measurement

Multiple terms surround the discussion of animal personalities, which has led to considerable debate in the literature. Table 1 lists many of the terms used in the study of animal personalities and defines how we use these terms in the current paper. The terms animal personality, temperament, and coping style have been used more or less synonymously (Réale et al. 2007). These terms describe consistent differences between individuals in behaviors across contexts over some period of time and are most often used to refer to general behavioral patterns (e.g., individual differences in boldness, activity, and aggressiveness and the relationships between them). We use the term behavioral type to describe an individual’s phenotype (e.g., bold versus shy; aggressive versus timid) relative to other individuals in the population.

One of the challenges in assessing personality traits in fish and other species is the fact that behavior can be extremely plastic; individuals often respond to changes in their environment by adjusting their behaviors to meet current conditions. Further, individuals may differ in their degree of behavioral plasticity, which at the individual level could affect the measurement of personality traits (Nussey et al. 2007; Dingemanse et al. 2010) and at the population level could affect stability and population persistence in response to environmental change (Dingemanse and Wolf 2013). A useful framework for examining personality traits in light of behavioral plasticity is the concept of behavioral reaction norms (Figure 2). The reaction norm framework illustrates how individual differences in personality traits can be assessed across multiple contexts to examine consistency of behavioral types and the extent of behavioral plasticity within and between individuals (Réale et al. 2007; Dingemanse et al. 2010). For example, Budaev and Brown (2011) provide a table of over a dozen measures that have been used to assess boldness in fish (e.g., predator inspection, foraging under predation risk, latency to emerge from cover, behavior in an open field, etc.). Measuring individuals in a number of these contexts would allow for the development of reaction norms that would more accurately characterize an individual’s personality type.

The behavioral reaction norm approach also provides an opportunity to identify which measures actually quantify the same personality trait. The few studies that have investigated correlations among different personality measures that were thought to quantify the same trait have found some surprising results (Carter et al. 2013; Garamszegi et al. 2013). For example, two measures that are often assumed to quantify aggression were investigated in yellow-bellied marmots (*Marmota flaviventris*). While both measures were repeatable over time, they were independent from each other (Blumstein et al. 2012). A similar result was found when investigating two measures that are often assumed to quantify boldness (i.e., response to a novel object and response to a predator; Carter et al. 2012). These results show how different ways of measuring a personality trait may not be interchangeable, providing additional justification for taking a reaction norm approach.

The application of behavioral reaction norms is still relatively new and not yet widely applied. Nevertheless, numerous studies of behavioral types in fish and other organisms provide results that allow us to explore the ecological consequences of personality variation, as long as we are careful in our interpretation and recognize that different ways of measuring a personality trait like "boldness" may in fact measure different things (Réale et al. 2007). Finally, while this review and prospectus focuses on personality variation in fish and its ecological consequences, we want to emphasize the wealth of studies that exist for other taxa. These studies provide a broader context in which to view the results from fishes, and we refer readers to publications by Réale et al. (2000), Dall et al. (2004), and Dingemanse and Réale (2005), as well as the review papers cited in the Introduction, as an entry point to the literature on animal personalities in birds, mammals, and other groups.

The evolution and maintenance of variation in behavioral traits

Fitness tradeoffs

The fitness consequences of behavioral traits are often context-dependent. For example, bold behavioral types may be less fit than shy behavioral types in an environment with high levels of predation, while the opposite may be true in an environment without predators. Behavioral ecologists have focused on fitness tradeoffs as an important mechanism to explain the generation and maintenance of variation in behavioral traits within a population, both on ecological and evolutionary time scales. For example, consider the situation where individuals that are bolder are more likely to encounter predators, resulting in higher mortality rates. Now imagine that these bolder individuals are also more likely to encounter more prey per unit foraging time and therefore experience higher feeding rates, resulting in higher energy gains and growth (Stamps 2007). As a result, under this hypothetical scenario, a potential tradeoff between energy gain and survival would exist that could maintain variation in boldness.

Mangel and Stamps (2001) developed a simple model to show how tradeoffs between growth and survival can result in a range of individual growth rates that all yield equivalent fitness (as measured by r in the Euler–Lotka equation), thus favoring the maintenance of multiple behavioral types within a population. Similarly, models for the evolution of interindividual differences in dispersal rates have been developed under the premise that the expected fitness of "dispersers" equals the expected fitness of "stayers", due to a growth–mortality tradeoff (Hamilton and May 1977; Frank 1986; Johst and Brandl 1999; Ronce et al. 2000).

Fish often face situations in nature where there is a potential tradeoff between increased feeding rate and reduced survival (Lima 1998; Mangel and Stamps 2001). For example, (*i*) open-water (pelagic habitats) may contain richer food resources but pose higher predation risk (e.g., Werner and Hall 1988; Gliwicz et al. 2006), (*ii*) daytime foraging may yield a higher feeding rate but greater risk of predation than nocturnal foraging (e.g., Fraser and Metcalfe 1997; Metcalfe et al. 1999; Ryer and Hurst 2008), and (*iii*) more active foragers may encounter more prey (or richer habitats) and grow faster but suffer higher mortality rates (e.g., Werner and Anholt 1993; Fraser et al. 2001; Biro et al. 2004, 2006; Sundström et al. 2004). Fitness tradeoffs can also occur spatially (e.g., one area of a habitat favors one behavioral type, while another area favors a different one), temporally within generations (e.g., across development or across genetically linked behavioral traits), and temporally between generations (e.g., frequency-dependent selection).

Inter-individual differences in metabolism and state

While fitness tradeoffs provide a powerful mechanism that may select for a variety of (equal fitness) behavioral types within a population, the maintenance of consistent behavioral types or personalities over time requires more than just a fitness tradeoff. To see this, consider the following question: what prevents individuals from continually shifting back and forth between different behavioral types that have equivalent fitness? Or, stated in another way — why do we find differential consistency (as defined in Table 1) in behavioral types within a population (e.g., individuals that are consistently bolder than others over time)? One possibility is that individuals differ in their relatively unchanging physiological traits (e.g., resting metabolic rate or the size of metabolically costly organs); therefore, the behavioral type that results in the optimal value of the growth–mortality tradeoff differs depending on physiological state. A number of authors (Stamps 2007; Biro and Stamps 2010; Houston 2010) have suggested that consistent individual differences in physiological state could be an important factor promoting the formation of individual differences in personality. The first step in examining this hypothesis is to determine whether individuals differ consistently in their physiological traits (e.g., resting metabolic rates or potential growth rate); the second step is to determine whether any such differences in physiological measures are correlated with behavioral traits (see Biro and Stamps (2010) for a review of the literature on resting metabolic rate and their relationship to behavioral traits).

Recently, it has been suggested that behavioral traits may covary with a whole suite of physiological and life-history traits, such that these covarying phenotypic traits can be effectively grouped under the umbrella of a "pace-of-life syndrome" (Réale et al. 2010). Figure 3 illustrates the potential integration of life-history, behavioral, and physiological traits along a pace-of-life continuum from "slow" to "fast". Evidence for a pace-of-life syndrome in fish or other organisms is still tentative (Adriaenssens and Johnsson 2009). However, Biro and Stamps (2008) show that behavioral traits are linked to life-history variation in a variety of organisms, including fish. For example, activity rates and boldness are positively related to growth rates in rainbow trout (*Oncorhynchus mykiss*; Biro et al. 2004, 2005, 2007), and boldness is positively related to growth, fecundity, and size at maturity in Atlantic silverside (*Menidia menidia*; Walsh et al. 2006). The pace-of-life syndrome provides a useful heuristic framework in which animal personality studies can be integrated to address how behavioral traits are maintained within populations, and how they may have ecological consequences affecting individual growth, survival, and reproductive success, as well as population dynamics and successful resource management. Moreover, viewing behavioral variation in the light of life-history traits and the pace-of-life syndrome allows us to consider the impacts of behavioral trait variation at different life stages and to better understand when and why personality types may be maintained over ontogeny (e.g., Schürch and Heg 2010; Chervet et al. 2011), even potentially across metamorphosis (e.g., Wilson and Krause 2012*a*, 2012*b*).

There are additional ways in which the “state” of an individual can affect the relative costs and benefits of different behavioral actions, leading to the generation and maintenance of adaptive behavioral trait variation within a population (Houston and McNamara 1999; Dingemanse and Wolf 2010; Luttbeg and Sih 2010; Wolf and Weissing 2010, Wolf and McNamara 2012). In the case of foraging boldness (where taking greater risk yields higher rewards), Luttbeg and Sih (2010) show how positive-feedback mechanisms can maintain differential consistency in behavioral traits. For example, if individuals having higher state (e.g., better condition, larger size, and more energy reserves) are better at defending themselves or fleeing from predators, then animals with higher state will have lower predation risk while being bold and should be bolder than low state individuals (Luttbeg and Sih 2010). Thus, small differences in initial state between individuals (e.g., due to parental provisioning and carryover from larval to adult stages) can lead to a positive-feedback loop between assets and behaviors, such that “….individuals that already have high state (assets) would be bold, and thus gain more resources that maintain their high state” (Luttbeg and Sih 2010: page 2979). In addition, positive feedbacks based on experience or learning can lead to differences in foraging efficiency in a habitat or on a particular prey type that can act to reinforce and maintain behavioral differences between individuals (Werner et al. 1981; Dingemanse and Wolf 2010).

Ecological Consequences Of Behavioral Types

“Surprisingly little attention has been paid to the ecological and evolutionary consequences of personality differences” (Wolf and Weissing 2012: page 452).

In the sections above, we examined the factors thought to drive the evolution and maintenance of animal personalities in fish and other organisms. These mostly theoretical studies seek to provide “ultimate” evolutionary explanations for the existence of consistent differences in behaviors between individuals. In the following sections, we focus on the ecological consequences of these behavioral differences. That is, given the presence of varying behavioral types within a population, how might this behavioral variation affect the ecology, management, and conservation of fishes. These ecological consequences may include effects on an individual’s survival and reproductive success, the dynamics of populations (through influences on species’ vital rates, e.g., growth, fecundity, and survival), effects on community structure and species diversity (through influences on species interactions), and impacts on the management and conservation of species and fish stocks (e.g., through hatchery rearing and supplemental stocking). We recognize that separating the factors thought to ultimately drive the evolution of behavioral types in fishes from the more proximate consequences of such behavioral types to the ecology of individuals and populations is a somewhat false dichotomy. Ecology and evolution go hand in hand. Still, this distinction is useful for highlighting how the existence of behavioral types within a population may impact various aspects of an individual’s ecology (e.g., growth, survival, diet, and habitat use), as well as population dynamics and species interactions.

Ecological consequences at the individual level

Growth and survival

Personality traits have the potential to affect an individual at nearly every stage of development, from a juvenile’s chances of surviving to adulthood to an adult’s reproductive success. We summarize what is known about the ecological consequences of behavioral trait variation in fish at the individual level in Table 2. Table 2 includes both laboratory and field studies. However, in the discussion below we focus on the results from natural and seminatural environments, as field studies provide the most direct tests of the ecological consequences of behavioral trait variation. Looking first at the impact of boldness, aggressiveness, and exploration on individual growth, dispersal, and survival, we find support for a hypothesized growth–mortality tradeoff, although the evidence from field studies is surprisingly limited.

Fraser et al. (2001) found that bolder individuals of the Trinidad killifish (*Rivulus hartti*) moved greater distances in the field. Moreover, movement distance was positively correlated with individual growth over a 19 month mark–recapture study in a section of river containing *Rivulus* predators. However, in a predator-absent zone, there was no correlation between movement and growth (Fraser et al. 2001). Our own studies with juvenile bluegill (*Lepomis macrochirus*) in ponds have shown a positive correlation between boldness measured in the laboratory and individual growth rates observed in the field over periods of 2–6 months (M. Kjelvik and G. Mittelbach, unpublished data). In studies comparing domestic and wild strains of salmon and trout, Sundström et al. (2004) and Biro et al. (2003*a*, 2003*b*, 2004) examined the growth, survival, and habitat use of fish in the presence and absence of predators. Sundström et al. (2004) found a tradeoff between growth and survival for strains of coho salmon (*Oncorhynchus kisutch*) transgenic for growth hormone (GH) relative to wild salmon. In seminatural stream channels, GH-transgenic coho fry grew faster than wild coho fry but suffered higher mortality from predators (nontransgenic coho juveniles) (Sundström et al. 2004). Higher mortality on GH-transgenic fry was most pronounced under low-food conditions. Other studies have documented increased risk-taking behavior in GH-transgenic salmon in the laboratory (Abrahams and Sutterlin 1999; Sundström et al. 2003), as well as increased movement by GH-enhanced trout in the wild (Sundt-Hansen et al. 2009). Thus, there appears to be an interaction between behavior, growth, and mortality when comparing salmon and trout strains modified with GH relative to wild populations (but see Johnsson and Björnsson 2001).

In a series of whole-lake experiments, Biro et al. (2003*a*, 2003*b*, 2004, 2006) compared the growth, survival, and habitat use of domestic (hatchery stock) and wild strains of rainbow trout (*Oncorhynchus mykiss*). In the presence of avian predators (loons, *Gavia immer*), age 1 domestic trout grained 20% more mass than wild trout (Biro et al. 2004), and age 0 domestic trout gained 100% more mass than wild trout (Biro et al. 2006). However, domestic trout (age 0 and age 1) suffered 50%–60% greater mortality than wild trout when predators were present (Biro et al. 2004, 2006). Behavioral differences between domestic and wild strains in the field were not specifically quantified in these experiments. However, in a subsequent field experiment (Biro et al. 2007), the authors examined more directly the behaviors of domestic and wild rainbow trout strains released into lakes that differed in predation pressure from loons. They found that fish from the domestic strain responded less to the presence of predators, used riskier habitats, and had higher catch rates during the day than did fish from the wild strain. Thus, Biro et al. (2007: page 894) conclude that "greater overall activity and greater daytime use of deep and pelagic habitats by the domestic genotype should lead to greater growth (given sufficient food) but higher predation mortality". These and other studies comparing the behaviors, growth, and survival of domestic versus wild stocks of salmonid fishes provide some of the clearest evidence for the ecological consequences of behavioral trait variation under a growth–mortality tradeoff.

Other studies in natural or seminatural environments provide no support for the expected link between behavioral traits and a growth–mortality tradeoff. Adriaenssens and Johnsson

(2011*a*) found that shy trout (individuals with low exploration tendency in the laboratory) actually grew faster than bold trout when released into a natural stream. In a subsequent study, they found no significant effects of activity or exploration measured in the laboratory on growth in the field, and if we can assume that recovery following release into the wild is an indication of survival, they found that more active individuals had higher survival (Adriaenssens and Johnsson 2011*a*). In both of the above studies, fish were collected from the wild, assayed for behavioral traits in the laboratory, and then released back into the wild at a site near where they were collected.

Höjesjö et al. (2011) also found no association between boldness measured on juvenile brown trout (*Salmo trutta*) reared in the laboratory and their growth and survival when released into the river that was the source of the parental stock. However, only about 4% of the released fish were recovered, which raises the question of whether the missing fish died or simply moved away. The inability to distinguish mortality from disappearance in release–recovery experiments into the wild (especially when recovery rates are low) greatly hinders the ability to assess the impact of behavioral traits on fish survival (and growth). In Höjesjö et al. (2011), the authors note that juvenile brown trout in their study population are very stationary (seldom moving further than 200 m). Thus, recapture should provide a good estimate of survival in the wild.

Following the pace-of-life syndrome (Figure 3), we might expect individuals with bold, active, and asocial behavioral types to have a higher propensity to explore their environment and disperse greater distances, which could give them an advantage in terms of finding richer habitats. As stated earlier, Fraser et al. (2001) found that individual killifish that were bolder in the laboratory dispersed greater distances when released into the field, and that individuals that moved greater distances in the field had higher growth rates (in stream sections with predatory fish). Bolder individuals of European roach (*Rutilus rutilus*) also showed a greater propensity to migrate (lake to stream) than shy individuals (Chapman et al. 2011), more asocial mosquitofish (*Gambusia affinis*) moved further from their social conspecifics when simultaneously introduced to experimental streams (Cote et al. 2010), and dominant brown trout moved longer distances and had larger home ranges in a radio telemetry study (Höjesjö et al. 2007). An individual's dispersal tendency is likely related to the strength of its social network (the number of social interactions an individual has and the strength of those interactions), which itself has been shown to be affected by an individual's behavioral type. A study with guppies investigating the relationship between boldness and social networks found a correlation between an individual's boldness (measured by predator inspection and shoaling tendency in the laboratory) and aspects of its social network in natural shoals in the field. Bolder individuals were found to have fewer total social connections and the average strength of the connections they had were weaker than those of shyer individuals (Croft et al. 2009). Again, following predictions of the pace-of-life syndrome, we might expect such traits of increased activity and dispersal to carry with them higher mortality costs, if migrating or dispersing greater distances increases exposure to predators. However, no studies that we are aware of have assessed these mortality costs in the field.

Reproductive success

Behavioral traits have the potential to affect the reproductive success of adults, with both intra- and intersexual selection likely to be influenced by interindividual variation in behavioral traits (Schuett et al. 2010). Numerous laboratory studies with a variety of fish species document positive relationships between boldness, dominance, and reproductive success. For example, in zebrafish (*Danio rerio*) the boldest and most aggressive males fertilized more of a female's eggs than the shyer and less aggressive males (Ariyomo and Watt 2012), and in guppies (*Poecilia reticulata*) the females have been shown to prefer to mate with bolder males (boldness measured by predator-inspection behavior; Godin and Dugatkin 1996). However, field studies examining the relationship between behavioral traits and reproductive success are still quite rare. Our own studies with largemouth bass (*Micropterus salmoides*) demonstrate that boldness measured in the laboratory is positively correlated with nesting success in the field (i.e., bolder males were more successful at building nests and receiving eggs when stocked into ponds with females than less-bold males; N. Ballew and G. Mittelbach, unpublished data). These apparent fitness benefits of being bolder and more aggressive may be offset in other situations (see Discussion).

Behavioral traits also have the potential to affect offspring survival during periods of parental care. For example, in fish species that build and guard redds or nests (e.g., Salmonidae and Centrarchidae), bolder, more aggressive individuals are likely to outcompete conspecifics to secure better nesting sites, and bolder, more aggressive individuals may be better at guarding their nests from potential predators (McPhee and Quinn 1998). A series of studies by D.P. Philipp and colleagues, using largemouth bass lines that originated from a single wild population and were selected over multiple generations for increased or decreased vulnerability to recreational angling during the non-nesting season, show that the high angling vulnerability line and low angling vulnerability line differ in parental care behavior and reproductive success (Philipp et al. 2009). Male bass from the high vulnerability to angling line displayed increased parental care activity and higher reproductive success in ponds with nest predators (juvenile bluegill) compared to males from the low vulnerability line (Cooke et al. 2007; Sutter et al. 2012). It is difficult to say how the trait of angling vulnerability relates to more commonly studied behavioral traits such as boldness and aggression. However, our own studies with largemouth bass show that male bass assayed as more aggressive towards conspecifics in the laboratory are more diligent at defending their nests from potential brood predators (bluegill) in the field and have higher reproductive success (N. Ballew and G. Mittelbach, unpublished data).

Habitat use and foraging specialization

Behavioral traits related to boldness or aggression, foraging styles, or predator avoidance have the potential to affect habitat use and resource consumption. For example, a study with bullheads (*Cottus perifretum*) found that less aggressive individuals (as assayed in the laboratory) showed a greater propensity to use complex habitats (i.e., branch jams) in the field (Kobler et al. 2011). In the same study, there was no correlation between habitat use in the field and individual differences in activity level measured in the laboratory. Functional linkages may also exist between behavioral traits that confer greater feeding efficiency in particular habitats and morphological traits, leading to complex polymorphisms (Wolf and Weissing 2012). In one of the first studies to look for an association between morphological and behavioral traits, Ehlinger and Wilson (1988) examined a foraging polymorphism in populations of bluegill sunfish. They found that bluegill collected from a small, Michigan lake clustered into two behavioral (foraging mode) types when allowed to feed on open-water prey (zooplankton) and benthic prey (damselfly nymphs) in the laboratory (Figure 4). These behavioral differences between individuals were stable across a 20 week testing period.

Differences in foraging behaviors (hover duration) between bluegill individuals corresponded to differences in feeding rate; fish exhibiting relatively short hover durations were more successful at capturing zooplankton, whereas fish displaying longer hover durations where more successful at capturing damselfly nymphs. When Ehlinger and Wilson (1988) compared the morphologies of these two behavioral types, they found that morphology and behavior were tightly correlated. Fish classified as "vegetation" (benthic) morphological types exhibited long hover durations, whereas fish classified as "open-water" morphological types exhibited short hover durations. Subsequent morphological analyses of sunfish (bluegill and pumpkinseed, *Lepomis gibbosus*) collected from open-water and littoral habitats in lakes confirmed the subtle but repeatable morphological distinctions between phenotypes associated with pelagic and littoral habitats (Robinson et al. 1993, 2000).

Selection on morphological and behavioral traits that increase feeding efficiency on certain prey types or within certain habitats is likely to go hand-in-hand. Therefore, an important question is how much does habitat or foraging specialization lead to the development of animal personalities and the generation of behavioral trait variation within a population? Further, might there be subtle differences in functional morphology between behavioral types within populations that have generally gone undetected? The morphological differences between bluegill behavioral types in the study of Ehlinger and Wilson (1988) were not visible to the naked eye, but they were detectable with morphometic analyses (e.g., Ehlinger and Wilson 1988; Robinson et al. 1993; Robinson and Wilson 1996). Other fish species (e.g., threespine stickleback) show distinct resource polymorphisms in morphology, behavior, and resource use in some populations (McPhail 1993) but continuous variation in others (Robinson 2000). Thus, how much subtle variation in morphology and physiology is associated with interindividual behavioral variation in populations is unknown. When viewed in the holistic framework of individual specialization (Bolnick et al. 2011), it is clear that interindividual differences in behavioral types and personalities may be an expected outcome of natural selection acting on populations in a complex environment.

Conclusions

At the individual level, variation in boldness, activity, and dispersal is often hypothesized to result from a tradeoff between growth and mortality (i.e., bolder fish are more active and grow faster but suffer higher mortality). Despite wide-spread acceptance of a growth–mortality tradeoff as a likely driver of behavioral variation in fish (Stamps 2007), to date there is little empirical evidence from field studies and the results are mixed (Table 2). Studies of other taxa (mammals, birds) also provide only mixed support for the hypothesis that bolder (more exploratory) individuals take more risks to gain food but may suffer higher mortality as a result (e.g., Réale and Festa-Bianchet 2003; Dingemanse et al. 2004; Réale at al. 2007; Quinn et al. 2012). The strongest support for a growth–mortality tradeoff associated with differences in boldness or aggression in fish comes from studies of wild versus domesticated strains of trout and salmon (e.g., Biro et al. 2003*a*, 2003*b*, 2004; Sundström et al. 2004).

Boldness and dominance appear to be positively associated with increased mating success in the laboratory and in the field, and individuals more vulnerable to angling exhibit more persistent nest guarding behavior and greater reproductive success in pond studies with largemouth bass. Boldness and aggression during reproductive events are likely to have negative consequences for adult energetics, survival and future reproductive success, and there are many examples of the high cost of parental care in fishes (e.g., Dufresne et al. 1990; Gillooly and Baylis 1999; Steinhart et al. 2005). To date, however, no field studies have examined the full set of correlations between boldness and aggression during the breeding season, offspring survival, and adult current and future reproductive success. In the Discussion section, we consider how the costs and benefits of various behavioral traits may differ at the adult and juvenile life stages. Such life-history asynchrony in the impact of behavioral traits may provide an additional mechanism for the maintenance of variation in behavioral traits within a population.

Field studies also suggest that habitat heterogeneity and habitat-specific foraging success may maintain phenotypic polymorphisms in fish populations that include both behavioral and morphological traits. For example, in sunfish and sticklebacks, behaviors associated with increased foraging success in limnetic versus benthic habitats are also associated with variation in morphological traits (fin placement, body shape), resulting in complex polyphenisms (e.g., Ehlinger and Wilson 1988; Robinson and Wilson 1996; Robinson 2000; Weese et al. 2012). Thus, there are many opportunities in nature for consistent individual differences in behavior to arise when individuals can exploit different habitats and resources. Ecologists, behaviorists, and evolutionary biologists have joined together to highlight the importance of studying how environmental heterogeneity and habitat selection may generate and maintain intraspecific variation in populations, including variation in behavioral traits (Adriaenssens and Johnsson 2011*a*; Araújo et al. 2011; Bolnick et al. 2011; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012).

Consequences at the population, community, and ecosystem level

Intraspecific variation in behavioral traits can have numerous effects at the population, community, and ecosystem levels. Recent reviews have highlighted the potential for behavioral traits to affect population dynamics, predator–prey interactions, species diversity, and ecosystem primary productivity (Sih et al. 2012; Wolf and Weissing 2012). However, when compared to consequences at the individual level, far fewer theoretical expectations have been proposed for population- and community-level consequences of multiple behavioral types within traits. Similarly, few empirical studies have examined the population- and community-level consequences of behavioral trait variation, and (to our knowledge) no studies have been conducted in natural conditions. We summarize in Table 3 the limited number of laboratory and mesocosm studies on the topic, looking first at studies that have investigated ecological consequences of variation in behavioral traits at the population level, then moving to studies examining consequences to species interactions, and concluding with studies on consequences to ecosystem functioning.

Population-level consequences of behavioral trait variation have been investigated in terms of group performance and population dynamics. For example, shoal composition for boldness affects foraging success in guppies. Fish from mixed shoals were found to feed more than fish from all bold or all shy shoals, indicating that shoals containing a mixture of boldness behavioral types may outperform all bold and all shy shoals (Dyer et al. 2009). In a study with shoaling European perch, the frequency of different risk-taking behavioral types within the shoal was shown to affect overall shoal risk-taking behavior, and bold individuals had an especially large effect on shoal behavior (Magnhagen and Bunnefeld 2009). A study with threespine sticklebacks investigated the effect of a population's composition of bold and shy individuals on population social structure. Individuals were assayed for boldness in the laboratory (measured as hesitancy to feed after being startled) and then artificial populations were formed based on the boldness scores. Populations were composed of either all shy individuals, all bold individuals, or a mix of bold and shy individuals. The all shy populations had stronger social structures (measured as the average number of interactions for all individuals in the population) than the all bold populations. Additionally, the all shy populations were more cliquish, meaning social subgroups appeared to form (Pike et al. 2008). A field study with roach, while not directly investigating population-level consequences, found that bold roach had a higher propensity to migrate (lake to stream) than shy roach (Chapman et al. 2011). Roach practice partial migration (only a fraction of the population migrates). Thus, it is easy to see how the frequency of bold behavioral types could impact the proportion of the population that migrates.

In terms of species interactions, the consequences of behavioral types to predator–prey interactions, interspecific competition, and invasive ability have been investigated in a single study for each type of interaction. In threespine stickleback, boldness was found to affect prey risk, with bolder sticklebacks feeding more heavily on chironomid larvae in laboratory trials (Ioannou et al. 2008). In a study that investigated interspecific competition between two species of sticklebacks (threespine and ninespine), bold behavioral types were found to consume more prey in heterospecific competitive foraging trials, regardless of species (Webster et al. 2009). The only study to date on behavioral traits and invasiveness found no relationship between the level of boldness and invasiveness for four *Gambusia* species (Rehage and Sih 2004).

A key (but unanswered) question in the study of the population-level consequences of animal personalities is whether the amount of variance in behavioral traits within a population matters. That is, if populations share the same mean value for a given behavioral trait but possess different mixtures of behavioral types, is there an effect on population dynamics? If behavioral types are nonrandomly distributed in space, such that they select different foraging habitats (e.g., Wilson et al. 1993), occupy different positions within a shoal (e.g., Ward et al. 2004), or preferentially associate with like behavioral types, then the mixture of behavioral types in a population will matter.

No studies to our knowledge have directly measured the effects of individual variation in behavioral traits on ecosystem functioning. However, a few studies suggest the possibility of such a relationship. An outdoor mesocosm study using guppies taken from natural streams that differ in predation pressure found that mesocosms containing guppies from the high predation environment contained fewer benthic invertebrates and more algae after a 28 day period than mesocosms with guppies from the low predation pressure environment (Bassar et al. 2012). In total, 9 of 13 ecosystem variables measured by Bassar et al. showed significant river-of-origin effects. The authors attributed these differential effects on ecosystem functioning, in part, to differences in foraging behavior by guppies adapted to the different stream environments. However, Bassar et al. (2012) measured only one behavioral variable directly in their experiment (pecking at the substrate), and this behavior did not differ in fish from high predation and low predation sites. Thus, it is possible that other phenotypic differences between the populations caused the observed ecosystem effects. Harmon et al. (2009) and Des Roches et al. (2013) conducted similar types of mesocosm studies comparing the ecosystem effects of two threespine stickleback morphotypes (benthic and limnetic; McPhail 1993; Schluter 2000) and found significant effects of stickleback type on a variety of ecosystem functions. Again, behavioral variation was not specifically measured in these ecosystem studies, but previous work on stickleback morphotypes has shown pronounced differences in foraging behaviors between benthic and limnetic forms (e.g., Schluter 1993). Thus, while no studies that we are aware of definitively link variation in behavioral traits to effects on ecosystem functioning, the potential for such effects clearly exists and there is abundant opportunity for both theoretical and empirical studies that specifically examine the consequences of interindividual variation in behavioral types to communities and ecosystems.

Management Implications of Behavioral Types

When considering the ecological consequences of behavioral trait variation at the individual and population level, it is important to remember that these consequences are dependent on environmental context. For example, bold behavioral types may be less fit than shy behavioral types in an environment with high levels of predation, while the opposite may be true in an environment without predators. Therefore, human impacts on the environment, such as recreational angling, commercial fishing, and hatchery-reared stocking programs, are likely to affect the ecological consequences of consistent interindividual behavioral variation. For example, bolder individuals may be more vulnerable to angling, which could decrease their fitness relative to more timid individuals and result in the population becoming less bold on average. In the same way, hatchery rearing programs may select for certain behavioral types or selectively alter the way behavioral traits develop with ontogeny, resulting in hatchery stocks that differ genetically from wild populations (Huntingford 2004; Fraser 2008). In addition, habitat modifications (e.g., adding structure to streams or lakes and construction of fishways) may selectively benefit certain behavioral types that have a higher propensity to use these new habitats than others (e.g., Kobler et al. 2011). Unfortunately, at this time there is too little evidence to evaluate many of these management practices in relation to their use by (and effects on) different personality types. Thus, we focus on two areas where data are available: fishing and hatchery rearing. We summarize in Table 4 what is known about the management implications of behavioral trait variation and discuss these implications in more detail below. We note that many of the studies summarized in Table 4 have compared behavioral traits of different groups of fish (high versus low angling vulnerability; domestic versus wild stock), as opposed to comparing behavioral differences among individuals within a population.

Fishing

It is increasingly recognized that fishing pressure (recreational angling and commercial fishing) may alter the individual-level consequences of behavioral types, which in turn could affect natural selection and the evolution of population characteristics (Uusi-Heikkila et al. 2008; Philipp et al. 2014). For example, in nonfished populations of species that build and guard nests or redds, aggressive individuals may defend their nests more vigilantly from potential predators, increasing egg and larval survivorship. However, the introduction of angling could turn high nest guarding aggressiveness into a detriment if aggressive nest guarding individuals are more likely to be caught than their less aggressive counterparts (e.g., as shown by Cooke et al. 2007). Removal of the nest-guarding parent (even short-term removal by catch-and-release angling), can greatly increase the probability of egg and larval mortality (Siepker et al. 2007). Personality traits also have the potential to affect angling vulnerability outside of the nesting season (though the traits may be different).

The hypothesis that recreational angling can affect the relationship between behavior and reproductive success is supported by the previously mentioned study on reproductive success and parental care in two largemouth bass lines selected for different vulnerabilities to angling during the non-nesting season. Bass from the line selected for high vulnerability to angling showed increased levels of aggression towards potential nest predators and greater diligence of parental care compared with bass from the low vulnerability line. Importantly, the high vulnerability line was also found to have the highest reproductive potential (Sutter et al. 2012). Thus, angling can reduce reproductive success and lower total reproductive output not only in current generations but also impact selection for traits associated with nest guarding behavior, potentially leading to reduced reproductive success and lower total reproductive output in future generations as well. Furthermore, as angling almost certainly selects for traits that reduce angling vulnerability, high levels of recreational angling are likely to impact the ability of the population to provide recreational angling opportunities in the future. This result was recently documented in bass populations that have historically been exposed to different levels of angling intensity (Philipp et al. 2014).

As discussed earlier, bolder individuals in some fish species have been found to forage more actively and grow faster than their shyer counterparts. Angling, however, could alter the ecological consequences of boldness, shifting the balance towards shyer foragers if bold fish are captured more frequently. A recent study by Nannini et al. (2011) compared the foraging behaviors of individual largemouth bass obtained from the two artificially selected high and low angling vulnerability lines previously described. Contrary to expectations, fish from the low vulnerability line actually captured more prey (and attempted to capture more prey) than fish from the high vulnerability line. The low vulnerability line also had higher prey rejection rates and was more efficient at converting consumed prey into growth than the high vulnerability line (Nannini et al. 2011). While this study clearly demonstrates the potential for angling during the non-nesting season to have a selective effect on foraging behavior, it also cautions against jumping to conclusions about what behavioral types are likely to be associated with increased angling vulnerability. Only one study that we are aware of has explicitly tested the link between an individual's boldness and angling vulnerability during the non-nesting season. Wilson et al. (2011) found that more timid bluegill were more likely to be caught by angling than their bolder counterparts in a natural lake — again, a result opposite of expectations. These early studies highlight the need for more research on the link between fish behavioral traits and responses to angling during the nesting and non-nesting seasons at the individual and population levels. However, unlike the relatively recent focus on the evolutionary impacts of recreational angling, the impacts of commercial fishing on fish life histories, growth rates, and behaviors have been studied for a much longer time.

Several studies over the last two decades have documented that the selective harvest of large individuals by commercial fisheries leads to decreased growth rates in future generations, as well as a variety of other effects (e.g., Conover and Munch 2002; Hutchings 2004; Reznick and Ghalambor 2005; Walsh et al. 2006). More recently, it has been proposed that commercial harvest may also selectively target individuals based on their behavioral type and growth rate, irrespective of size. As Uusi-Heikkila et al. (2008; page 419) note, “fishing-induced selection directly acting on behavioral rather than on life-history traits per se can be expected in all fisheries that operate with passive gears such as trapping, angling, and gill-netting”. Biro and Post (2008) found exactly this result in an experimental study of rainbow trout in Canadian lakes, where faster-growing individuals were found to be more vulnerable to harvest by gill nets irrespective of their size. The authors attributed the greater vulnerability of faster-growing individuals to differences in their behaviors, as faster-growing fish were more active and bolder than their slower-growing, nonharvested counterparts (Biro and Post 2008). Even if commercial fishing does not select directly on behavioral traits, early findings from the pace-of-life syndrome suggest that direct selection on one trait, be it a behavioral trait like boldness or a life-history trait like growth rate, likely leads to indirect selection on a whole suit of correlated traits. These effects can have important impacts on species performance as well as community dynamics, making it essential that fisheries managers consider these effects when making management decisions.

Hatchery rearing

Hatchery rearing and fish stocking represent the opposite-side of the coin from fish harvest (i.e., they add rather than remove fish from a population). But, like selective harvest, hatchery programs designed to supplement the abundance of wild populations can impact behavioral variation and may have important ecological consequences. It is well known that fish raised in hatchery environments often perform poorly when stocked into the wild (Araki et al. 2008) and there is a long-standing debate on whether or not hatchery stocking demographically boosts wild populations. For this reason, there is a wealth of literature examining how hatchery selection, including both purposeful selection on desirable traits (such as increased growth rate) and unintentional selection resulting from rearing experiences, may affect fitness (see reviews by Huntingford 2004; Huntingford and Adams 2005; Araki et al. 2008; Fraser 2008). Changes in behavioral phenotypes due to domestication selection have been suggested to be a major factor contributing to the poor performance of hatchery-reared fish stocked into the wild (Fraser 2008).

Effects of artificial selection on behavior

Common garden studies demonstrate that offspring from hatchery-reared adults are often bolder and (or) more aggressive than those from wild stocks (e.g., Berejikian 1995; Einum and Fleming 1997), and laboratory studies comparing the behaviors of domestic and wild strains have found that domestic strains tend to be bolder and more aggressive than their wild counterparts (Budaev and Brown 2011; Conrad et al. 2011). For example, fry from sea-ranched brown trout parents initiated feeding sooner and bit at a novel object more often than fry from wild brown trout parents (Sundström et al. 2004). Domesticated strains of fishes often undergo selection aimed at increasing production traits such as rapid growth (Huntingford 2004). However, selection for increased growth rate can have unintentional consequences on behavioral trait variation, either by altering variation in the behavioral traits themselves (e.g., selecting for individuals that are more bold, aggressive, and active in their feeding behaviors) or selecting on metabolic traits that may cause individuals to act more boldly to fulfill their metabolic needs (see prior discussion on this topic).

Behavioral traits that confer an advantage to individuals in a hatchery environment may carry a cost in nature. The most obvious examples involve feeding behaviors in the absence or presence of predators. The work by Biro and colleagues discussed earlier nicely documents how domesticated trout strains grow faster but suffer higher mortality than wild fish when stocked into natural lakes with predators (Biro et al. 2006, 2007). Looking at foraging behavior in a different context, Adriaenssens and Johnsson (2011*b*) assayed hatchery-reared and wild-origin brown trout for cognitive tasks such as cryptic prey discovery and maze solving. They found that hatchery-reared trout had higher feeding rates than wild fish, but they did so with less accuracy. In the wild, lowered accuracy in foraging may incur energetically costly errors such as prey misidentification, whereas higher foraging rates may be advantageous in hatchery settings with consistent food dispersal (Adriaenssens and Johnsson 2011*b*).

The behavioral syndromes approach has been applied to determine whether selection on behaviors in the hatchery environment (e.g., propensity to feed in a predator-free environment) may influence the distribution of behaviors expressed in other environments (e.g., aggressiveness and boldness under predation risk). For example, Lee and Berejikian (2008) found that juvenile rockfish (*Sebastes auriculatis*) that fed at high rates in the absence of a predator also tended to feed at higher rates when a model predator was present. However, they found the behaviors of individuals were inconsistent across two assay periods (8–12 days apart), suggesting plastic responses and behavioral flexibility. In contrast, a study using rainbow trout found that individuals were consistent in their behaviors over 2–3 days and across safe and unsafe contexts (Conrad and Sih 2009). Behavioral flexibility may be important when determining whether selection on fast growth rates has unintentional consequences on associated behaviors. Selection may be limited if individuals are capable of changing behavior in response to their environment. Alternatively, if behaviors are tightly correlated and not plastic, selection for high growth rates is likely to also select for bold and aggressive individuals. Determining the degree to which individuals (or species) differ in their behavioral plasticity (e.g., Figure 2) and understanding how early development and rearing environment may affect levels of behavioral plasticity are important areas for future research (Dingemanse et al. 2010; Dingemanse and Wolf 2013).

The effects of hatchery rearing environments on behavior

The process of raising juvenile fish in hatchery environments has been shown to affect the cognitive pathways that influence behavior (Huntingford and Adams 2005). Enrichment strategies, such as providing physical structure, decreasing fish densities, feeding with live prey, and introducing simulated predator attacks, have been suggested to better prepare hatchery fish for stocking (Brown et al. 2003; Lee and Berejikian 2009; Brockmark and Johnsson 2010; Brockmark et al. 2010; see review in Huntingford 2004). All of these modifications to current hatchery conditions have been shown to benefit hatchery-reared fish in ways that could increase post-stocking survival. For example, brown trout assigned to “low” and “moderate” rearing density treatments showed superior skills when tested for their ability to locate food, identify novel prey as resources, and respond to predators (Brockmark et al. 2010). Additionally, when stocked into an experimental stream, fish that were reared in high densities showed decreased survival relative to individuals reared at low or medium density (Brockmark et al. 2010). Individuals that were reared with physical structure, fed natural prey, and subjected to simulated attacks showed decreased boldness relative to individuals raised under conventional hatchery conditions (Roberts et al. 2011). When comparing both the presence and stability of physical structure during rearing, Lee and Berejikian (2009) found that stable structures (the "unstable" treatment moved structure throughout the experiment) were important for individuals to assess current risk and display behaviors accordingly. Individuals reared with stable structure were more explorative without predators but showed reduced exploration under predation threat. Thus, there is accumulating evidence suggesting that the hatchery experience itself affects behaviors that can have important implications for fish stocked into the wild (e.g., impacts on habitat use, growth, and survival). Encouragingly, these studies demonstrate that carefully considering rearing experiences of hatchery fish and attempting to replicate natural environments could be a promising direction for hatcheries.

Although a number of studies have investigated individual behaviors of hatchery-reared fish in laboratory settings and others have compared how hatchery strains compare to wild strains, comparatively few studies have evaluated how the behaviors of hatchery-reared fish influence their fitness in natural environments. In one recent study, Moore et al. (2012) examined the field survival and migration rates of hatchery-reared steelhead trout compared to fish of wild origin across three years. As an additional component, the hatchery-reared fish were from two hatcheries that differed in rearing environment (stocking density and shape of tanks). Fish that were reared under lower densities and in circular tanks (thought to decrease effects of density), survived as well as wild steelhead. However, fish raised at higher densities and in rectangular raceways showed decreased survival relative to wild fish. Moore et al. (2012) also found that migration ranges for steelhead from both hatcheries were less than those of wild steelhead. This study suggests that changes in conventional hatchery rearing may facilitate behavioral flexibility and the development of cognitive skills fish need upon entering natural systems. However, to justify these changes, more field studies examining fitness of individuals subjected to various enrichment regimes are needed to understand how these changes affect survival in natural conditions.

Discussion

Fish have proven to be excellent model organisms for the study of animal personalities, providing some of the earliest demonstrations of behavioral trait variation in any species (e.g., Huntingford 1976, 1982; Ehlinger and Wilson 1988) and continuing today as one of the most studied taxonomic groups (Stamps 2007; Toms et al. 2010; Budaev and Brown 2011; Conrad et al. 2011). Still, as Wolf and Weissing (2012) and others have noted, relatively little attention has been paid to the ecological consequences of varying behavioral types. This comment applies to all species, not just fish, and is especially true of studies conducted in natural or seminatural environments. Our review has sought to summarize what ecologists, behaviorists, and managers know about the ecological consequences of behavioral types at the individual, population, and community or ecosystem levels, including implications for fisheries management and conservation. Important research foci in this area include the impacts of behavioral trait variation on individual growth and survival, nesting behaviors and reproductive success, habitat use, diet, and ontogenetic niche shifts, migration and dispersal, commercial and recreational fishing, and hatchery rearing for supplemental stocking. We discuss these different research foci below and suggest avenues for future research.

Examining behavioral type effects on growth and survival

The concept of a growth–mortality tradeoff is firmly entrenched in the ecological literature (e.g., Werner and Anholt 1993; Lima 1998), and a growth–mortality tradeoff provides much of the theoretical underpinning for the maintenance of behavioral variation in boldness in fish and other organisms (e.g., Stamps 2007). Thus, it is surprising that evidence from natural or seminatural environments documenting the effects of variation in boldness and (or) aggression on fish growth or survival is limited and is almost entirely based on comparisons of wild and domesticated stocks (e.g., Biro et al. 2003*a*, 2003*b*). This is not to question the reality of a growth–mortality tradeoff, or to doubt its relationship to fish behavioral traits, but only to note that much more work is needed to examine how individual variation in boldness affects growth and survival. Archard and Braithwaite (2010) discuss some of the challenges involved in studying the consequences of behavioral traits in wild animals. They note that a particularly hard nut to crack is the effect of behavioral type on the survival of free-living individuals, as the recovery of marked individuals is often very low (see Höjesjö et al. 2011 for an example with fish). The low recovery of marked individuals leaves us wondering, are missing individuals dead? Or, have they simply dispersed from the study area? Studies conducted in closed, seminatural environments (experimental ponds, outdoor raceways, or fenced reaches of streams), where all surviving individuals can be recovered post-stocking, can provide useful experimental systems for testing the growth–mortality tradeoff and its relationship to boldness variation in fishes.

Diet, habitat use, and ontogenetic niche shifts

Fish, like many other organisms, show pronounced changes in diet and habitat as they grow. For example, most piscivorous fish begin life feeding on zooplankton and benthic invertebrates before reaching a size where they can switch to feeding on other fish (Mittelbach and Persson 1998). Many other fishes occupy protective habitats (e.g., littoral zone vegetation) when small and vulnerable to predators, and then they shift to feeding in more open and riskier habitats when they reach sizes that are less vulnerable to predation (Werner et al. 1983; Werner and Hall 1988). These ontogenetic niche shifts have important consequences for population dynamics and species interactions (de Roos and Persson 2013). However, a completely unexplored question is — what role does personality play in determining the timing and extent of ontogenetic niche shifts, in fish or other organisms?

Studies documenting ontogenetic niche shifts in diet and habitat invariably show considerable variation amongst individuals (e.g., Mittelbach 1981; Werner and Hall 1988; Hjelm et al. 2000). How much of this individual variation in the timing and extent of ontogenetic niche shifts is due to differences in personality? Consider for example the study by Post (2003), who examined the factors contributing to the onset of piscivory in a cohort of young-of-year (YOY) largemouth bass. In bass and other piscivores, becoming piscivorous in the first summer of life greatly increases fitness by increasing the probably of surviving through the winter (Buijse and Houthuijzen 1992; Post et al. 1998). Post (2003) found that only the largest individuals in the YOY bass cohort from Paul Lake, Michigan, were able to successfully transition to feeding on YOY bluegill during their first summer. Further, all bass that grew large enough to become piscivorous in their first year were born early in the spring (Figure 5). Birth date, however, was by itself a poor predictor of either bass size in August or the propensity to shift to piscivory (i.e., many early-born bass did not get large enough to become piscivorous; Figure 5). Why did some early-born bass grow quickly and become piscivorous in their first summer, whereas others did not? Chance could of course played a role (e.g., some individuals may have been lucky enough to find and consume a large number of energetically rewarding prey early in life and get a jump on their less-fortunate fellows). However, it is tempting to speculate that differences in personality may contribute as well. In this case, the hypothesis would be that those early-born bass growing large enough to become piscivorous are individuals that are relatively bold and take greater risks to increase their feeding rate, either by being more active or by using riskier habitats. No studies to date have examined the impact of behavioral types on the timing of ontogenetic niche shifts. This seems a particularly ripe area for future research.

Consistency in behavioral traits across life stages and fitness tradeoffs

Numerous studies have documented consistency in behavioral traits or behavioral syndromes in fish and other organisms over relatively short time periods of days and weeks (Bell et al. 2009; Conrad et al. 2011). However, much less is known about consistency in behaviors across longer time periods or across life stages. Wilson and Godin (2009) found that shy–bold behavioral types showed differential consistency in bluegill sunfish over a 1–3 month period (measured in the field). However, Bell and Stamps (2004) and Edenbrow and Croft (2011) observed little differential consistency in individual behavioral types between life stages (e.g., juveniles to adults) in threespine sticklebacks and mangrove killifish (*Kryptolebias marmoratus*), respectively (measured in the laboratory). In a study of how behavioral consistency changed across ontogeny in an Africa cichlid (*Steatocranus casuarius*), Budaev et al. (1999) found that behaviors (response to a novel environment, a novel fish, and a mirror) were not consistent in juveniles (4 and 4.5 months of age), but they were consistent in adults (12–13.5 months). Bell and Stamps (2004) measured three types of behavior (activity, aggression toward a conspecific, and boldness under predation risk) at three developmental stages (juvenile, subadult, and adult) and found that individual behavioral types were not stable over ontogeny. In one stickleback population, the boldness-aggression behavioral syndrome was stable over ontogeny (showed structural consistency), but in another population it was not. Edenbrow and Croft (2011) also found that behavioral types of boldness and exploration were highly plastic during ontogeny, but that correlations between these two behaviors (i.e., bold types were more exploratory) were maintained from juvenile to adulthood (structural consistency; see also Schürch and Heg 2010; Chervet et al. 2011).

Studies with aquatic organisms other than fish have observed differential consistency in activity traits across life stages (e.g., tadpole to adult frog (*Rana ridibunda*; Wilson and Krause 2012*a*) and nymph to adult damselfly (*Lestes congener*; Brodin 2009)). In general, however, we know very little about the differential consistency of behavioral traits across life stages (e.g., juvenile to adult) or in individuals undergoing ontogenetic niche shifts (e.g., freshwater to marine, benthic to pelagic, and insectivorous to piscivorous). Clearly, such long-term differential consistency in behavioral traits has important implications for fitness and for the maintenance of variation in behavioral traits in populations (Adriaenssens and Johnsson 2013).

If behavioral traits have differential consistency only over short intervals, then the potential for fitness tradeoffs to contribute to the maintenance of variation in behavioral traits is rather limited. However, if behavioral traits are consistent across life stages or across ontogenetic niche shifts, then there are many more opportunities for tradeoffs to occur. For example, it is commonly assumed that boldness may have a positive effect on individual fitness through increased energy gain and (or) reproductive success, but it may have a negative effect due to reduced survival (Stamps 2007; Smith and Blumstein 2008). If fish that are relatively bold as juveniles are also relatively bold as adults, then boldness could positively affect fitness at the juvenile stage through higher feeding rates or energy gains and at the adult stage through greater reproductive output (per breeding event), but boldness could negatively affect fitness at the juvenile stage due to decreased survival, and (or) negatively affect reproductive success at the adult stage due to decreased survival during a reproductive event and (or) reduced probability of surviving to reproduce again. Thus, there are multiple ways in which boldness effects on growth, fecundity, and survival could trade off to affect lifetime fitness.

To date, studies that have investigated the relationship between behavioral traits and fitness have generally focused on a single measure of fitness (such as survival) at a specific life stage (Dingemanse and Réale 2005; Smith and Blumstein 2008). However, fitness tradeoffs across life stages or between different components of selection (sexual and viability) provide a potentially powerful mechanism for the maintenance of adaptive variation in behavioral traits. Moreover, if behavioral traits are heritable, the fitness consequences of animal personalities can extend across generations.

Heritability of behavioral traits and consequences arising from the release of domesticated fish

Although there are relatively few estimates of the heritability of behavioral traits in fishes, the evidence suggests that many behavioral traits are heritable, with levels of heritability that are generally lower than those for morphological traits, but roughly comparable to those measured for life-history traits (Bakker 1986; Stirling et al. 2002; Bell 2005; Brown et al. 2007; Dingemanse et al. 2009; Chervet et al. 2011). Additionally, researchers have been able to artificially select stocks of largemouth bass to express high and low vulnerability to recreational angling (Sutter et al. 2012), demonstrating again a strong genetic component to certain aspects of fish behavior. The heritability of behavioral traits has many important ecological implications, particularly with regard to the mixing of wild and domestic fish stocks.

Fisheries biologists have long been concerned with potential consequences of interbreeding between wild, hatchery-reared, or escaped farmed fish, particularly salmon, (e.g., Gross 1998). This concern has been largely focused on the detrimental effects of introducing nonadaptive life-history traits into wild stocks (causing, for example, a mismatch in the timing or orientation of migration and reproduction). However, interbreeding between wild and domestic stocks (e.g., farmed or hatchery-reared fish) could influence behavioral traits as well, with unknown consequences for wild fish. A number of studies comparing domestic and wild strains of salmon and trout show that hatchery-reared fish, or fish that have been genetically modified for faster growth, may be bolder, more aggressive, and (or) more risk-prone in their habitat use, resulting in higher growth rates but reduced survival in nature (e.g., Abrahams and Sutterlin 1999; Sundström et al. 2003, 2004; Biro et al. 2003*a*, 2003*b*, 2004, 2007; Sundt-Hansen et al. 2009). To the extent that behavioral traits are heritable and adaptive, the interbreeding of domestic and wild fish stocks has the clear potential to reduce the fitness of locally adapted stocks.

Commercial and recreational fishing may also select on heritable behavioral traits that have unanticipated evolutionary consequences. One possibility discussed earlier is the association between boldness and aggression in nest guarding behavior and angling vulnerability in largemouth bass. Male bass that are bolder and more aggressive are more diligent in guarding their young and have higher reproductive success (Cooke et al. 2007). However, bolder, more aggressive bass may also be more vulnerable to recreational angling (Suski and Philipp 2004). Thus, if boldness and aggression are heritable traits (see Bell 2005), then increased fishing pressure may lead to the evolution of reduced boldness and aggression in the population, a reduction in angling vulnerability, and the unwelcome consequence of a reduction in average reproductive success (Sutter et al. 2012). Here again, an understanding of the correlations among behaviors, combined with a knowledge of their heritabilities and their ecological consequences at different life stages, is an important avenue for future research for the effective management of fish stocks.

Summary

Interindividual variation in behavioral traits is now recognized to be an important feature of most animal populations, including fish. In this review, we have sought to highlight some of the mechanisms driving the evolution and maintenance of variation in behavioral traits within fish populations, as well as the ecological consequences of this variation. Field evidence for the ecological consequences of behavioral trait variation is still quite limited in any group of organisms. However, there is little doubt that behavioral trait variation plays an important role in the growth, survival, and reproductive success of individuals, as well as having potential impacts on species interactions and ecosystem functioning. It is also clear, however, that we must be careful not to jump to conclusions about the universality of the causes and consequences of behavioral trait variation (e.g., the growth–mortality tradeoff) without more evidence from nature. In fishes, behavioral trait variation has added implications for conservation, harvest, and resource management. Biologists and managers recognize the importance of environmental context to the evolution of behavioral traits and the role that behavioral variation among individuals and between populations (wild versus domestic stocks) may play in successful stocking and conservation. Again, more data from field studies, especially with tagged or marked individuals of known behavioral types, is crucial.

Acknowledgments

We thank Alison Bell, Pat Hanly, David Philipp, and two anonymous reviewers for their comments and suggestions, and for sharing unpublished manuscripts. Our special thanks to Judy Stamps for her many insightful comments on an early version of the manuscript. Grants from the U.S. National Science Foundation supported portions of this research (IOS-1210438 to M.K.K. and G.G.M.; IOS-1311455 to N.G.B. and G.G.M.). This is contribution number 1732 from the W.K. Kellogg Biological Station.

APPENDIX

Table 1. Glossary of terms related to animal personality.

Term	Definition
Behavior/ behavioral response	An individual's action or response at a given time in a given context
Behavioral/personality trait	A behavioral pattern that characterizes consistent individual differences in behavior in a given type of situation. For example, boldness characterizes consistent individual differences in behavior in situations that involve risk.
Behavioral/personality type	An individual's consistent response over a given period of time relative to other individuals for one or more behavioral traits. For example, an individual could be relatively bold or shy in situations that involve risk-taking.
Animal personality	A behavioral pattern that can describe multiple behavioral traits and the relationship between those traits across time (Caspi et al. 2005; Réale et al. 2007; Stamps and Groothuis 2010).
Differential consistency	Consistency between individuals in a particular behavior (or behavioral trait) across time (Stamps and Groothuis 2010).
Contextual generality	Consistency between individuals in behaviors measured in different contexts (e.g. activity in a safe, familiar environment and activity in an unfamiliar environment).
Structural consistency	Consistency across time in the correlation between two behaviors in a group (Stamps and Groothuis 2010).
Behavioral syndrome	Correlated suites of behaviors. Such correlations may occur within an individual (i.e., an individual's tendency to behave in a certain way may be correlated across contexts or over time). In this sense, behavioral syndromes and animal personalities describe similar phenomena. Behavioral syndrome also may describe a correlation between two or more behavioral traits between individuals in a population (e.g., boldness and aggression are commonly correlated when examined in a group of individuals; Sih and Bell 2008).
Pace-of-life syndrome	A suite of covarying behavioral, physiological, and life-history phenotypic traits arrayed on a continuum from "slow" to "fast" life-styles.

Note: Definitions refer to how terms are used in the current text and are not meant to resolve disputes in meaning. See also: Stamps and Groothuis (2010); Wolf and Weissing (2012).

Table 2. Summary of evidence for the ecological consequences of behavioral types in fishes at the individual level.

Consequence	Trait(s)	Result	Study	Study Method
Dispersal	Boldness	Bolder fish had a higher propensity to disperse.	Fraser et al. 2001	Field
	Sociability	More asocial fish had a higher tendency to disperse.	Cote et al. 2010	Lab
Social network	Boldness	Bolder fish had fewer total social connections and the average strength of the connections were weaker.	Croft et al. 2009	Field
Growth	Exploration and Aggressiveness	Slow explorers grew faster. Aggressiveness was not related to growth.	Adriaenssens and Johnsson 2011	Field
	Boldness	No correlation between boldness and growth.	Höjesjö et al. 2011	Field
	Boldness	Bolder fish grew faster.	Ward et al. 2004	Lab
Survival	Boldness	Bolder fish were preyed on more.	Dugatkin 1992	Lab
	Exploration and Aggressiveness	Neither exploration nor aggressiveness was related to survival.	Adriaenssens and Johnsson 2011	Field
	Exploration	More exploratory individuals had higher survival.	Adriaenssens and Johnsson 2012	Field
	Boldness	No correlation between boldness and survival.	Höjesjö et al. 2011	Field
	Activity, Boldness, and Exploration	More active, bold, and exploratory individuals survived longer with predators.	Smith and Blumstein 2010	Lab
Social status	Boldness	Bolder fish were more dominant.	Dahlbom et al. 2011	Lab
	Aggressiveness, Boldness, and Activity	Males that were more aggressive, bolder, and more active had higher positions in the dominance hierarchy.	McGhee and Travis 2010, Colleter and Brown 2011	Lab Lab
Reproduction	Boldness	Females chose bolder males as mates.	Godin and Dugatkin 1996	Lab

Table 2 (cont'd)

	Aggressiveness	Females chose low or moderately aggressive males, whereas highly aggressive males were rarely chosen.	Ward and Fitzgerald, 1987	Lab
	Boldness and Activity	Assortative mating based on personality type.	Budaev et al. 1999	Lab
	Boldness and Aggressiveness	Bolder and more aggressive male zebrafish fertilized more eggs.	Ariyomo and Watt 2012	Lab
Survival of offspring during parental care	Aggressiveness	Females that were more aggressive in guarding their nests from threats were found to stay on their nests longer.	McPhee and Quinn 1998	Field
Habitat use	Aggressiveness, Boldness, and Activity	More aggressive individuals were found more frequently in open water whereas less aggressive individuals were found in or near structured refuge. No relationship between boldness and aggression was found.	Kobler et al. 2011	Field
Migration	Boldness	Bolder fish were found to have a higher propensity to migrate.	Chapman et al. 2011	Field

Table 3. Summary of evidence for the ecological consequences of behavioral types in fishes at the population and community level.

Consequence	**Trait(s)**	**Result**	**Study**	**Study Method**
Population performance	Boldness	Full bold and mixed shoals approached food more than full shy shoals. Mixed shoals fed most.	Dyer et al. 2009	Lab
	Boldness	Shoal group behavior was impacted by the frequency of boldness types within the shoal. Bold individuals especially impacted shoal behavior.	Magnhagen and Bunnefeld 2009	Lab
Social structure	Boldness	Populations of all shy fish had stronger social structures and were more cliquish than populations of all bold fish.	Pike et al. 2008	Lab
Predator-prey	Boldness	Prey where more heavily preyed upon by bolder fish.	Ioannou et al. 2008	Lab
Interspecific competition	Boldness	Bolder behavioral types were found to consume more prey regardless of species in heterospecific competitive foraging trials.	Webster et al. 2009	Lab
Invasiveness	Boldness	No relationship between boldness and invasiveness.	Rehage and Sih 2004	Lab

Table 4. Summary of the effects of angling and hatchery rearing on behavioral traits in fishes and their ecological consequences.

Comparison	**Variables**	**Result**	**Study**	**Study Method**
Between individuals	Angling vulnerability with reproductive success, anti-predator aggression, and parental care	Male bass that have a high vulnerability to angling demonstrated greater anti-predator aggression and parental care and have higher reproductive success.	Sutter et al. 2012	Field
Between individuals	Angling vulnerability and foraging behavior	Bass that have a low vulnerability to angling foraged more frequently and also had more successful foraging attempts.	Nannini et al. 2011	Field
Between individuals	Boldness, angling vulnerability	Bolder bluegill were found to be less vulnerable to angling than more timid bluegill.	Wilson et al. 2011	Lab and Field
Domestic, wild strains	Boldness, dominance	Domestic fish initiated feeding sooner, but no difference in latency to approach novel object. Domestic fish bit at novel object more. All bold fish, regardless of origin were socially dominant.	Sundström et al. 2004	Lab
Hatchery vs wild rearing, low vs. high density hatcheries	Survival, Migration	Hatchery-reared fish showed similar survival over three years, but only for fish reared in lower densities and circular tanks. Migration ranges smaller for hatchery fish.	Moore et al. 2011	Field
Between individuals	Boldness	Behavioral syndromes found between behavior with and without predators, behaviors plastic during 16 weeks in hatchery environment.	Lee and Berejekian 2008	Lab
Availability and stability of structure	Exploration	Individuals reared with stable structure increased exploration without predators, but no difference in structure treatments with predators.	Lee and Berejikian 2009	Lab
Conventional vs. enriched rearing	Boldness	Individuals subjected to simulated predator attacks, physical structure, and natural prey showed decreased boldness than conventional rearing.	Roberts et al. 2011	Lab
Low, medium, conventional rearing densities	Survival, Exploration, Boldness	Fish from lower densities consumed more prey, increased predator response, located food in a maze faster, and increased survival in field.	Brockmark et al. 2010	Lab and Field

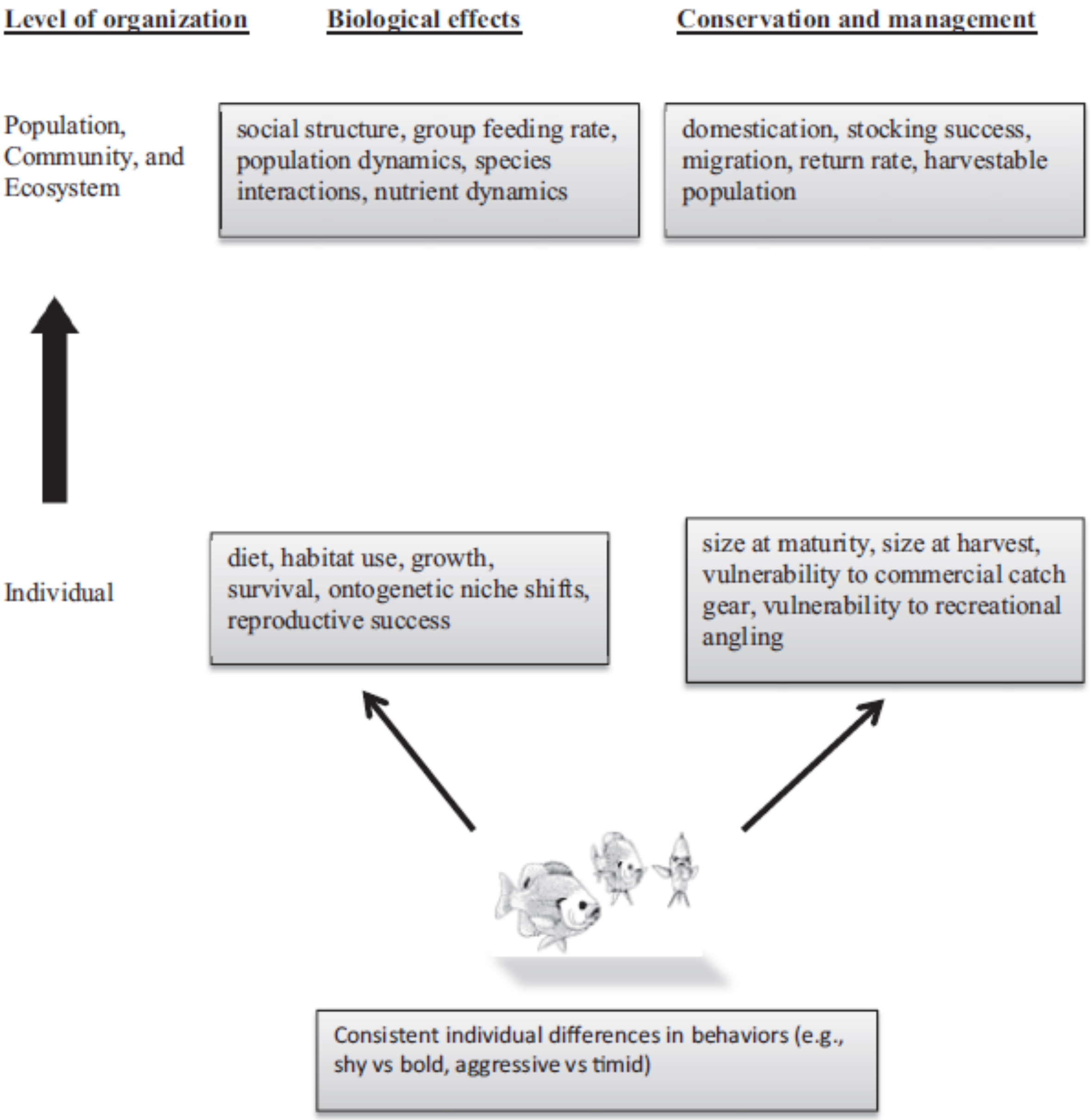

Figure 1. The consequences of variation in fish behavioral types may be expressed at different levels of ecological organization (from individuals to ecosystems) and have implications for conservation and management, as well as basic biology.

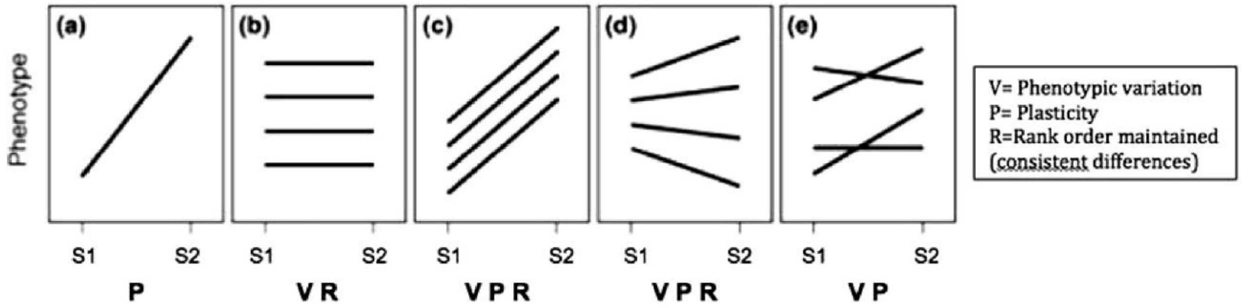

Figure 2. Five scenarios (a-f), each depicting the behavior of four individuals (solid horizontal lines) in two different situations (S1 and S2). In scenario a, all individuals display the same phenotypes in both S1 and S2 and there is plasticity between situations. In scenario b, there is phenotypic variability in situations S1 and S2 (equal between situations), no plasticity between situations, and consistent inter-individual differences in phenotype between situations. In scenario c, there is phenotypic variability in situations S1 and S2 (equal between situations), plasticity between situations, and consistent inter-individual differences in behavior. In scenario d, there is unequal phenotypic variation between situations S1 and S2 (S2 has much more), inter-individual differences in plasticity between situations (the individual with the smallest phenotype has high individual plasticity while the individual with the second smallest phenotype demonstrates a lower level of plasticity), and consistent inter-individual differences in behavior (perfect consistency in rank order but less in the raw values). In scenario e, there is phenotypic variability in situations S1 and S2, plasticity between situations, but individuals do not show consistent differences in behavior across situations because of differential directionality in responses. Modified from Dingemanse et al. (2010).

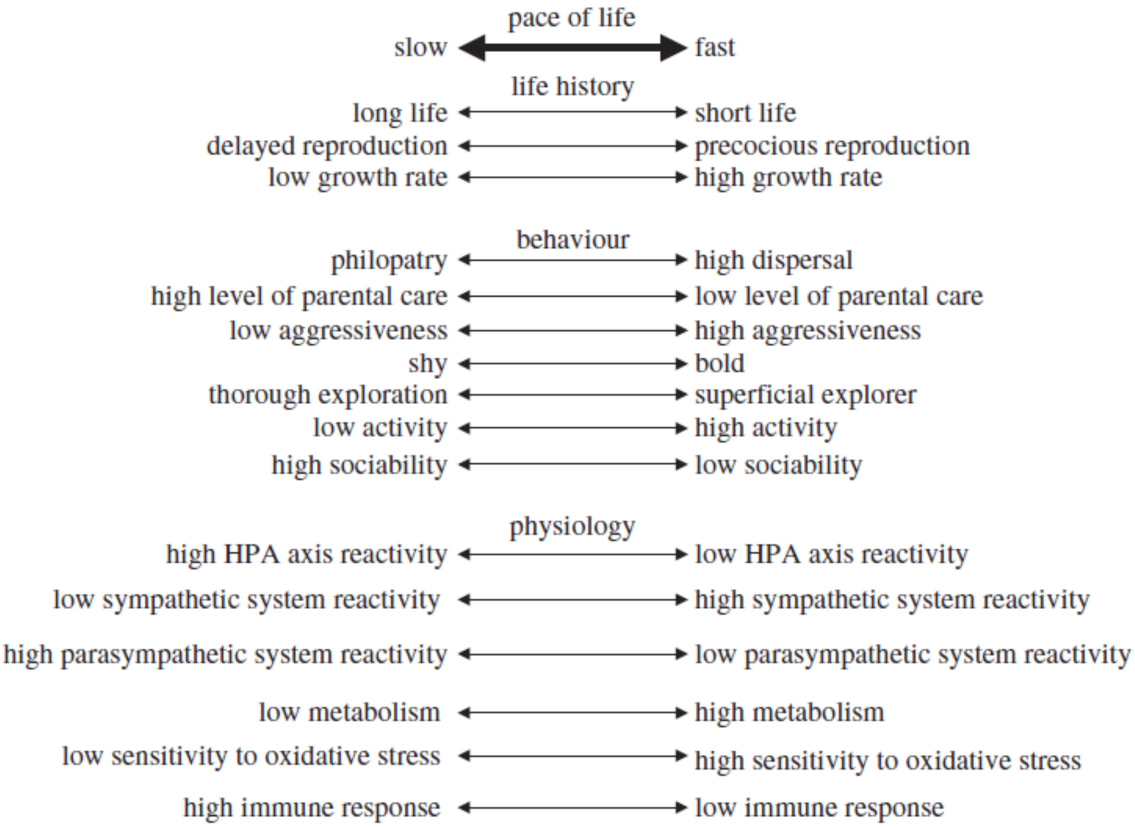

Figure 3. Representation of the potential integration of different phenotypic traits along a pace-of-life continuum. Double arrows illustrate presumed continuous variation in a trait, with traits categorized under life-history strategies, behavior, and physiology, and distributed along the pace-of-life continuum from “slow” to “fast”. From Réale et al. (2010).

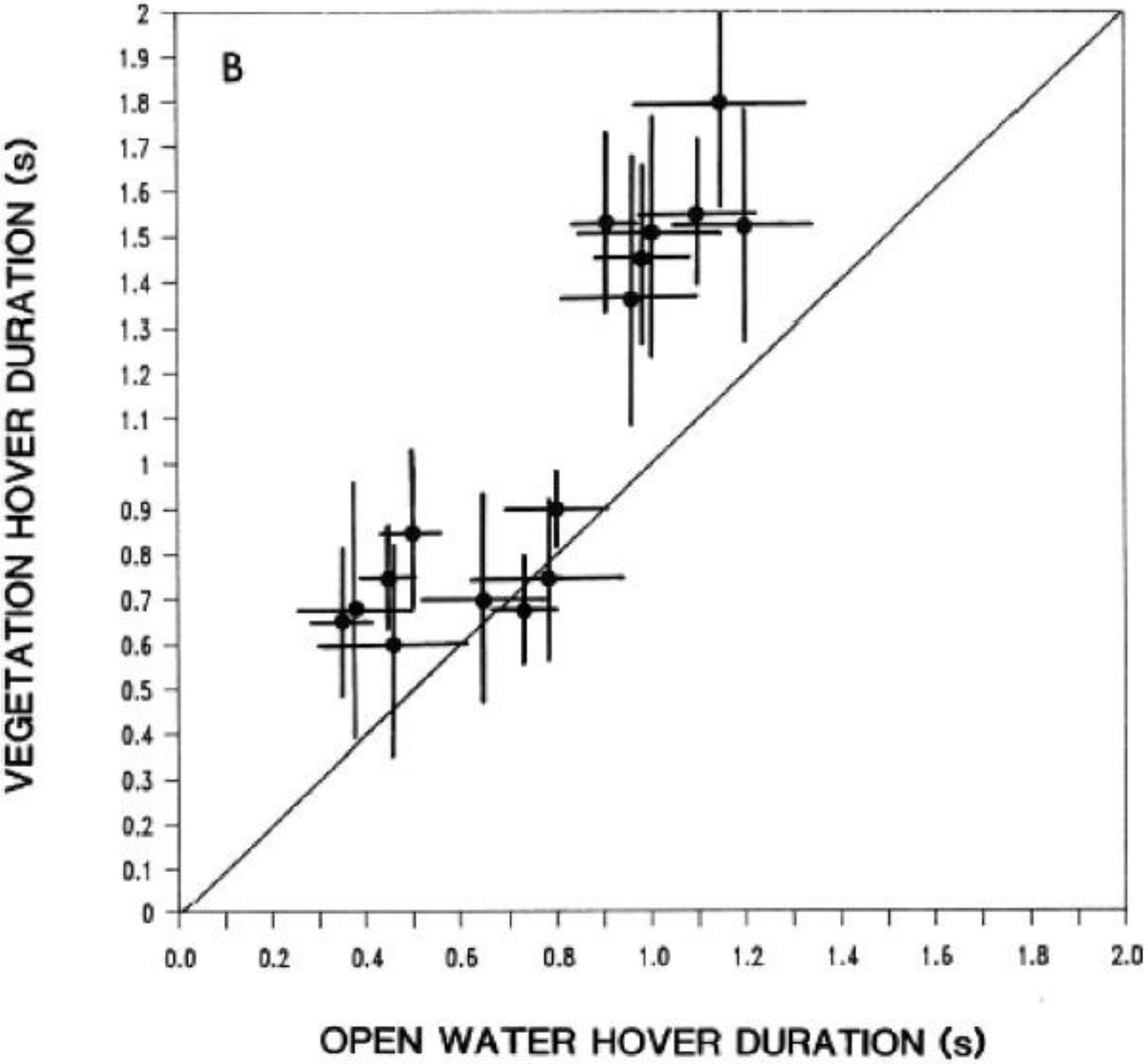

Figure 4. Bluegill sunfish display two distinct behavioral types when foraging on open-water versus vegetation-dwelling prey in the laboratory. Graphed are the foraging behaviors (hover duration when searching) used by individual bluegill when searching for damselfly nymphs in the vegetation and when searching for zooplankton (*Daphnia*) in the open-water habitat of aquaria. Each point represents the mean hover time (± SE) of six feeding trials for a given fish in each habitat. The diagonal line represents equal hover duration in each habitat. From Ehlinger and Wilson (1988).

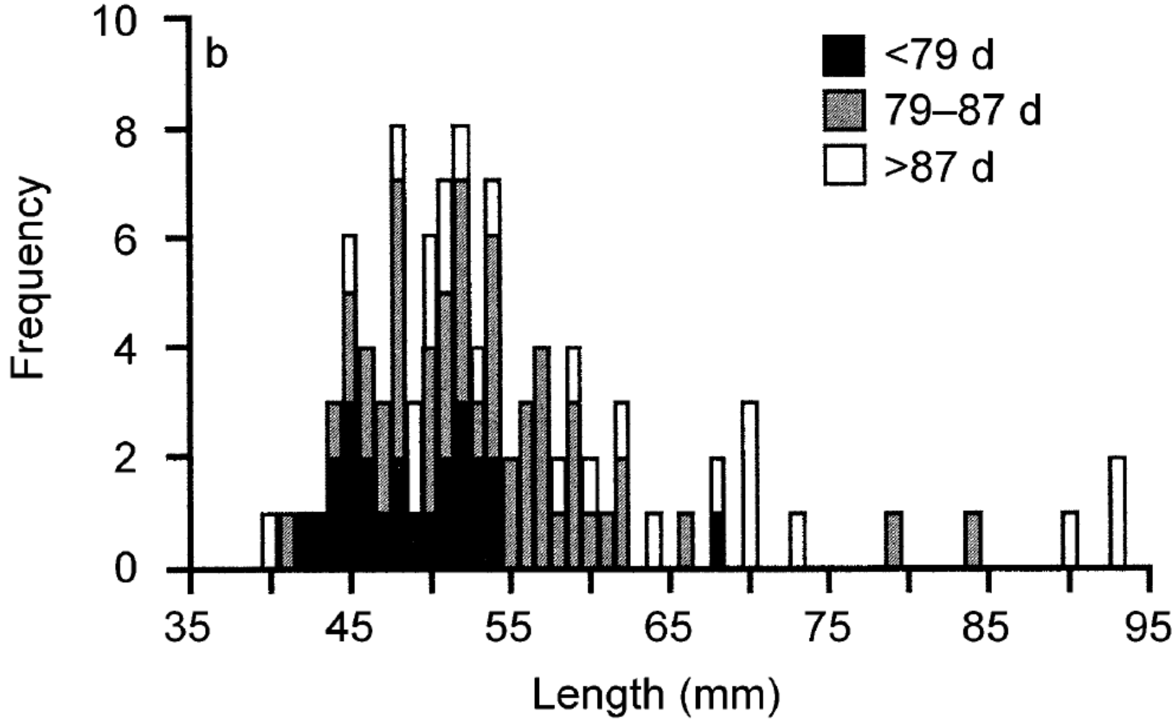

Figure 5. Length frequency distribution of young-of-year largemouth bass collected from Paul Lake, Michigan in late August 1994. Fish were categorized by age (age determined using daily rings from otoliths). Age categories represent roughly the youngest 25%, the central 50%, and the oldest 25% of fish collected. Only fish >85 mm in length become piscivorous during their first summer of life. From Post (2003).

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CHAPTER TWO

REPEATABILITY AND THE EFFECT OF SOCIAL CONTEXT ON FORAGING BOLDNESS IN JUVENILE BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS)*

Introduction

Within the past decade, studies examining inter-individual behavioral variation within a species have shown that many animal taxa show consistent differences in behavior, despite the presence of behavioral plasticity across contexts (Dall et al. 2004; Sih et al. 2004*a*,*b*; Dingemanse and Réale 2005; Bell and Sih 2007; Réale et al. 2007; Smith and Blumstein 2008; Dingemanse and Wolf 2013). Consistent individual differences in behavior within a population have been labeled as animal personalities (Dall et al. 2004), behavioral profiles (Groothuis and Trillmich 2011), temperaments (Réale et al. 2007), and coping styles (Koolhaas et al. 1999). A wide variety of taxa exhibit behavioral types associated with aggressiveness, boldness, and exploratory activity (Dingemanse and Réale 2005; Smith and Blumstein 2008), and in some cases these behavioral types have been shown to be heritable (Boake 1994; Stirling et al. 2002; Kölliker 2005; Van Oers et al. 2005; Sinn et al. 2006; Réale et al. 2007).

It is often assumed that behavior is more flexible than other phenotypic traits and that individuals can respond optimally to varying contexts within their environment. However, the fact that we are now documenting consistent responses at the individual level within and across various contexts for several behavioral types suggests that behavioral plasticity is often limited. The mechanisms for the maintenance of these inter-individual differences in behavior have not been determined, but if behavioral types have adaptive significance, there must be a genetic component that corresponds to some level of behavioral consistency. Therefore to fully understand and interpret the ecological and evolutionary implications of consistent behavioral variation, researchers must first assess the structure and repeatability of behavioral measures of interest.

Fish have proven to be popular organisms for the study of animal personalities (Toms et al. 2010; Conrad et al. 2011; Mittelbach et al. 2014). There is also ample evidence showing that in field conditions, fish often face tradeoffs in energy gain and predation risk (e.g., Werner and Gilliam 1984; Werner and Hall 1988; Byström et al. 2004). Such tradeoffs may allow for the development of multiple foraging strategies. For example, mortality risk in juvenile bluegill sunfish (*Lepomis macrochirus*) is higher in the open-water than in near-shore habitats (Werner and Hall 1988). However, foraging in the open-water on zooplankton (e.g., *Daphnia*) may yield greater energy gain than feeding on littoral-zone invertebrates (Mittelbach 1981; Werner and Hall 1988; Kjelvik 2015 Chapter 4). These tradeoffs set up opportunities for differences in the costs and benefits of behavioral traits such as boldness in juvenile bluegill. As a strategy to decrease this high vulnerability to predators as juveniles, bluegill commonly school in small groups (shoals) when foraging. These associations with conspecifics have the potential to introduce complex interactions between an individual's behavioral type, social dynamics, and environmental cues. It is important to understand how differences in behavior can lead to the formation of groups and in return how group composition can affect individual behaviors (Laskowski and Bell 2014) and foraging success. Explorative tendency is often correlated with boldness (Magnhagen 2007; Wolf et al. 2007), along with the propensity to consume novel food items (Wilson et al. 1993; Magnhagen and Staffan 2003; Sneddon 2003; Frost et al. 2007). Bolder fish are typically more proactive, less responsive to the behavior of conspecifics, and exhibit higher foraging initiative, whereas shier individuals are more reactive and attentive to the behavior of individuals around them (Wilson et al. 1993; Frost et al. 2007; Harcourt et al. 2009*a*; Pascual and Senar 2014). Shy fish tend to be followers, a behavior that assists the formation of shoals (Harcourt et al. 2009*a*). In group formation, both shy and bold three-spined stickleback (*Gasterosteus aculeatus*) prefer to associate with bolder individuals (Harcourt et al. 2009*b*), perhaps in recognition that bold shoals may be more successful than shy shoals (guppies, *Poecilia reticulate,* Dyer et al. 2009).

Here, I examine whether juvenile bluegill have consistent individual differences in foraging boldness that could align with varying adaptive social and foraging strategies. Specifically, differences in boldness may affect the previously described tradeoff in energy acquisition and predation risk. Additionally, differences in boldness may results in “leaders” and “followers” during foraging, where bold fish will make riskier decisions to obtain prey, while shy fish may watch bolder males and follow when foraging is considered safe. First, I document the behavioral structure of foraging boldness under the context of predation threat in laboratory experiments with several cohorts of bluegill sunfish over a four-year period. Then, I tested the temporal repeatability of foraging boldness when individuals were with familiar or unfamiliar conspecifics to examine whether repeatability was affected by group composition. I hypothesized that bluegill foraging with a familiar group would have higher levels of repeatability as the group composition and behavioral types would be maintained. In addition, I addressed how social dynamics might impact individual foraging success by examining whether focal individuals were more likely to consume prey if their conspecifics had also consumed prey during the assay.

Methods

Study population and collection

In 2008, a bluegill brood pond was established at the Kellogg Biological Station by stocking adult bluegill collected from nearby Wintergreen Lake (Kalamazoo County) and was maintained in the absence of piscivorous fish. Juvenile bluegill from this brood pond were used in a pilot study conducted in 2008 (young-of-year fish, YOY) and in a 2010 pond experiment (age 1+ fish). In 2011, I again collected adult bluegill from Wintergreen Lake and stocked them into one of the KBS experimental ponds to obtain juvenile bluegill for experiments conducted in 2011 (YOY fish), 2012 (age 1+ fish) and 2015 (age 1+ fish). In each of the years, juvenile bluegill were collected from the brood pond and then transported to large outdoor holding tanks (1000 L). Individuals were transferred to aquaria in the laboratory in batches of up to 160 fish and were kept at a maximum density of 20 fish per 110 L. Bluegill were fed daily with zooplankton collected from an on-site pond. Over the first three days in the lab, thawed commercial bloodworms (Chironomids) were gradually introduced to the diet.

Upon acclimation to the lab, each fish was given a unique identification code using visible implant elastomer, a pliable biocompatible polymer (Northwest Marine Technology, Goldsmith et al. 2003), injected subcutaneously. Four colors of elastomer were used and each fish was marked in four out of five possible locations to allow for thousands of potential combinations for individual identification. Prior to marking, fish were sedated by transferring them to a container with tricaine methanesulfonate (MS-222, 40 mg/L) in solution. I observed the fish for signs of moderate sedation (e.g., decreased fin and gill movement, lowered responses to stimuli, loss of equilibrium) and then gave each individual a series of assigned marks for identification. The fish were allowed to recover in a bucket of water and then transferred back to an aquarium after showing increased awareness and recovery from the sedative.

Behavioral assays

Behavioral assays were performed in a 120 x 30 x 50 cm (180 L) aquarium, filled to 42 cm water height and partitioned into three zones: a refuge area at one end of the aquarium, a predator housing area at the opposite end of the aquarium, and a larger open-water area in the middle. The predator housing area (25 cm in length) contained one piscivorous largemouth bass (*Micropterus salmoides*) and was separated from the open-water area by a nylon mesh partition, which allowed the test fish to perceive the bass through visual and chemical cues, along with any movement from the bass during the assay. The refuge area (30 cm in length) was located at the opposite end of the tank and contained artificial vegetation, the only physical structure in the tank. The middle 65 cm of the aquarium served as an open-water foraging environment located between the predator and the refuge. To track position and movement of the test fish during assays, the aquarium was marked every 10 cm along the bottom for reference.

In nature, juvenile bluegill school together in small groups. Therefore, bluegill behaviors were assayed in groups of three fish. This group size allowed two observers to monitor the behavior of the three fish simultaneously, while at the same time providing the fish with a normal social environment. Before the initiation of the assay, the observers selected which fish they would track and gave them the temporary label as Fish 1, 2, or 3 for data transcription purposes. Prior to the behavioral assays, bluegill were starved for 24 h to standardize hunger and increase motivation for foraging. At the start of each behavioral trial, three bluegill were haphazardly selected from their holding tank and placed into the refuge area of the test aquarium. The test fish were allowed to swim freely in the refuge and open-water areas for a 15 minute acclimation period. An opaque partition prevented the test fish from seeing the bass for the first 10 minutes of acclimation, reducing the amount of perceived threat to allow initial exploration of the foraging arena. The partition was then removed to visually expose the predator and five minutes later, the assay was initiated by dropping 10-15 bloodworms (Chironomid larvae) at the edge of the open-water foraging environment, proximal to the predator. When the bloodworms were dropped into the aquarium, most fish retreated back to the vegetation. If individuals started the assay outside of the refuge, their location within the open-water foraging area was noted. For the next ten minutes, behaviors of the three test fish were simultaneously recorded onto an audio recorder by two observers. The data from each foraging assay was later transcribed into JWatcher version 1.0, a free event recording program commonly used to develop ethograms and record animal behavior (Blumstein and Daniel 2007).

Quantifying boldness

The behavior of each test fish was quantified by recording when it left and re-entered the refuge, its activity within the open-water foraging area, the latency to consume bloodworm, and all distances it traveled while foraging. These behaviors were then used to construct ecologically relevant behavioral variables (see Table 5). Time spent in the refuge reflects hesitance to investigate novel environments, and was calculated by summing the total time an individual spent in the vegetated refuge zone. Individuals with higher values spent less time in the open-water foraging zone and are therefore interpreted as being less explorative of novel environments. Willingness to assume risk in order to obtain prey was measured as the latency to consume bloodworms and was quantified as the time elapsed from the beginning of the trial until the first bloodworm was consumed (recall that prey, bloodworms, were placed directly in front of the predator housing area). Lower values of this behavioral measure indicate increased risk-taking tendencies, in which individuals quickly left the refuge to forage. As an estimate of willingness to approach or investigate a risky encounter, I calculated the average distance traveled into the open-water foraging zone before turning back towards the refuge. Using the average position of each fish before it retreated to the refuge (changed orientation away from the predator and towards the refuge), risk-taking was measured through this variable in a way that was not dependent on whether test fish consumed bloodworm during the trial. The final behavioral measure recorded was the activity rate during the assay. Activity was calculated by summing the total distance traveled from retreats and advances (orientation change away from refuge and towards predator in open-water zone) during bouts in the open-water foraging zone.

I ran correlation tests on the four behavioral variables obtained from the foraging assays, and they consistently showed strong pairwise correlations in each year (Spearman rank test, $p < 0.001$, Table 6). Therefore, I conducted a principal components analysis (PCA) on the correlation matrix of the four behavioral traits to condense the variation into uncorrelated principal components, a common technique in studies of complex animal behaviors (e.g Martin and Réale 2008; Šíchová et al. 2014). The behaviors of a total of 323 (2010), 283 (2011), 512 (2012), and 206 (2015) bluegill were used in the Principal Components Analysis.

PCA scores in the current context represent behaviors measured for fish foraging under predation threat, which I interpret as a composite measure of foraging boldness. I confirmed the appropriateness of the data for PCA analysis using Bartlett’s sphericity test and the Kaiser-Meyer-Olkin measure of sampling adequacy (data considered adequately sampled if $KMO>0.7$, Budaev 2010). Component retention was tested with the Kaiser-Guttman rule of retaining components with an eigenvalue greater than one (Kaiser 1991).

Structural validity of behavioral assays

In 2015, I assessed the repeatability of the boldness scores obtained from the behavioral assays. In total, the behaviors of 207 juvenile bluegill (age 1+) were measured twice. The second round of behavioral assays occurred three days after the initial round. During the initial round, I alternated the assignment of each set of three individuals to one of two social treatments for the second round: “familiar” or “unfamiliar”. In the second round, the groups assigned to the “familiar” treatment were composed of the same three individuals used in the first round of behavioral assays. Individuals in groups assigned to the “unfamiliar” treatment were randomly chosen in groups of three for the second round (i.e., as was done in the initial round of assays). In the interim period between assays, bluegill were housed at a density of 20 fish per 110 L tank, with each tank consisting of individuals in either the familiar or unfamiliar treatment. These treatments allow a comparison of the consistency of behaviors with varying social dynamics (unfamiliar or familiar) while foraging under the context of simulated predation threat. Of the 207 fish assayed in 2015, 102 and 105 fish were assigned to the familiar and unfamiliar treatment, respectively. Separate PCA analyses were conducted for the two time periods in 2015 (round 1 and round 2). One fish was excluded from the 2015 repeatability analysis due to an issue that occurred with the recording program.

I also examined the impact of social context on the likelihood of obtaining prey by determining the probability that a focal fish was successful at foraging, given the foraging success of its two group members. To do this, I randomly selected one focal fish using a random number generator for each trial in the 2012 data. I then determined the number of group members that had consumed prey (bloodworm) during the foraging assay (0, 1, 2) for each focal fish and created a binary response variable (0, 1) for individual foraging success to indicate whether the focal fish had consumed at least one bloodworm during the assay.

Statistical analyses

All statistics were performed using R version 3.2.2 (R Core Team 2015). To assess the temporal repeatability of an individual's boldness score, I conducted an intra-class correlation analysis (ICCest function, ICC package, Wolak et al. 2012) on the standardized boldness scores from the first and second rounds of the behavioral assays. Intra-class correlation analysis coefficients represent the fraction of total variation that is attributable to the variance among and within individuals (Wolak et al. 2012). Boldness scores from Round 1 and Round 2 were scaled to have a mean of 0 and showed similar levels of variance, an assumption of the intra-class correlation coefficient analysis. Behaviors that are close to 1 have high precision and repeatability. In a meta-analysis examining the repeatability of behavioral traits in animals (Bell et al. 2009), the average intra-class correlation coefficient spanning all animal taxa was 0.37. In addition, I performed Spearman's Rank correlations on the boldness scores from the two time periods as another mechanism to determine whether individuals were maintaining similar ranks from round 1 to round 2. I conducted both of these analyses at two levels; 1) with all individuals included and 2) separated by social treatment (familiar and unfamiliar). To assess the impact of social context on individual foraging success, contingency tables were created from the 2012 data to examine success of the focal fish given the number of successful group members (0, 1, or 2). I used Pearson's Chi square test to examine whether conspecific success had an effect on the probability that a focal individual was successful.

Results

Behavioral structure

Prior to running PCAs for each year, I confirmed that the four behavioral variables measured in the laboratory assays (Table 5) were adequately sampled (KMO, 2010: 0.75, 2011: 0.77, 2012: 0.79, 2015 Round 1: 0.80, 2015 Round 2: 0.78; Budaev 2010) and that the correlation matrices were appropriate for PCA (Bartlett's sphericity 2010-2012, 2015: $p<0.001$; Budaev 2010). The four behaviors loaded evenly and with similar weights onto principal component axis-1 in and across each year (PC1; Table 7) and their loadings are readily interpretable. High PC1 scores represent individuals that spent more time in the open (away from the refuge), moved closer to the predator on average, initiated feeding on bloodworm sooner, and were more active. Low PC1 scores indicate more time in the refuge, shorter distances traveled away from the refuge, an increased latency to consume bloodworm (if at all), and low activity. PC1 explained 69% (2010), 73% (2011), 72% (2012), 72% (2015: Round 1) and 73% (2015: Round 2) of the variation observed in the four behavioral measures. Based on the Kaiser-Guttman rule, PC1 was the only component retained for further analysis in all years (Eigenvalues, $PC1>1.0$, $PC2< 1.0$ for 2010-2012, 2015; Kaiser 1991). From here on, I will use PC1 score as a composite measure of an individual's boldness in the context of foraging under predation threat.

Histograms of boldness scores across years consistently show right-skewed distributions, with the exception of 2015 Round 1 (Figure 6). Some fish never left the refuge during the behavioral assay, resulting in identical boldness scores, which produced a bounded limit on the lower range of observed scores. The degree of skewedness depends on both the number of these inactive individuals along with the level of variation seen within the cohort. For example, in

2011, there were fewer individuals that remained in the refuge for the entire trial, and fish that did forage showed more variation than in years such as 2010 and 2012 (Figure 6). In 2015 Round 1, the PCA scores resemble a normal distribution, indicating that few bluegill remained in the refuge for the entire duration of the behavioral assay (Figure 6). However, the second round of assays in 2015 showed the more-commonly observed right-skewed distribution of boldness scores (Figure 6).

Repeatability

Intra-class correlation coefficients and Spearman rank tests were significant across the first and second rounds of behavioral assays in 2015; however, the overall estimate of repeatability with both treatments combined was low (ICC estimate= 0.21, rho=0.18, Table 8). When separated by treatment (familiar vs. unfamiliar), the repeatability estimates for bluegill grouped with unfamiliar conspecifics was much higher (Unfamiliar: ICC=0.42, rho=0.38, Table 8). When group composition was maintained between rounds 1 and 2 (familiar treatment), repeatability was not significantly greater than 0.

Social context

The number of conspecifics consuming prey during the behavioral assay had a positive effect on the probability of a focal fish consuming prey in the 2012 foraging assays (2012: n=138, df=2, $\chi 2$ = 15.9, p<0.001). If no group mates consumed bloodworms, there was a lower probability the focal individual consumed (2012: probability=0.18, n=71). The probability of the focal fish consuming prey increases as 1 (2012: probability=0.40, n=48) or 2 (2012: probability=0.63, n=19) of its group members fed during the trial.

Discussion

The evidence for consistent inter-individual differences in behavior within populations is steadily growing (Dall et al. 2004; Dingemanse and Réale 2005; Bell and Sih 2007; Stamps and Groothuis 2010), and in a number of cases behavioral type variation has been linked to fitness (Dingemanse and Réale 2005; Smith and Blumstein 2008), with a heritable component (Boake 1994; Stirling et al. 2002; Kolliker 2005; Van Oers et al. 2005). To better understand the ecological and evolutionary impacts of different behavioral types, it is important to know the degree of variation and the strength of repeatability obtained from behavioral tests designed to capture personality traits. Consistency in observed behaviors is an important prerequisite to determine the adaptive significance of behavioral variation among individuals. Here, I examined the behavioral structure, repeatability, and effects of social dynamics on boldness in the context of foraging under predation threat in juvenile bluegill sunfish.

Results from behavioral assays conducted over four years showed that the distribution of boldness scores is not normally distributed and instead, most individuals were relatively shy. The right-skewed nature of the boldness scores is largely due to the fact that many of the fish did not leave the vegetated refuge area during the behavioral assay. In Michigan lakes and ponds, small bluegill are commonly restricted by predation risk to the vegetated littoral-zone (Werner and Hall 1988), where their risk of mortality by largemouth bass is lower compared to the open-water (Werner et al. 1983). Thus, it is perhaps not surprising that many of the fish remained in the vegetation refuge during the behavioral assay, even though they came from a population naïve to piscivorous predators. However, there was still considerable individual variation in boldness, and within each year there are several individuals that were much bolder then their conspecifics. Interestingly, Werner et al. (1983) found that individuals exhibited a great deal of variation in their use of open water and vegetated habitats in a field experiment that had treatments with and without bass predators.

The estimate for repeatability of boldness in the context of foraging under predation threat with unfamiliar social groupings (ICC=0.42) is in line with the average intra-class correlation coefficient Bell et al. (2009) found in their meta-analysis of 114 studies using 98 species (mean ICC=0.37). Approximately 50% of published studies examining the repeatability of behaviors had ICC estimates between 0.10-0.50 (Bell et al. 2009). The lower bound for acceptable repeatability estimates from test-retest studies has not yet been agreed upon within the field. However, studies with repeatability estimates as low as 0.14 (Neumann et al. 2013) have been used to document consistent behavioral differences in personality types. Although these temporal correlations of behaviors may be significant at low values, researchers should be cautious when interpreting the adaptive significance with low repeatability estimates. Given the broad range of accepted repeatability estimates, I consider the intra-class correlation coefficient estimates in my test-retest design to be moderately high for individuals that were in the unfamiliar treatment (ICC=042).

Although I found that foraging boldness measured in my behavioral assays was repeatable, there was substantial variation in the responses between the two rounds. Unlike many other studies examining repeatability and consistency of inter-individual behavioral variation, I assayed individuals in groups to accommodate natural social environments. The performance of individuals during the assays represents their behavioral type coupled with their reaction to the given social context. In a study with stickleback, Bell and Laskowski (2014) found no difference in the repeatability of behaviors amongst familiar (housed together) or unfamiliar groups in a foraging environment. Rather, the behavioral type of the individual measured in isolation in several contexts was the best predictor of their behavior in groups (Bell and Laskowski 2014). While behavioral traits may be plastic in response to social environments, there is evidence that rank order is generally maintained across isolated and group contexts, (Magnhagen and Bunnefeld 2009; Bell and Laskowski 2014). In this study, I tested whether being with familiar or unfamiliar conspecifics during a second round of assays affected the repeatability of behaviors observed in the first round. I found that when juvenile bluegill were assayed with the same individuals in both the first and second round, boldness scores were not repeatable. However, when individuals were again in a novel group composition, as they were in the first round, repeatability estimates increased dramatically (ICC=0.42, Table 8).

There was also a significant effect of conspecific consumption on a focal fish's behavior. A focal fish was more likely to consume prey during the behavioral assay as the number of group-mates consuming prey increased. This pattern could be a result of increased foraging rates given the propensity of individuals within the group to form shoals. Alternatively, individuals that are more reactive (typically shy fish) may be observing proactive (typically bold) individuals to obtain information on foraging success or predation threat (Koolhaas 1999; Harcourt et al. 2009*a,b*). Similarly, in a study examining the foraging success of groups (4 individuals) with varying social composition (4 bold, 4 shy, or 2 bold and 2 shy), Dyer et al. (2009) found shy fish followed bolder fish into the foraging zone. Interestingly, the mixed group composition was beneficial for both shy and bold individuals, with increased foraging success over groups containing one behavioral type.

Differences in behaviors based on social context seen here and in other studies undoubtedly increase the variability in observed behaviors. However, it is important to consider the interaction of individual behavioral types along with social dynamics for species that form shoals in nature. In natural populations, it is likely that shyer fish react to risky foraging environments by forming shoals with surrounding conspecifics, perhaps selectively associating with bolder individuals to benefit from their inherently explorative nature. The results from these experiments show that foraging boldness in juvenile bluegill sunfish is consistent and could have adaptive significance in natural settings. However, the differences in repeatability estimates based on social composition (e.g. familiar vs. unfamiliar) suggests that group dynamics may influence observed behaviors. More research documenting social dynamics and schooling behaviors of juvenile bluegill in field settings is necessary to determine the interplay of individual behavioral types and social associations.

APPENDIX

Table 5. Definition of behavioral variables used to determine boldness scores (PC1) of individual bluegill sunfish in the laboratory experiments.

Behavioral Variable	Behaviors Used	Definition
Latency to Consume Prey	Consume	The amount of time that had passed in the assay before the individual consumed bloodworm. A measure of willingness to approach the predator to obtain prey.
Activity	Retreats, Advances	Total activity in the open-water area during the assay. Calculated by summing the distance traveled from retreats and advances.
Average Distance Traveled	Retreats	Mean distance at which an individual retreated. Represents the average distance an individual will venture towards the predator before turning back towards the refuge.
Time in Refuge	Emerge, Vegetation	Total time spent within the vegetation as a measure of willingness to explore novel/risky environments. High values indicate less time exploring.

Table 6. Spearman's Rho correlation matrix for behavioral variables obtained from behavioral assays of juvenile bluegill sunfish at five time periods. 2015 Round 1 and Round 2 assays are from a test-retest scenario with the same set of individuals. ** indicate significant Spearman Rank correlations at p<0.001. See Table 5 for definitions of behavioral variables.

Behavioral Variable	C	D	A	R
2010 (n=323)				
Latency to Consume Prey (C)	1	-0.47**	-0.48**	0.47**
Average Distance Traveled (D)		1	0.87**	-0.77**
Activity (A)			1	-0.90**
Time in Refuge (R)				1
2011 (n=283)				
Latency to Consume Prey (C)	1	-0.64**	-0.68**	0.59**
Average Distance Traveled (D)		1	0.76**	-0.72**
Total Activity (A)			1	-0.92**
Time in Refuge (R)				1
2012 (n=512)				
Latency to Consume Prey (C)	1	-0.61**	-0.61**	0.65**
Average Distance Traveled (D)		1	0.75**	-0.82**
Total Activity (A)			1	-0.95**
Time in Refuge (R)				1
2015 Round 1 (n=206)				
Latency to Consume Prey (C)	1	-0.60**	-0.56**	0.64**
Average Distance Traveled (D)		1	0.72**	-0.73**
Total Activity (A)			1	-0.86**
Time in Refuge (R)				1
2015 Round 2 (n=206)				
Latency to Consume Prey (C)	1	-0.53**	-0.62**	0.65**
Average Distance Traveled (D)		1	0.83**	-0.79**
Total Activity (A)			1	-0.95**
Time in Refuge (R)				1

Table 7. Directionality and loading weights from the Principal Component Analysis on the four behavioral variables.

Behavioral Variable	2010	2011	2012	2015.1	2015.2
Latency to Consume Prey	-0.47	-0.47	-0.47	-0.46	-0.49
Average Distance Traveled	0.46	0.47	0.46	0.49	0.41
Activity	0.55	0.53	0.53	0.51	0.54
Time in Refuge	-0.52	-0.52	-0.53	-0.53	-0.55
Proportion Variance Explained	**0.69**	**0.73**	**0.72**	**0.72**	**0.73**

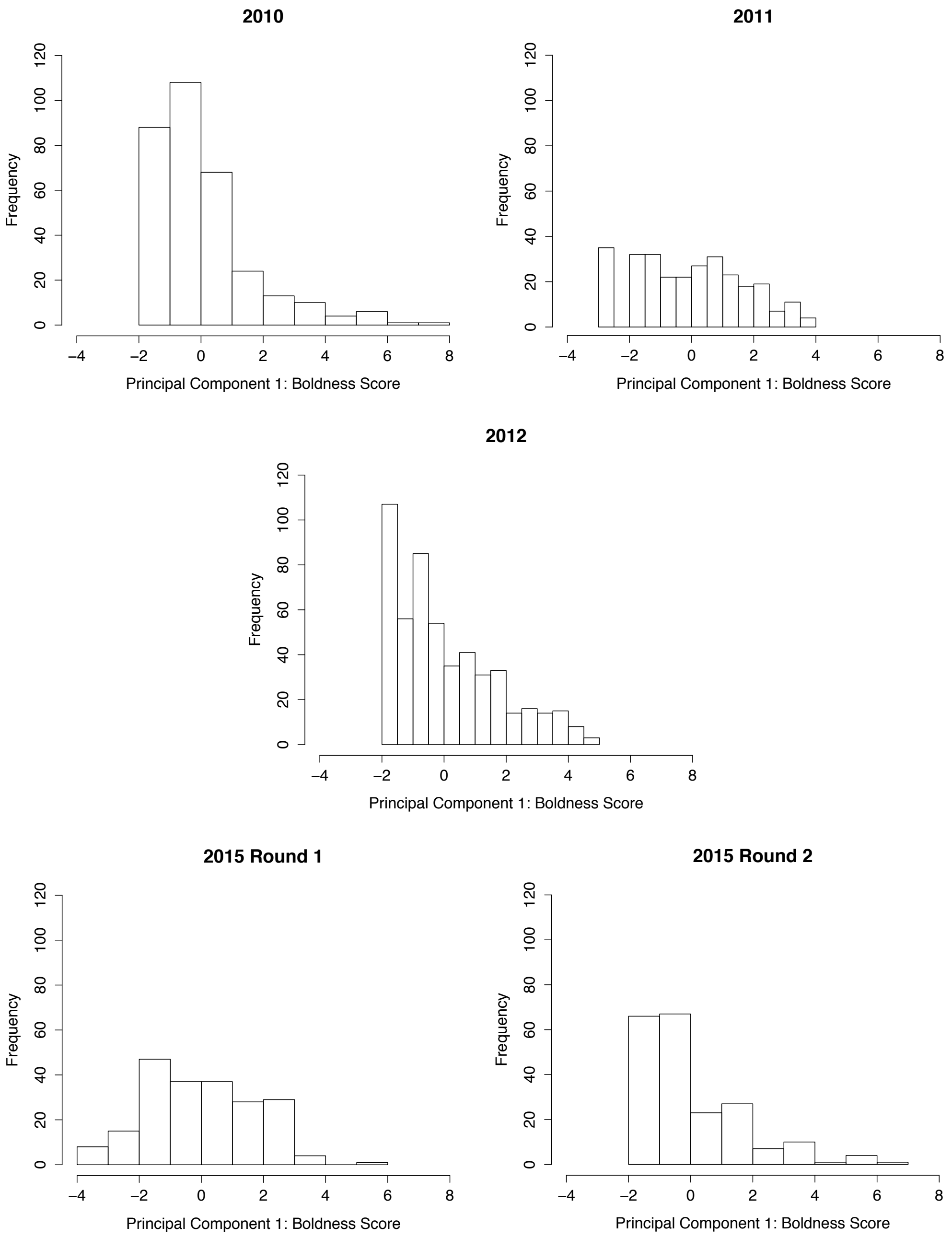

Figure 6. Histograms showing the frequencies of boldness scores (PC1) measured for juvenile bluegill sunfish in four different experiments. Separate PCA analyses were conducted at each time point on behaviors measured during a standard foraging boldness assay.

Table 8. Repeatability measures for boldness in the context of foraging under perceived predation threat. Intra-class correlation coefficient analysis (ICCest) and Spearman's Rank (Rho) tests were conducted at two levels; 1) all individuals included and 2) separated by social treatment (familiar and unfamiliar conspecifics).

	ICCest	Lower C.I.	Upper C.I.	Rho
2015: All Individuals (n=206)	**0.21***	0.07	0.33	**0.18***
Unfamiliar (n=104)	**0.42***	0.24	0.56	**0.38***
Familiar (n=102)	-0.01	-0.20	0.18	0.00

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CHAPTER THREE

ECOLOGICAL AND PHYSIOLOGICAL COSTS AND BENEFITS OF FISH BEHAVIORAL TYPES IN THE FIELD

Introduction

Inter-individual differences in behavioral types (often referred to as “animal personalities”) are common across taxa and knowledge of their contributions to ecological and evolutionary dynamics is rapidly expanding (Sih et al. 2004*a,b*; Réale et al. 2007; Smith and Blumstein 2008; Wolf and Weissing 2012; Mittelbach et al. 2014). However, surprisingly little research has examined the ecological consequences of these inter-individual behavioral differences in natural or semi-natural environments (Wolf and Weissing 2012; Mittelbach et al. 2014). Variability in behavioral types within a population may represent different adaptive strategies to complex environments (Wolf and McNamara 2012), and the maintenance of behavioral variation has often been ascribed to fitness tradeoffs (Dall et al. 2004; Stamps 2007; Wolf et al. 2007; Smith and Blumstein 2008). For example, an early study by Wilson et al. (1993) suggested that a foraging gain-predation risk tradeoff may help maintain behavioral type variation in boldness within a population of pumpkinseed sunfish (*Lepomis gibbosus*), in which individuals from the same population differed in their diet, habitat use, and parasite loads. Mangel and Stamps (2001) developed a simple theoretical model to show that a tradeoff between growth and survival can result in similar fitness for individuals with different growth and mortality rates. In this case, individuals with higher growth rates had decreased survival or lifetime longevity compared to individuals with slower growth rates (Mangel and Stamps 2001).

Thus, if foraging behaviors, such as boldness, affect individual growth and survival (e.g., bolder individuals have higher growth rates, but lower survival), behavioral variation could be maintained along a bold/shy continuum.

Fish have proven to be model organisms for the study of animal personalities (Toms et al. 2010; Conrad et al. 2011; Mittelbach et al. 2014). There also is ample evidence showing that fish respond to tradeoffs in energy gain and predation risk under field conditions (e.g., Werner and Gilliam 1984; Eklöv and Persson 1995; Biro et al. 2003). For example, mortality risk in juvenile bluegill sunfish (*Lepomis macrochirus*) is higher in the open-water than in near-shore habitats (Werner and Hall 1988), where vegetation structure provides protection from the bluegill's main predator, the largemouth bass (Micropterus salmoides). However, foraging in the open-water on zooplankton (e.g., *Daphnia*) may yield a greater energetic gain than feeding on littoral-zone invertebrates (Mittelbach 1981; Werner and Hall 1988; Kjelvik 2015 Chapter 4). Thus, juvenile bluegill (like many other fish species; de Roos and Persson 2013) may experience a foraging gain-predation risk tradeoff during their early life history, before reaching a size at which the risk from gape-limited predators declines. This conflict between taking risks to acquire more energy to grow and using refuge habitat to avoid predators creates an ideal system to explore the consequences of inter-individual variation in foraging boldness, or propensity to accept risks to find prey. Bolder individuals may forage in the risky, but more profitable open-water habitats. The increased energetic intake may lead to increased growth rates, allowing bolder individuals to escape predation by growing into a size refuge. Alternatively, shier individuals may remain in the refuge habitats as a mechanism for reducing the risk of predation. Although this may delay the ontogenetic transition into the profitable open-water habitat, chances of survival may be higher.

In this study, I tracked growth and survival of individually marked bluegill stocked into an experimental pond along with largemouth bass as predators to explore whether a predation risk-foraging gain tradeoff is present and capable of maintaining behavioral strategies across a boldness continuum. In a series of similarly executed experiments repeated over three years, I manipulated several factors: 1) bluegill age (young of year, 1+), 2) predator abundance, and 3) experimental duration, to examine how boldness (measured in the laboratory) affected juvenile bluegill growth and survival in experimental ponds. Consistent with the predation risk-foraging gain tradeoffs described above, I predicted that shier individuals would have higher survival rates, but lower growth over the course of the experiments compared to bold conspecifics.

The simultaneous examination of behavioral and physiological differences is recognized as a potentially powerful way to determine how these tradeoffs contribute to the maintenance of inter-individual variability (Biro and Stamps 2008; Careau and Garland 2012; Biro et al. 2014). The pace-of-life syndrome (POLS) hypothesis suggests that inter-individual variation in behavioral phenotypes is one component of a suite of covarying physiological, ecological, and life-history traits (Réale et al. 2010). Although support for specific components of the POLS hypothesis has been mixed (Careau et al. 2010; Galliard et al. 2013; Niemelä et al. 2013), it provides a useful conceptual framework to examine the causes and consequences of behavioral types. To examinepotential tradeoffs and differential energetic investment into physiological processes, I compared fin regrowth after clipping as a proxy of investment into injury repair. In alignment with the POLS hypothesis, I predicted that shier fish would invest more energy into injury repair than bolder fish.

Methods

Study population and collection

In 2008, a bluegill brood pond was established at the Kellogg Biological Station by stocking adult bluegill collected from nearby Wintergreen Lake (Kalamazoo County) and was maintained in the absence of piscivorous fish. Juvenile bluegill from this brood pond were used in a pilot study conducted in 2008 (young-of-year, YOY) and in a 2010 pond experiment (age 1+ fish). In 2011, I again collected adult bluegill from Wintergreen Lake and stocked them into one of the KBS experimental ponds to obtain juvenile bluegill for experiments conducted in 2011 (YOY fish) and 2012 (1+ fish). In each of the years, juvenile bluegill were collected and then transported to large outdoor holding tanks (1000 L). Individuals were transferred to aquaria in the laboratory in batches of up to 160 fish and were kept at a maximum density of 20 fish per 110 L. Bluegill were fed daily with zooplankton collected from an on-site pond. Over the first three days in the lab, thawed commercial bloodworms (Chironomids) used in the foraging assays, were gradually introduced to the diet to increase bluegill familiarity of this prey type.

Upon acclimation to the lab, each fish was given a unique identification code using visible implant elastomer, a pliable biocompatible polymer (Northwest Marine Technology, Goldsmith et al. 2003), injected subcutaneously. Four colors of elastomer were used and each fish was marked in four out of five possible locations to allow for thousands of potential combinations for individual identification. Prior to marking, fish were sedated by transferring them to a container with tricaine methanesulfonate (MS-222, 40 mg/L) in solution. I observed the fish for signs of moderate sedation (e.g., decreased fin and gill movement, lowered responses to stimuli, loss of equilibrium) and then gave each individual a series of assigned marks for identification. The fish were allowed to recover in a bucket and then transferred back to an aquarium after showing increased awareness and recovery from the sedative.

Behavioral assays

Behavioral assays were performed in a 120 x 30 x 50 cm (180 L) aquarium, filled to 42 cm water height and partitioned into three zones: a refuge area at one end of the aquarium, a predator housing area at the opposite end of the aquarium, and a larger open-water area in the middle. The predator housing area (25 cm in length) contained one piscivorous largemouth bass (*Micropterus salmoides*) and was separated from the open-water area by a nylon mesh partition, which allowed the test fish to perceive the bass through visual and chemical cues during the assay. The refuge area (30 cm in length) was located at the opposite end of the tank and contained artificial vegetation, the only physical structure in the tank. The middle 65 cm of the aquarium served as an open-water foraging environment located between the predator and the refuge. To track position and movement of the test fish during assays, the aquarium was marked every 10 cm along the bottom for reference.

In nature, juvenile bluegill school together in small groups. Therefore, bluegill behaviors were assayed in groups of three fish. This group size allowed two observers to monitor the behavior of each fish, while at the same time providing the fish with a normal social environment. Prior to the behavioral assays, bluegill were starved for 24 h to standardize hunger and increase motivation for foraging. At the start of each behavioral trial, three bluegill were selected from their holding tank and placed into the refuge area of the test aquarium. The test fish were allowed to swim freely in the refuge and open-water areas for a 15 minute acclimation period. An opaque partition prevented the test fish from seeing the bass for the first 10 minutes of acclimation, reducing the amount of perceived threat to allow initial exploration of the foraging arena. The partition was then removed to visually expose the predator and five minutes later, the assay was initiated by dropping 10-15 bloodworms (Chironomid larvae) at the edge of the open-water foraging environment, proximal to the predator. When the bloodworms were dropped into the aquarium, most fish retreated back to the vegetation. If individuals started the assay outside of the refuge, their location within the open-water foraging area was noted. For the next ten minutes, behaviors of the three test fish were simultaneously recorded onto an audio recorder by two observers. The data from each foraging assay was later transcribed into JWatcher version 1.0, a free event recording program commonly used to develop ethograms and record animal behavior (Blumstein and Daniel 2007).

Quantifying boldness

The behavior of each test fish was quantified by recording when it left and re-entered the refuge, its activity within the open-water foraging area, the latency to consume bloodworm, and all distances it traveled while foraging. These behaviors were then used to construct ecologically relevant behavioral variables (see Table 9). Time spent in the refuge reflects hesitance to investigate novel environments, and was calculated by summing the total time an individual spent in the vegetated refuge zone. Individuals with higher values spent less time in the open-water foraging zone and are therefore interpreted as being less explorative of novel environments. Willingness to assume risk in order to obtain prey was measured as the latency to consume bloodworms and was quantified as the time elapsed from the beginning of the trial until the first bloodworm was consumed (recall that prey, bloodworms, were placed directly in front of the predator housing area). Lower values of this behavioral measure indicate increased risk-taking tendencies, where individuals quickly left the refuge to forage. As an estimate of willingness to approach or investigate a risky encounter, I calculated the average distance traveled into the open-water foraging zone before turning back towards the refuge was calculated. Using the position of each fish before it retreated to the refuge (changed orientation away from the predator and towards the refuge), risk-taking was measured in a way that was not dependent on whether test fish consumed bloodworm during the trial. The final behavioral measure recorded was the activity rate during the assay. Activity was calculated by summing the total distance traveled from retreats and advances (orientation change away from refuge and towards predator in open-water zone) during bouts in the open-water foraging zone.

The four behavioral variables obtained from the foraging assays showed strong pairwise correlations (Spearman rank test, $p < 0.001$). Therefore, I conducted a principal components analysis (PCA) on the correlation matrix of the four behavioral traits to condense the variation into uncorrelated principal components, a common technique in studies of complex animal behaviors (e.g., Martin and Réale 2008; Šíchová et al. 2014). The data from a total of 323 (2010), 283 (2011), and 254 (2012) were used in the Principal Components Analysis to obtain individual measures of PC1. PCA scores in the current context represent behaviors measured for fish foraging under predation threat, which I interpret as a composite measure of foraging boldness. I confirmed the appropriateness of the data for PCA analysis using Bartlett's sphericity test and the Kaiser-Meyer-Olkin measure of sampling adequacy (data considered adequately sampled if KMO>0.7, Budaev 2010) Component retention was tested with the Kaiser-Guttman rule of retaining components with an eigenvalue greater than one (Kaiser 1991).

Pond experiments

I conducted a pilot study in the summer of 2008, in which I ran young-of-year (YOY) bluegill through behavioral assays as described above. However, in this pilot experiment I did not individually mark fish and instead classified bluegill into two general behavioral types. "Bold" individuals were fish that ventured to the predator side of the tank and consumed at least one bloodworm during the behavioral assay, whereas "shy" individuals consumed no prey during the assay. Immediately following the assays, I clipped either the right or left pelvic fin to distinguish the two behavioral types (right fin clip = bold, left fin clip = shy). I then stocked 100 bold and 100 shy fin-clipped bluegill along with two largemouth bass as predators into one of the ponds (30 m diameter, 2 m deep) at the Kellogg Biological Station Experimental Pond Facility. The pond provided a natural environment with two distinct habitats; emergent vegetation (cattails, *Typha*) around the pond perimeter and open water in the pond center. Zooplankton (e.g., *Daphnia*) were the dominant bluegill prey in the open-water of the pond, whereas aquatic insect larvae (Ephemeroptera, Odonata, Trichoptera) were abundant prey for bluegill in the near-shore vegetation. The experiment ran for 60 days. At the end of the experiment, the pond was drained and all surviving juvenile bluegill were collected, identified as either bold or shy, and measured for total length and wet mass.

In September of 2010, I stocked 323 juvenile bluegill (year 1+) that had been individually marked, assayed for boldness (PCA score), and measured for total length (mean initial length = 52 mm, range = 35-76 mm) along with 2 largemouth bass (Table 10). I drained the pond after 60 days and collected all surviving bluegill, which were then identified and measured for total length and wet mass. In 2011 and 2012, I repeated the experiments, but changed experimental conditions slightly to increase predation risk and (potentially) decrease survival (see Table 10). In 2011, I stocked 283 assayed and individually marked young-of-year (YOY) bluegill (mean total initial length = 48 mm, range = 31-82 mm, mean initial wet mass = 1.6 g, range 0.3-6.8) into the same pond as 2010 in September. I extended the duration of the experiment to eight months and stocked an additional largemouth bass (three total) to increase predation riskthroughout the experiment. In 2012, I increased predation pressure by stocking nine largemouth bass, a threefold increase over the three I had placed in the pond in 2011. I stocked 254 year 1+ bluegill (mean total initial length = 44 mm, range = 31-73 mm, mean initial wet mass=0.96, range= 0.2-5.2) into the same pond as the previous years. The 2012 experiment began in August and ran for 60 days. As in 2010, individuals were identified and total length and wet mass were measured at the conclusion of the 2011 and 2012 experiments.

Fin regeneration

At the end of the 2008 pilot study, I noted that the fin-clipped fish had regrown their fins to varying extents. To assess energetic investment into fin repair, I compared the length of the clipped and unclipped pelvic fins. The proportion of clipped fin that was regrown was calculated by dividing the regenerated (clipped) fin length by the full (unclipped) fin length, both measured at the longest axis of the fins. I found that shy fish had regrown more of their fins than bold fish (bold fish mean fraction regrowth = 0.686, 1 SE = 0.030, n=66; shy fish mean fraction regrowth = 0.841, 1 SE = 0.014, n=74; t-test, df=91.83, t=-4.67, $p < 0.001$). To better test the hypothesis that foraging boldness affects the energy individuals invest into fin regeneration, I removed a consistent fraction of the left pelvic fin from all individuals using scissors prior to stocking the 2011 pond experiment. The fish were clipped blind to their boldness scores to eliminate potential bias. Upon termination of the pond experiment, I again measured the clipped and unclipped fins and calculated the proportion regenerated for each fish.

Statistical analyses

All statistics were performed using R version 3.2.2 (R Core Team 2015). For the 2008 pilot experiment, I used the Mann-Whitney U test to compare the distribution of final lengths of surviving bold and shy individuals. For the three experiments conducted in 2010, 2011, and 2012, I analyzed growth and survivorship separately for each year using the statistical methods described below. I first standardized initial length and boldness (group-mean=0, S.D.=1) to facilitate comparison of parameter estimates within and across years. Growth was calculated as the difference between final and initial total lengths for each surviving individual. I used multiple linear regression to examine the effect of initial length and boldness score (PC1) on growth observed during the experiment. Interaction terms were included to account for differences in the relationship between growth and initial length due to variation in boldness scores. I tested for collinearity in the models by calculating a correlation coefficient of determination (R^2) and variance inflation factors (VIF) for boldness, initial length, and the interaction.

To examine whether survival was affected by boldness, I used generalized linear models (family=binomial, link= logit) with survival (0, 1) as the response variable with standardized initial lengths and boldness scores along with the associated interaction as predictor variables. To test whether boldness affected fin regeneration in the 2011 experiment (where fish were fin clipped, individually marked, and scored for boldness along the continuous PCA axis), I used beta regression models (betareg function, betareg package; Cribari-Neto and Zeileis 2010) to accommodate the bounded proportion data as a dependent variable. I included standardized individual growth to assess whether a tradeoff in energetic investment (fin repair vs. body growth) was present. The beta regression models examined the effects of standardized boldness and growth, along with the interaction, on the proportion of fin regenerated. I tested for collinearity of growth and PCA score by calculating a correlation coefficient of determination (R^2) and then using variance inflation factors (VIF) for growth, PCA score, and the interaction.

Results

Boldness scores

Prior to running PCAs for each year, I confirmed that the four behavioral variables measured in the laboratory assays were adequately sampled (KMO, 2010: 0.75, 2011: 0.77, 2012: 0.79; Budaev 2010) and the correlation matrices were appropriate for PCA (Bartlett's sphericity 2010-2012, $p<0.001$; Budaev 2010). The four behaviors (Table 9) loaded with similar weights onto principal component axis-1 in each year (PC1; Table 11) and their loadings are readily interpretable. High PC1 scores represent individuals that spent more time in the open (away from the refuge), moved closer to the predator on average, initiated feeding on bloodworm sooner, and were more active. Low PC1 scores indicate more time in the refuge, shorter distances traveled away from refuge, an increased latency to consume bloodworm (if at all), and low activity. PC1 explained 69% (2010), 73% (2011), and 72% (2012) of the variation observed in the four behavioral measures (Table 11). Based on the Kaiser-Guttman rule, PC1 was the only component retained for further analysis (Eigenvalues, PC1>1.0, PC2< 1.0 for 2010-2012; Kaiser 1991). From here on, I use PC1 score as a composite measure of an individual's boldness in the context of foraging under predation threat.

Growth

The distribution of final lengths in the bold and shy groups from the 2008 experiment were different (final length: bold mean = 68.6, S.E. = 0.8, shy mean = 65.6, S.E. = 0.8, Mann-

Whitney U p = 0.01), with the bold distribution shifted towards higher values compared to shy fish. Thus, boldness had a general positive effect on growth in my pilot experiment. Prior to conducting multiple linear regression analysis with data from 2010, 2011, and 2012, I first tested whether boldness was correlated with initial length. Boldness and initial length were weakly correlated in all years and these correlations were significant in 2011 and 2012 (2010: n=323, r=0.02, p = 0.68; 2011: n= 283, r=0.43, p <0.001; 2012: n=254, r=0.29, p < 0.001). An analysis of Variance Inflation Factors (VIFs) showed little impact of these weak correlations on the degree of multicollinearity in multiple regression models with both predictor variables included. VIF thresholds for multicollinearity are often set at 10, where all variables greater than this threshold are considered collinear and may affect model results (O'Brien 2007, Zuur et al. 2010). In my models with both initial length and boldness scores explaining variation in growth, VIFS were substantially less than the standard threshold of 10, and in fact were all under 2, a highly conservative threshold for multicollinearity (VIFs, 2010: boldness = 1.00, initial length = 1.00, 2011: boldness = 1.30, initial length = 1.48, interaction = 1.24, 2012: boldness = 1.10, initial length = 1.15, interaction = 1.08; Zuur et al. 2010). Therefore, I proceeded with analyzing both initial length and boldness scores in multiple regressions for each year.

In all years, there was a significant positive effect of initial length on growth (Table 10, Figures 9). Over the course of the pond experiments, bluegill that started large exhibited a greater growth increment than those that started small (Figure 9). In 2012, a quadratic term was also significant in the model explaining growth with initial length as a predictor variable, indicating that growth leveled off (Figure 9). In 2010, boldness score had an additive positive effect on growth that was marginally significant (β = 0.23, p = 0.08). In 2011, boldness score again had a positive effect on growth (β = 0.31, p = 0.05), and the interaction term was also significant (β = 0.03, p = 0.02). Figure 7 shows that the positive interaction between boldness and initial length in 2011 is the result of boldness increasing the effect of initial length on growth. Bolder fish that started the experiment large exhibited the highest growth. In addition, bolder fish that started small had relatively lower growth than shier conspecifics that started small (Figure 7). In contrast, the 2012 interaction term was negative (β = -0.52, p = 0.04), indicating the magnitude of the relationship with initial length and growth decreases as boldness increases (Figure 8). Here, shyer fish that started small had a lower growth increment than bolder fish that started small (Figure 8). The direct effect of boldness was not significant in 2012, but boldness affected the relationship with initial length and growth through the interaction.

Survivorship

Bluegill survivorship varied across years, with 91% survivorship in 2010, 81% survivorship in 2011 and 73% in 2012. Survivorship was lowest in the year with the highest number of bass predators. Total length at the beginning of the experiment had a positive effect on survivorship in 2010 and 2012, but not in 2011 (Table 10). With the resulting increased predation pressure in 2012, the cost of being small (<50 mm) was noticeably higher than in 2010 (Figure 10). Surprisingly, and not in support of my initial hypothesis, boldness had no effect on juvenile bluegill survival in any of the experiments.

Fin regeneration

Standardized growth, boldness, and their interaction all had significant effects on the extent of fin regeneration in 2011 (beta regression model, pseudo R^2=0.11, boldness score β =

-0.24, standardized growth β = -0.26, interaction β = 0.14, all variables $p<0.001$). Standardized growth and boldness had negative effects on the proportion of the clipped fin that was regenerated. Bolder fish regenerated a lower proportion than shier conspecifics. Individuals that grew more during the pond experiment also showed decreased energetic allocation to repairing the clipped fin. The interaction term is positive, indicating that the negative effect of growth on the proportion of fin regenerated becomes more negative with boldness. Bolder fish that show high growth do so at an increased cost to fin regeneration than shy fish (Figure 11). The correlation coefficient of determination between the two independent variables, PCA score and total growth, was relatively small ($R^2 = 0.173$), and the VIFs were all less than 2 (VIFs, standardized boldness score = 1.27, standardized growth = 1.42, interaction = 1.37).

Discussion

More than two decades ago, Wilson et al. (1993) helped pioneer research on the ecological consequences of inter-individual differences by examining the behaviors of sunfish in ponds and lakes. The ensuing 20 years has witnessed a rapid expansion of the study of animal personalities in a wide variety of taxa (see reviews in Sih et al. 2004*a*,*b*; Réale et al. 2007; Stamps and Groothuis 2010; Wolf and Weissing 2012). Although these studies have greatly increased our understanding of the potential ecological and evolutionary consequences of behavioral variation, most of this work has been conducted in the laboratory and relatively few studies have emulated Wilson et al.'s (1993) original study by examining behavioral variation in natural or semi-natural environments. Recently, there have been several calls to increase the number of studies conducted in the field to better assess the fitness consequences of different behavioral types (Bolnick et al. 2011; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012; Mittelbach et al. 2014).

There are many challenges to studying the fitness consequences of behavioral traits in wild or semi-wild animals (Archard and Braithwaite 2010). Particularly challenging is estimating the effects of inter-individual differences in behavior on the survival of free-living individuals. The recovery of marked individuals in the wild is often very low (see Höjesjö et al. 2011 for an example with fish); thus, researchers are often left wondering whether missing individuals are dead or have simply emigrated from the study area. In my pond studies, I was able to recover all the surviving marked fish at the end of the experiment by draining the ponds. Thus, I could determine with a great deal of certainty an individual's growth rate and probability of survival in relation to its behavioral score. The experimental ponds are of course small compared to most natural lakes in which bluegills occur. However, the habitat structure and prey resources (e.g., zooplankton, benthic invertebrates) are very similar to those in nearby natural lakes (Mittelbach 1984), and a number of pond experiments demonstrate that bluegill respond to size-specific foraging gain and predation risk in the ponds in the same way they do in natural lakes (e.g., Werner et al. 1983; Turner and Mittelbach 1990; Kjelvik 2015 Chapter 4).

Fitness tradeoffs are often hypothesized as a mechanism for the maintenance of behavioral variation within a population (Mangel and Stamps 2001; Dall et al. 2004; Stamps 2007; Wolf et al. 2007; Smith and Blumstein 2008; Mittelbach et al. 2014). Previous research with bluegill documents that foraging in open-water habitats increases foraging returns, but predation pressure from piscivorous bass restricts small bluegill to the protection of the vegetated littoral zone (Werner et al. 1983). Thus, juvenile bluegill experience a foraging gain-predation risk tradeoff until they reach sizes that gape-limit their predators. As bluegill grow to sizes where mortality risk decreases, they may shift to feeding primarily on open-water zooplankton (Mittelbach 1981, 1984; Werner and Hall 1988). I hypothesized that behavioral differences in foraging boldness would affect foraging decisions (e.g., use of risky habitats, see Kjelvik 2015 Chapter 4) that would lead to observable differences in growth and survival. In two of the three pond experiments (2010, 2011) as well as in the 2008 pilot experiment, bolder fish showed increased growth compared to their shier conspecifics. In the third experiment (2012), boldness had an effect on growth through its negative interaction with initial length, but not a direct additive effect.

Several factors may have contributed to the differences in bluegill growth observed in the 2010 and 2012 experiments, and in turn contributed to the different effects of boldness on growth in the two years. Average growth in 2012 was more than double that of 2010 (2010: mean change in length = 20.3 mm, range = 9-32 mm; 2012: mean change in length = 45.3 mm, range = 30-71 mm). Average daily air temperatures were similar for the time periods of each experiment (2010: 15.4 degrees Celsius, 2012: 15.0 degrees Celsius), suggesting the observed increased growth rates were not explained by temperature differences across years. However, higher growth in 2012 could be the consequence of reduced density-dependence over the course of the experiment. Fewer bluegill were stocked in 2012 compared to 2010 (254 fish compared to 323 fish) and survival was 18 percent lower in 2012 than 2010 due to a greater number of bass predators in 2012. Bluegill abundances at the end of the experiment were 294 in 2010 and 186 in 2011, thus, per-capita resource availability was greater in 2012 than 2010. Bluegill undergo an ontogenetic niche shift that typically corresponds to sizes that reduce vulnerability to predation, and has been documented to occur as total length approaches 90-100mm (Mittelbach 1984, Kjelvik Chapter 4). In 2012, the majority of surviving bluegill had final lengths that were greater than this range, therefore high growth rates may have allowed individuals to grow into a safe size refuge more quickly, affecting their use of the open water habitat and allowing the use of increased resources to reallocate energy from growth to reproduction.

In 2012, I observed an asymptotic relationship between initial length and growth (Figure 9). Individuals that started the 2012 experiment at relatively large sizes (>50 mm) had very similar growth rates over the course of the experiment. In contrast, in both 2010 and 2011, growth was a linear, positive function of initial length (Figure 9). I hypothesize that individuals starting the 2012 experiment at relatively large sizes may have reached sexual maturity during the experiment and thereby shifted their allocation of energy from growth to gonadal development. Dissection of a subset of the individuals from 2012 showed that most (around 80%) had begun development of reproductive organs (M. Kjelvik, unpublished data). This investment in reproductive organs could explain the leveling of growth for initially large fish. It is possible that as individuals grew into a size refuge, they transitioned from allocating energy to growth in body size to development of gonads.

My results indicate that the costs and benefits of inter-individual differences in behavior and associated foraging strategies may be complex, with both short and long-term ramifications. The pace-of-life syndrome framework aligns a suite of covarying behavioral, physiological, and life history traits (Réale et al. 2010) that allow the comparison of costs and benefits of various behavioral phenotypes throughout ontogeny (e.g., Schürch and Heg 2010; Chervet et al. 2011). Similarly, Mangel and Stamps (2001) suggest that increased growth rates may come at a cost to decreased body quality or investment into injury repair. Although I did not find significant differences in survival based on boldness as predicted, I did find a repeated (2008, 2011) pattern of higher investment into regeneration of clipped fins by shier fish compared to bolder fish.

Results from the 2011 experiment show that individuals with higher growth rates allocated less energy to fin repair, but the cost of increased growth rates was higher for bolder fish (Figure 11C). In other words, shy fish always invested more into fin regrowth, even if they had high growth in body size (Figure 11A,B,C). Bold fish tend to allocate minimal energy to fin repair (Figure 11F) regardless of their growth in body size, whereas this tradeoff is very apparent in shy fish (Figure 11D). An increase in fin regeneration at a cost of lower body growth is indicative of an energy allocation tradeoff. The fact that boldness has an effect above this tradeoff hints that there may be varying life history strategies associated with boldness that align with ideas in the pace of life syndrome framework.

As adults, bluegill have been shown to exhibit variation in morphological, physiological, and behavioral traits that are associated with alternative male reproductive strategies (e.g. sneaker males vs. parental care males, Gross and Charnov 1980). It could be that the behavioral differences and resulting physiological effects (growth, injury repair) that I present here for juvenile bluegill are components of complex adaptive life history strategies that correspond with adult reproductive strategies. Perhaps the faster growing bold fish are able to reach reproductive sizes earlier than shier fish. Larger, bold fish may also be more aggressive during the acquisition of nest sites, giving them better territories to attract more females and secure higher egg deposition. Shier males may take on sneaker reproductive strategies, as they may be unable to acquire adequate nest territories. Therefore, they could utilize their smaller body sizes to mimic females and fertilize eggs in another male's nest.

Although fitness tradeoffs have been proposed as a mechanism for the maintenance of behavioral variation, very little research has examined the costs and benefits of variation in behavioral phenotypes in field settings. Understanding how behavioral variation affects the growth and survival of juvenile individuals under varying ecological regimes is crucial for understanding the role of fitness tradeoffs in natural environments. I show that studies conducted in the field may yield complex results; however, incorporating the natural history and ecology of an organism helps to elucidate patterns. Individual behavioral types are likely a complex combination of responses to physiological and social cues along with genetic predisposition and lifetime learning. Quantifying both long and short-term fitness proxies can uncover the complex causes and consequences of inter-individual behavioral variation.

APPENDIX

Table 9. Definition of behavioral variables used to determine boldness scores (PC1) of individual bluegill sunfish in the laboratory experiments.

Behavioral Variable	Behaviors Used	Definition
Latency to Consume Prey	Consume	The amount of time that had passed in the assay before the individual consumed bloodworm. A measure of willingness to approach the predator to obtain prey.
Activity	Retreats, Advances	Total activity in the open-water area during the assay. Calculated by summing the distance traveled from retreats and advances.
Average Distance Traveled	Retreats	Mean distance at which an individual retreated. Represents the average distance an individual will venture towards the predator before turning back towards the refuge.
Time in Refuge	Emerge, Vegetation	Total time spent within the vegetation as a measure of willingness to explore novel/risky environments. High values indicate less time exploring.

Table 10. Design parameters and model results from three pond experiments (2010, 2011, 2012). Growth was estimated using multiple regression models. Survival was estimated with general linear models (family="binomial", link="logit"). Standardized coefficients and significance are shown for each model.

	2010	2011	2012
Study Design			
Duration	2 months	8 months	2 months
Season	Aug-Oct	Sept-Apr	Aug-Oct
Bluegill Age	1+	YOY	1+
Stocked, Survived	323, 294	283, 229	254,186
Piscivore Numbers	2	3	9
Bluegill Data			
Initial Lengths (mm)	35-76	31-82	31-73
Growth (mm)	9-32	8-36	30-71
Percent survived	91%	81%	73%
Growth Models			
Initial Length (β)	**2.86****	**0.26****	**4.31****
Boldness (β)	**0.23#**	**0.31***	0.09
Interaction (β)	n.s.	**0.03***	**-0.52***
Survivorship Models			
Initial Length (β)	**0.65****	n.s.	**0.84****
Boldness (β)	n.s.	n.s.	n.s.
Interaction (β)	n.s.	n.s.	n.s.

p ≤0.10 *p ≤0.05 **p ≤0.001

Table 11. Directionality and weight of loadings for the four behavioral variables onto Principal Component 1 for each experiment.

Behavioral Variable	2010	2011	2012
Latency to Consume Prey	-0.47	-0.47	-0.47
Average Distance Traveled	0.46	0.47	0.46
Activity	0.55	0.53	0.53
Time in Refuge	-0.52	-0.52	-0.53
Proportion Variance Explained	**0.69**	**0.73**	**0.72**

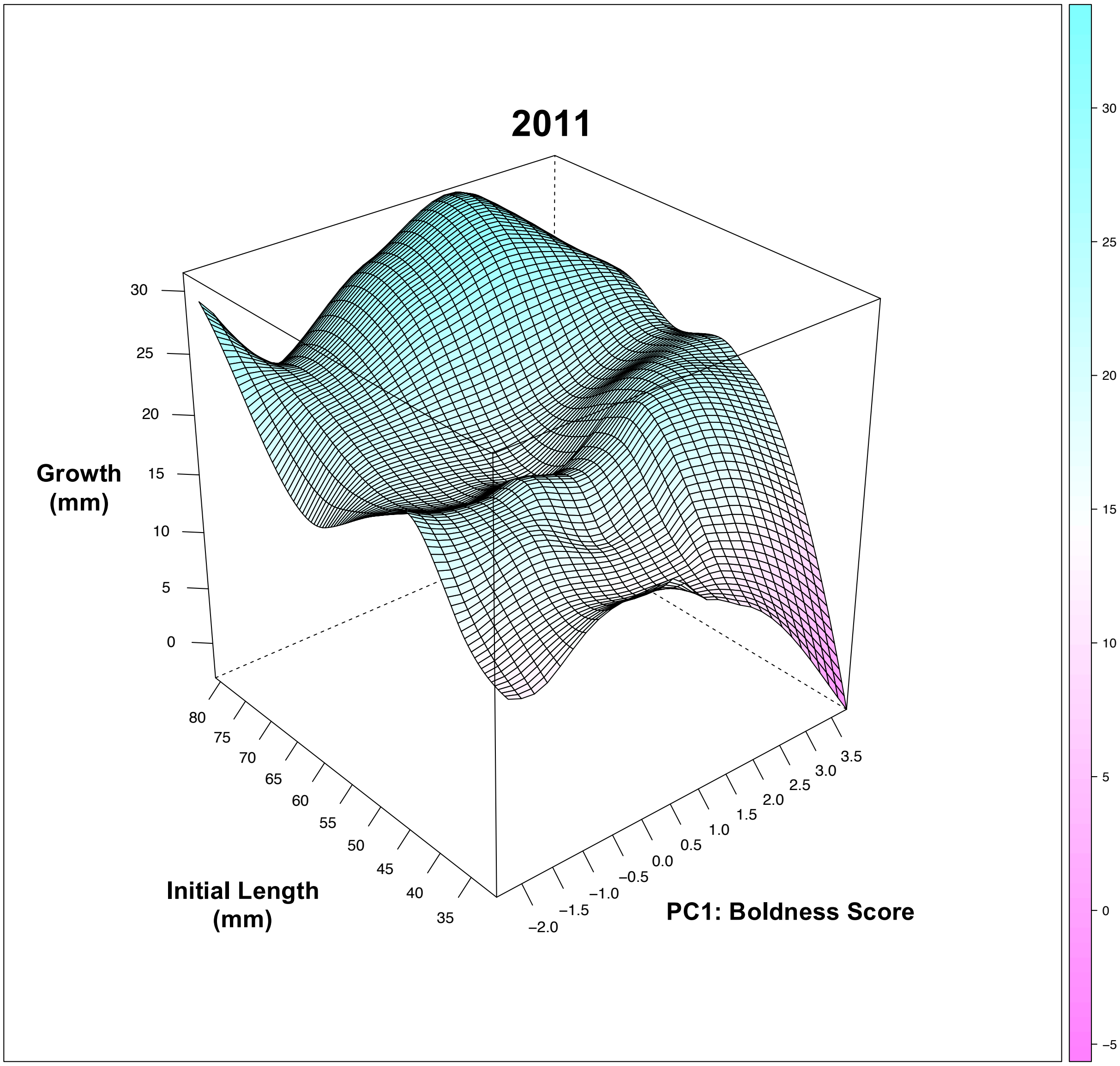

Figure 7. Surface plot showing the effects of initial total length (mm) and boldness score on juvenile bluegill growth increment from the 2011 pond experiment.

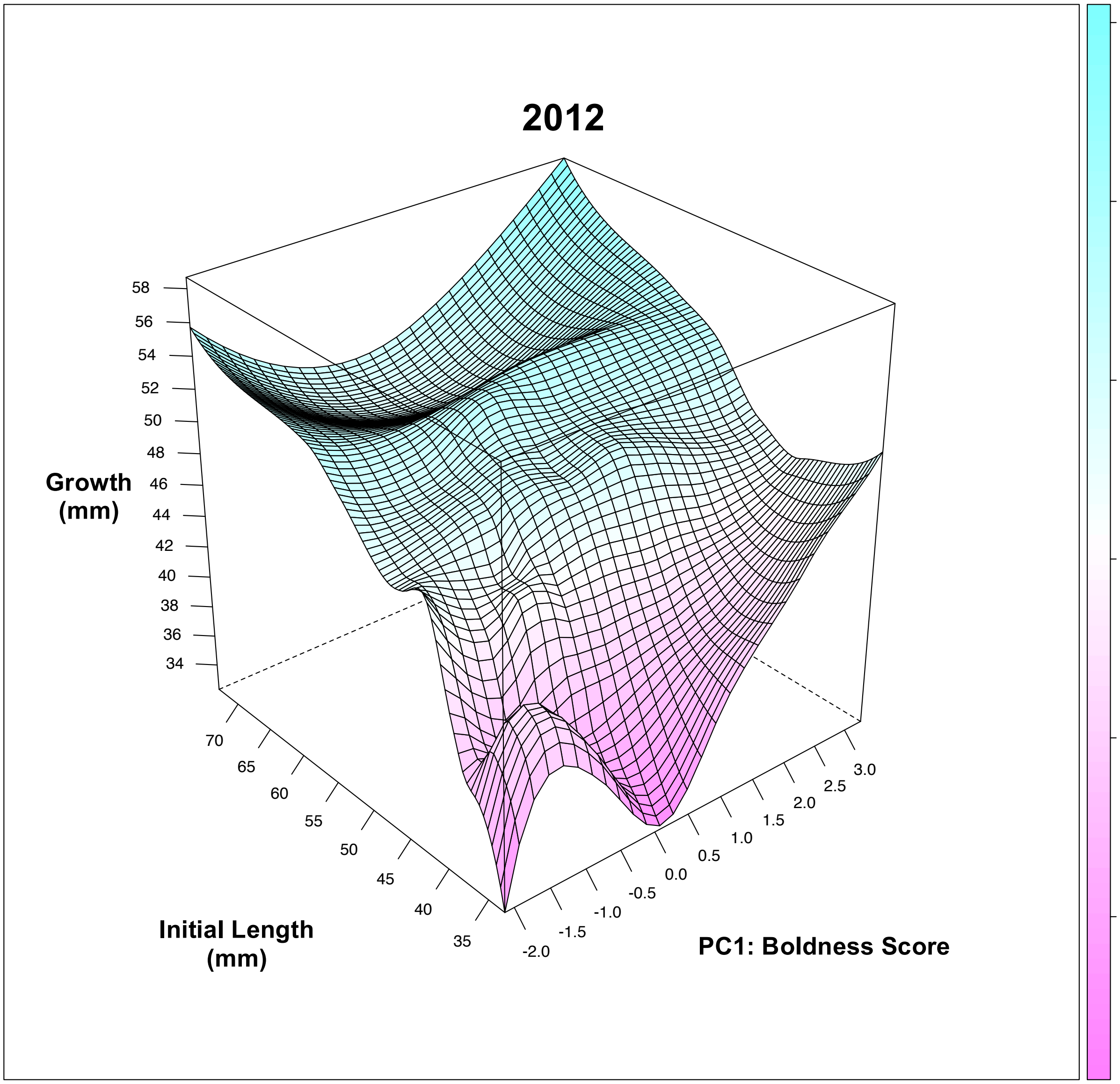

Figure 8. Surface plot showing the effects of initial total length (mm) and boldness score on juvenile bluegill growth increment from the 2012 pond experiment.

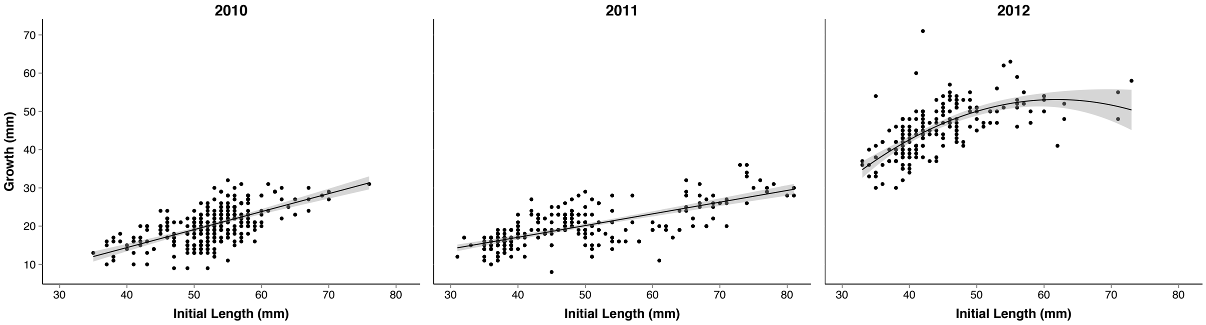

Figure 9. Relationships between individual total length at the beginning of an experiment (2010-2012) and change in total length (growth, mm) over the course of the experiment. Regression lines are presented with 95% confidence bands. In 2012, the model included a significant quadratic term.

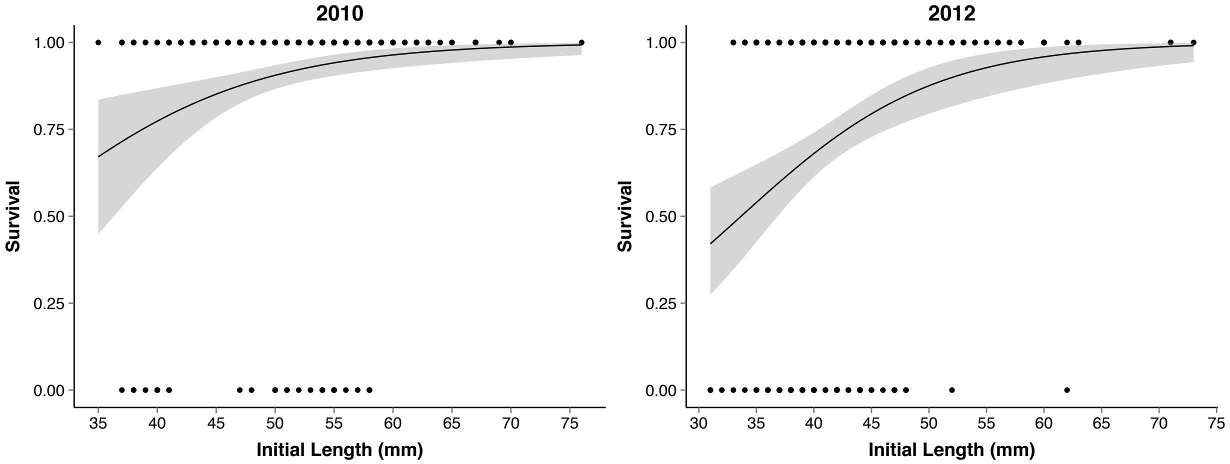

Figure 10. Logistic curves fit to the probability of survival in the 2010 and 2012 pond experiments with initial total length (statistically significant, Table 10) as the predictor variable.

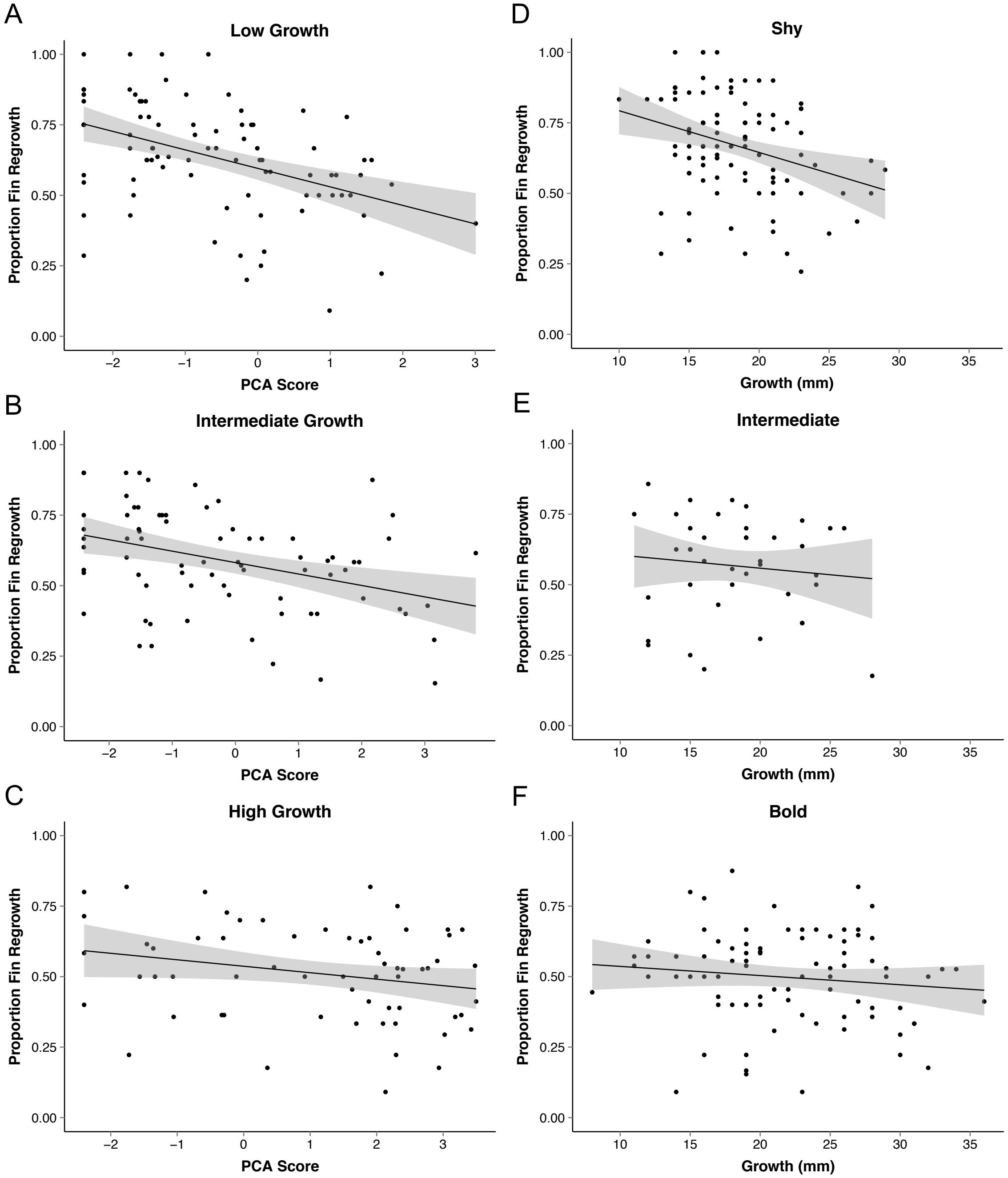

Figure 11. The effect of boldness (PCA score) on proportion fin regrowth for juvenile fish grouped into three classes based on growth from the 2011 pond experiment (first column) and the effect of growth on proporation fin regrowth for juvenile bluegill in three boldness classes (second column). To visualize the effect of boldness on proportion fin regrowth, fish were grouped based on centered and standardized values into three levels of growth;

Figure 11 (cont'd) s.d. values < -0.50: "Low" (Panel A: n = 87, growth = 8-17 mm), s.d. values -0.50-0.50: "Medium" (Panel B: n = 75, growth = 18-22 mm), s.d. values >0.50: "High" (Panel C: n = 62, growth = 23-36 mm) and boldness; "Shy" (Panel D: n = 99, PCA score = -2.40- -0.50), "Intermediate" (Panel E: n = 41, PCA score = -0.50-0.50) and "Bold" (Panel F: n = 84, PCA score = 0.50-3.81).

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LITERATURE CITED

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CHAPTER FOUR

FORAGING BOLDNESS AFFECTS DIET SPECIALIZATION AND ONTOGENETIC NICHE SHIFTS IN BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*)

Introduction

Ontogenetic niche shifts, defined as a change in an organism's resource use over its lifetime (Werner and Gilliam 1984; de Roos and Persson 2013), are well documented in a wide variety of taxa. Organisms that grow significantly in size from birth through adulthood are especially likely to exhibit ontogenetic niche shifts, as body size influences an individual's ability to capture and consume different sizes and kinds of resources, as well as its vulnerability to predators. Much theoretical and empirical work has focused on how a foraging gain-predation risk tradeoff may drive ontogenetic niche shifts in diet and habitat use, particularly when habitats differ in their associated risks and rewards (Werner and Gilliam 1984; de Roos and Persson 2013). Recently, it has been suggested that individual differences in the perception of risk tradeoffs (Matich and Heithaus 2015), as well as consistent individual differences in behavior (Mittelbach et al. 2014) may affect the timing and/or extent of ontogenetic niche shifts. Here, I examine how individual differences in "shy" versus "bold" behavior affect ontogenetic niche shifts in the bluegill sunfish (*Lepomis macrochirus*).

Bluegill provide a classic example of an ontogenetic niche shift driven by a foraging gain-predation risk tradeoff (Mittelbach 1981, 1984; Werner et al. 1983; Werner and Hall 1988). Small (juvenile) sunfish are very vulnerable to predation and as a consequence occupy near-shore (littoral) habitats, where dense vegetation provides protection from predators such as largemouth bass (*Micropterus salmoides*). The open-water (pelagic) habitat, however, often provides a higher foraging return, as the bluegill's morphology is well-adapted to feeding on zooplankton (e.g., *Daphnia*, Mittelbach 1981). Thus, a foraging gain-predation risk tradeoff exists when choosing to forage in the two habitat types (littoral or pelagic). This tradeoff is strong for small bluegill (which are very vulnerable to predation), but becomes less so for large fish. With increased length, mortality risk decreases by gape-limiting predators. Thus, bluegill undergo an ontogenetic niche shift from littoral to pelagic habitats as they grow from juveniles to adults - a pattern that has been repeatedly documented in natural lakes (Figure 12; see also Mittelbach 1981, 1984; Werner and Hall 1988).

Tradeoffs may also function to maintain multiple behavioral strategies within a population. For example, Mangel and Stamps (2001) show how tradeoffs between growth and survival can result in a range of growth rates that yield similar fitness. If behavior is either the cause or consequence of variation in growth rates, these tradeoffs may promote behavioral variation within a population. Additionally, if individuals differ in their behavioral response to a foraging gain/predation risk tradeoff, then variation in the timing and extent of ontogenetic niche shifts would be expected. In fact, bluegill sunfish show considerable variation in the size at which individuals shift from feeding in the vegetation to feeding in the open-water (Figure 12, Mittelbach 1984; Werner and Hall 1988). Variation in size at which individuals switch to foraging in open-water habitats could simply be noise around an adaptive mean. Alternatively, the observed variation could be related to phenotypic differences between individuals that result in different foraging strategies. To date, the factors underlying this individual variation have not been investigated.

In situations that pose costs and benefits to taking risks, such as the previously described foraging gain-predation risk tradeoff, an individual's foraging boldness could affect its perception of and interactions with its environment. To explore whether individual differences in risk-taking behavior affects ontogenetic niche shifts in bluegill sunfish, I examined the diets and associated habitat use of juvenile bluegill in the field. I hypothesized that bolder individuals may be more willing to use risky habitats, regardless of vulnerability associated with their current length, and/or that bolder fish may perceive danger differently by taking risks at smaller sizes than shier fish. These two hypotheses would result in observable differences in habitat use and associated prey types in diet. As part of a larger field experiment, I examined these hypotheses by quantifying the diets of marked individuals that differed in their foraging boldness. At the population level, I expected juvenile bluegill to undergo an ontogenetic niche shift from feeding primarily on vegetation-dwelling prey to feeding mostly on open-water zooplankton. However, I predicted that bolder fish would take more risks to obtain open-water prey resources and thus have a higher proportion of zooplankton in their diets at all lengths. At smaller lengths, I expected bolder fish to forage primarily in the littoral habitat, but that they would supplement their foraging with bouts into the open-water. As bluegill approach lengths that begin to gape-limit their predators, I predicted that increased risk-taking behavior would lead to bolder fish shifting to open-water as their primary foraging habitat at smaller lengths than shier fish.

Methods

Study population and collection

Juvenile bluegill were obtained from a brood pond at the Kellogg Biological Station that was stocked with adult bluegill in 2008 and maintained in the absence of piscivorous fish. From this population, 323 year 1+ bluegill sunfish were collected and transferred to large outdoor holding tanks (1000 L) in May 2010. Individuals were transferred to aquaria in the laboratory in batches of up to 160 fish and were kept at a density of 20 fish per 110 L. Bluegill were fed daily with zooplankton collected from an on-site pond. Over the first three days in the lab, thawed commercial bloodworms (Chironomid larvae) were gradually introduced to the diet.

Upon acclimation to the lab, each fish was given a unique identification code using visible implant elastomer, a pliable biocompatible polymer (Northwest Marine Technology, Goldsmith et al. 2003) injected subcutaneously. I used four colors of elastomer and each fish was marked in four out of five possible locations to allow for thousands of potential combinations for individual identification. Prior to marking, I sedated the fish by transferring them to a container with tricaine methanesulfonate (MS-222, 40 mg/L) in solution. I observed the fish for signs of moderate sedation (e.g., decreased fin and gill movement, lowered responses to stimuli, loss of equilibrium) and then gave each individual a series of assigned marks for identification. The fish were then allowed to recover in a bucket containing water from their home tank. After the fish showed increased awareness indicating recovery from the sedative, they were transferred back to an aquarium.

Behavioral assays

Behavioral assays were performed in a 120 x 30 x 50 cm (180 L) aquarium, filled to 42 cm water height and partitioned into three zones: a refuge area at one end of the aquarium, a predator housing area at the opposite end of the aquarium, and a larger open-water area in the middle. The predator area (25 cm in length) contained one piscivorous largemouth bass (*Micropterus salmoides*) and was separated from the open-water area by a nylon mesh partition, which allowed the test fish to perceive the bass through visual and chemical cues during the assay. The refuge area (30 cm in length) was located at the opposite end of the tank and contained artificial vegetation, the only physical structure in the tank. The middle 65 cm of the aquarium served as an open-water foraging environment located between the predator and the refuge. To track position and movement of the test fish during assays, the aquarium was marked every 10 cm along the bottom for reference.

In nature, juvenile bluegill school together in small groups. Therefore, I assayed bluegill behaviors in groups of three fish. This group size allowed the monitoring of each fish's behavior, while at the same time providing the fish with a normal social environment. Prior to the behavioral assays, bluegill were starved for 24 h to standardize hunger and increase motivation for foraging. At the start of each behavioral trial, three bluegill were selected from their holding tank and placed into the refuge area of the test aquarium. The test fish were allowed to swim freely in the refuge and open-water areas for a 15 minute acclimation period. An opaque partition prevented the test fish from seeing the bass for the first 10 minutes of acclimation, reducing the amount of perceived threat to allow initial exploration of the foraging arena. The partition was then removed to visually expose the predator and five minutes later, the assay was initiated by dropping 10-15 bloodworms (midge larvae) at the edge of the open-water foraging environment, proximal to the predator. When the bloodworms were dropped into the aquarium, most fish retreated back to the vegetation. If individuals started the assay outside of the refuge, their location within the open-water foraging area was noted. For the next ten minutes, behaviors of the three test fish were simultaneously recorded onto an audio recorder by two observers. The data from each foraging assay was later transcribed into JWatcher version 1.0, a free event recording program commonly used to develop ethograms and record animal behavior (Blumstein and Daniel 2007).

Quantifying boldness

The behavior of each test fish was quantified by recording when it left and re-entered the refuge, its activity within the open-water foraging area, the latency to consume bloodworm, and all distances it traveled. These behaviors were then used to construct ecologically relevant behavioral variables (see Table 12). Time spent in the refuge reflects hesitance to investigate novel environments, and was calculated by summing the total time an individual spent in the vegetated refuge zone. Individuals with higher values spent less time in the open-water foraging zone and are therefore interpreted as being less explorative of novel environments. Willingness to assume risk in order to obtain prey was measured as the latency to consume bloodworms and was quantified as the time elapsed from the beginning of the trial until the first bloodworm was consumed (recall that prey, bloodworms, were placed directly in front of the predator holding area). Lower values of this behavioral measure indicate increased risk-taking tendencies, where individuals are quickly leaving the refuge to forage. As an estimate of willingness to approach or investigate a risky encounter I calculated the average distance traveled into the open-water foraging zone before turning back towards the refuge. Using the position of each fish before it retreated to the refuge (changed orientation away from the predator and towards the refuge), I am able to measure risk-taking in a way that was not dependent on whether test fish consumed bloodworm during the trial. The final behavioral measure recorded was the activity rate during the assay. Activity was calculated by summing the total distance traveled from retreats and advances (orientation change away from refuge and towards predator in open-water zone) during bouts in the open-water foraging zone. A total of 323 juvenile bluegill were run through the behavioral assays, with each fish assayed only once.

The four behavioral variables measured in the foraging assays showed strong pairwise correlations (Spearman rank test, $p < 0.001$, Table 13). Therefore, I conducted a principal components analysis (PCA) on the correlation matrix of the four behavioral traits to condense the variation into uncorrelated principal components, a common technique in studies of complex animal behaviors (e.g., Martin and Réale 2008; Šíchová et al. 2014). PCA scores in the current context represent behaviors measured for fish foraging under predation threat, which I interpret as a composite measure of foraging boldness. I confirmed the appropriateness of the data for PCA analysis using Bartlett's sphericity test and the Kaiser-Meyer-Olkin measure of sampling adequacy (data considered adequately sampled if $KMO>0.7$, Budaev 2010). Component retention was tested with the Kaiser-Guttman rule of retaining components with an eigenvalue greater than one (Kaiser 1991).

Diet analysis

The impact of boldness on bluegill diets in the field was assessed as part of a larger study examining how boldness affects juvenile bluegill growth and survival. In August 2010, I stocked 323 individually marked bluegill, along with two adult largemouth bass into one of the experimental ponds (30 m dia., 2 m deep) at Michigan State University's Kellogg Biological Station. The pond provided a natural environment with two distinct habitats; emergent vegetation (e.g., cattails, *Typha*) around the pond perimeter and open water in the pond center. Zooplankton (e.g., *Daphnia*) were the dominant bluegill prey in the open-water of the pond, whereas aquatic insect larvae (Ephemeroptera, Odonata, Trichoptera) were abundant prey for bluegill in the near-shore vegetation.

Bluegill stocked into the pond were individually marked, measured for total length, and had been assayed in the laboratory for boldness. The experiment ran for 60 days. Fish were collected for diet analysis on the final day of the experiment using a beach seine. Sampling began about three hours after sunrise allowing the diurnal foragers time to feed, but before prey in the stomachs were extensively digested. Fish were euthanized with an overdose of MS-222, measured for total length and wet mass, and then frozen for later diet analysis. I then drained the pond to recover all remaining individuals (fish collected later were not used in the diet analysis).

For diet analysis, I determined the identities of all the seined fish and selected 23 individuals that were at the extremes of the boldness continuum (the 13 lowest and the 10 highest boldness scores). I then randomly selected an additional 15 fish for analysis that had boldness scores spanning across the boldness continuum. A preliminary analysis of the diets of these 38 fish indicated an ontogenetic niche shift from feeding on vegetation-dwelling prey to open-water prey at about 70-80 mm total length (broadly consistent with previous bluegill studies; see Figure 12 and Mittelbach 1984). However, only a few of the fish in my initial sample for diet analysis were at or above the size at which the shift to feeding on zooplankton occurred (70-80 mm total length; Figure 12, Mittelbach 1984). Therefore, to better characterize the ontogenetic niche shift and its relation to bluegill behavioral type (boldness), I analyzed the diets of all fish collected in the beach seine that were >80 mm total length. In total, 54 fish were analyzed for diets.

Bluegill stomach contents were quantified using methods employed in previous pond experiments (e.g., Werner et al. 1983; Olson et al. 1995). Prey items were identified to the lowest taxonomic level possible (typically family or genus), enumerated, and measured (first ten per prey category per fish). Prey lengths were converted to dry masses using length-mass regressions (G. Mittelbach, unpublished data), and prey mass was used to characterize bluegill diets. Prey types were assigned to two habitat categories; “littoral” (e.g., invertebrates found in vegetation or sediments) or “pelagic” (e.g., open-water zooplankton). *Daphnia* and *Chaoborus* were the two pelagic prey consumed in abundance by the bluegill. However, because *Chaoborus* is found in the water column mostly at night and migrates into the pond sediments during the day (a mechanism to avoid fish predation; Garcia and Mittelbach 2008), I used percent *Daphnia* in the diet as the most reliable indicator of feeding in the open-water.

Statistical analyses

All statistics were performed using R version 3.2.2 (R Core Team 2015). I used fish length at capture as a covariate in all models examining the effect of boldness on diet and habitat use, as previous studies showed that bluegill diets and ontogenetic niche shifts are strongly related to body length (e.g., Mittelbach 1984; Werner and Hall 1988). To test whether the open-water habitat provided a higher foraging return than the littoral zone, I examined how gut fullness (total biomass of prey in the stomach, response variable) was related to the proportion of *Daphnia* in the diet (predictor variable) using multiple regression with body size as a covariate. Maximum likelihood estimation (bbmle package in R, Bolker and R Development Team 2014) was used to develop logistic regression models examining the effects of boldness and body length on the proportion of pelagic prey found in an individual’s diet. Akaike Information Criterion (AIC) was used to compare the fit of several models: full (boldness, length, and interaction), two variable (boldness, length), single variable (length), and null model (no predictors).

To quantify inter-individual diet variation, I used the Proportional Similarity Index (PSi, Bolnick et al. 2002). I used the RInSp package in R (Zaccarelli et al. 2013) to calculate the population diet in terms of the mean proportion of each of 16 prey types from all fish analyzed. Using the population diet as a baseline, similarity indices were used to assign each individual a score along a generalist-specialist continuum. The PSi scores were calculated by comparing the overlap of each individual's diet with the mean diet composition of the population (54 individuals sampled). The output of the RInSp functions gives an estimate of the average level of specialization within the population as well as individual-level scores. The scores range from 0-1, where lower values signify individuals with high diet specialization (different from mean population diet) and values approaching 1 indicate high diet generalization (similar to mean population diet). Due to the use of proportions for this index and the associated bound data with values between 0 and 1 as a dependent variable, a beta regression model was used to determine the effects of boldness, length, and the associated interaction on variation in PSi scores. I used the betareg package in R (Cribari-Neto and Zeileis 2010) to fit the model and used maximum likelihood estimation and Wald tests on the coefficients and covariance matrix for significance (Cribari-Neto and Zeileis 2010). Petraitis's W, another diet specialization index (Bolnick et al. 2002), was similarly calculated and analyzed. Both indices yielded similar results and thus I proceed with results from the PSi scores.

Results

Composite boldness scores

Prior to running the PCA, I found the four behavioral variables measured in the laboratory assays were adequately sampled (KMO=0.75, Budaev 2010) and the correlation matrix was appropriate for PCA (Bartlett's sphericity $p<0.001$). The four behaviors loaded with similar weights onto principal component axis-1 (PC1; Table 14) and their loadings are readily interpretable. High PC1 scores represent individuals that spent more time in the open (away from the refuge; loading -0.52), moved closer to the predator on average (loading 0.46), initiated feeding on bloodworm sooner (loading -0.47), and were more active (loading 0.55). Low PC1 scores indicate more time in the refuge, shorter distances traveled away from refuge, an increased latency to consume bloodworm (if at all), and low activity. PC1 explained 69 percent of the variation observed in the four behavioral measures. Based on the Kaiser-Guttman rule, PC1 was the only component retained for further analysis (Eigenvalues, PC1: 2.74, PC2: 0.63, PC3: 0.40, PC4: 0.23; Kaiser 1991). From here on, I will use PC1 score as a composite measure of an individual's boldness in the context of foraging under predation threat.

Habitat profitability

In the pond experiment, bluegill that were larger and consumed a higher proportion of *Daphnia* also had significantly more prey biomass in their stomachs (beta=0.32, $p<0.01$, beta=0.51, $p<0.001$, respectively). Larger fish are expected to consume more prey per foraging bout. After accounting for the effect of fish length on gut fullness, I found a strong positive relationship between the proportion of *Daphnia* in the diet and the total biomass of prey consumed (Figure 13). The most common prey types in the diets of pond bluegill have similar caloric densities per dry mass unit (e.g. *Daphnia* 21 J/mg, Chironomidae 23 J/mg, other vegetation prey 21 J/mg, Mittelbach 1981). Thus, feeding in the open-water on *Daphnia* provides a higher foraging return than feeding in the littoral zone, supporting increased foraging gain in pelagic areas(Werner et al. 1983; Werner and Hall 1988).

Ontogenetic niche shifts

Bluegill in the pond experiment exhibited an ontogenetic niche shift (Figure 14) that was very similar to that previously shown for bluegill in natural lakes (Figure 12; Mittelbach 1984). Fish smaller than 70-80 mm in total length consumed mostly vegetation-dwelling invertebrates, whereas larger fish consumed a combination of open-water prey (*Daphnia*) and prey from vegetation. Fish total length and boldness score (PC1) each had significant effects on the proportion of *Daphnia* in bluegill diets, and a logistic model including both fish total length and boldness score best fit the data (Table 15). The differences in AIC values from the best model (FL+BS) and all alternative models were greater than 2 AIC units (a standard criterion for model support, Burnham and Anderson 2002). A model including an interaction term for fish length and boldness score was within 2.1 AIC units of the best model; however, these two models had identical log likelihood values, indicating the addition of the interaction term is not supported (Table 15; Burnham and Anderson 2002; Arnold 2010). I accept the simpler model as the best fit. Thus, as expected from previous research, larger bluegill had more open-water prey in their diets. After accounting for the pattern of increased *Daphnia* with increasing fish length, there is a significant positive effect of individual foraging boldness on the proportion of *Daphnia* in the diet.

To better illustrate the effect of boldness on the size at which bluegill shift from feeding in the vegetation to feeding in the open water, I separated the continuous boldness variable into three classes: “bold”, “intermediate”, and “shy”. “Shy” individuals were those that did not leave the refuge during the behavioral assay and had the lowest behavioral scores (boldness score = -1.75, n=23). “Intermediate” fish were classified as those with scores between -1.75 and 1.75, whereas “bold” were individuals with scores > 1.75. As Figure 14 shows, small individuals fed primarily on littoral prey, regardless of their boldness score. At larger sizes, bold individuals had higher proportions of *Daphnia* in their diet, but still consumed some prey from the littoral zone, indicating the use of multiple habitats (littoral and pelagic). Although some shy fish had *Daphnia* in their diets, only two had over 50% of their diet from *Daphnia,* indicating that shy fish used this habitat less frequently even at large body sizes.

Diet specialization

Individual fish varied widely in the diversity of prey types eaten, as the proportional similarity index (PSi) scores ranged from 0.05-0.94 (maximum range 0-1). Individuals consuming prey items in direct proportion to the population as a whole will have a PSi equal to 1, whereas individuals specializing on a single diet item will have a PSi near 0 (Bolnick et al. 2002). Population-wide dietary specialization is measured by the average of all individual PSi scores, which was 0.62 for this bluegill population. Thus, individuals with PSi scores >0.62 have a greater degree of diet generalization than the population on average. In this study, individuals with a generalized diet (compared to the population average) are likely feeding in two habitats (pelagic and littoral), or feed on many prey types from the littoral habitat. Dietary specialists have PSi scores <0.62. Specialization relative to the population as a whole could be the result of decreased number of prey items in the diet and/or a high proportion of a diet item that is “rare” in the population’s mean diet.

Body length and boldness score both had a significant positive effect on PSi scores (from beta regressions, Wald’s test: final length pr(z)<0.001, boldness score pr(z)=0.007). Thus, smaller and less-bold individuals tended to be more specialized than the calculated population diet as a whole (Figure 15). This pattern of dietary specialization is linked to the ontogenetic niche shift shown in Figure 14. Larger individuals are more likely to forage in the open-water habitat and include *Daphnia* in their diet. However, very few individuals fed entirely on *Daphnia* and even large fish included some vegetation-dwelling prey in the diet, resulting in a diet that is more generalized. Additionally, as boldness scores increase, PSi increases, supporting the idea that bolder fish are utilizing more habitats (pelagic and littoral) and/or more prey types. In fact, of the nine individuals that are considered "specialists" (PSi value <0.50), only two scored high in boldness (Figure 16). One of these bold fish consumed >90% *Daphnia* and the other consumed >90% Odonata (dragonfly larvae, Figure 16). Additionally, of the nine specialists, five had a single prey type that contributed >50% to the diet as a whole. Each of these fish specialized on a unique prey type from the other specialists, suggesting there may be learned foraging efficiencies or individual foraging preferences.

Discussion

A rich literature documents consistent inter-individual differences in behavior in fish and other taxa, yet very few studies have examined the ecological consequences of this behavioral variation in natural or semi-natural environments (Wolf and Weissing 2012; Mittelbach et al. 2014). In particular, the effects of individual variation in behavior on the timing and extent of ontogenetic niche shifts is essentially unknown. I found that behavioral differences among juvenile bluegill assessed in the laboratory explained variation in diet specialization, habitat use, and ontogenetic niche shifts in the field. Juvenile bluegill exhibiting increased boldness in their foraging behavior under predation threat in the laboratory made greater use of the (risky) open-water habitat in the field, thereby increasing their foraging gain (biomass of prey eaten).

Ontogenetic niche shifts are common in fishes and other size-structured taxa, where both foraging ability and mortality risk are strongly related to body size (Werner and Gilliam 1984; de Roos and Persson 2013). Experimental and observational studies in ponds and lakes document that predation risk by piscivorous bass restricts small bluegill to the vegetated littoral zone (Werner et al. 1983), but after reaching sizes where mortality risk declines, bluegill often shift to feeding on open-water zooplankton (Mittelbach 1981, 1984; Werner and Hall 1988). Considerable variation exists among individuals, however (e.g., Figure 12). Werner and Hall (1988) showed that in lakes with more piscivorous bass, bluegill shifted to using the open-water habitat at larger body sizes (range in switch sizes was 51-82 mm standard length). Thus, variation in the *average* population niche shift appears to be driven by differences in overall predation risk. Understanding what drives variation between individuals within a population in the timing and extent of ontogenetic niche shifts is another question. In this study, bolder individuals showed higher use of pelagic habitats among fish that were at early stages of an ontogenetic niche shift (total length >78 mm). Only two shy fish (both over 90 mm) had >50% *Daphnia* in their diet, suggesting shy fish shift more gradually than bolder fish. My results show that differences in boldness among individuals can affect foraging behavior and ontogenetic niche shifts when a tradeoff exists between foraging gain and mortality risk in the field.

In natural lakes, the distance separating littoral and limnetic habitats is much greater than in the experimental pond. Thus, it wouldn't be surprising if the diets of bluegill from the pond exhibited a less distinct separation between littoral and limnetic prey compared to previous studies conducted in lakes. Comparing Figures 12 and 14, there are a large number of lake bluegill with diets >80% plankton, whereas only one of the pond bluegill was feeding on >80% plankton. In general, separation in diets (pelagic vs. littoral) was less distinct among larger fish in the pond study than in lakes (see also Werner and Hall 1988). However, smaller bluegill fed almost exclusively on littoral prey in both the pond and the lakes. The absence of *Daphnia* in the diets of small bluegill is evidence that there is strong reinforcement to remain in littoral habitats. This suggests that predation risk increases substantially when small individuals leave the vegetation, even over short distances.

Araújo et al. (2011) suggest that predation could either increase or decrease individual specialization within a population. Predators that restrict vulnerable individuals to foraging in a refuge habitat may reduce the extent of individual specialization within the population. My study suggests that if habitats differ in predation risk and foraging gain, differences in boldness can affect individual-level specialization (see also Coleman and Wilson 1998). In the case of the pond bluegill, bold fish had higher generalization, resulting from the use of both littoral and pelagic habitats. With increased habitat separation, lake bluegill may show higher specialization across the boldness continuum. With predators present, bolder fish may be willing to accept risks in order to specialize on pelagic prey types, while shier fish remain in the refuge until the risk to forage in open-water is minimal.

Although bolder fish may receive a benefit from higher prey biomass intake through increased use of pelagic habitats, they are likely still vulnerable to predation when they first begin the niche shift. Bolder individuals could be accepting the risk of increased predator encounter rates, have an inflated perception of their body size relative to the average predator's gape, or be better able to assess and respond to predation threat than shier individuals (Rodríguez-Prieto et al. 2011; Niemelä et al. 2012; Pascual and Senar 2014). In this study, bolder fish had higher diet generalization than the population mean. The proximity of the habitats in the ponds could have made it easier for bolder fish to forage along the edge, potentially assessing risk before leaving the littoral habitats. In lakes, there is greater separation between habitats where *Daphnia* is found, making the choice to forage in the pelagic a greater commitment. The shape of the relationship between length and diet specialization (e.g. PSi, Figure 15) may resemble a hump-shaped curve in lake ecosystems, where individuals at small lengths specialize on vegetative prey, then incorporate multiple habitats and prey types to become more generalized, and finally become more specialized again at larger lengths as they primarily consume open-water prey. Approximately 10% of individuals in the pond experiment showed very high specialization (>50%) on a single prey type, whereas most of the diets resemble opportunistic foraging. Each highly specialized individual isolated a unique prey type, indicating a strong preference while foraging. Other researchers have suggested that individuals may have varying optimization criteria while foraging (Schoener 1971; Araújo et al. 2011), leading to differences in diet composition. Additionally, positive feedback loops initiated at young ages by learning how to become efficient at foraging certain prey may maintain differences in habitat use and associated behaviors (Sih et al. 2015). Bluegill have complex morphological phenotypes associated with habitat specialization, such that some mouth and body shapes are best suited for littoral or pelagic foraging (Ehlinger and Wilson 1988). It could be that behavioral differences such as foraging boldness are associated with these morphological traits leading to complex adaptive strategies to accommodate heterogeneous environments. While my results show some patterns in specialization, more research on diet composition over time is needed to understand the interplay between behavioral variation, habitat selection, and complex adaptive life history strategies. .

Very little research has examined how individual differences in behavior affect resource and habitat use in the field. Knowledge of how behavioral variation affects habitat use, distribution of individuals within a population, and resource use is imperative for our understanding of ecological processes such as intraspecific competition, predator-prey dynamics, and dispersal. I show that dietary analysis and associated specialization indices can be powerful techniques to facilitate the understanding of how inter-individual differences in behavior interacts with ecological processes. In this study, I combined a well-established, ecologically relevant pattern (shifting habitats across ontogeny), with observations of how individuals react to the driving mechanism behind this pattern (foraging gain-predation risk tradeoff). The outcome is an understanding of how behavioral variation contributes to and explains noise surrounding previously established ecological patterns.

APPENDIX

Table 12. Definition of behavioral variables obtained from laboratory foraging assays used to determine boldness scores (PC1) of individual bluegill sunfish.

Behavioral Variable	**Behaviors Used**	**Definition**
Latency to Consume Prey	Consume	The amount of time that had passed in the assay before the individual consumed bloodworm. A measure of willingness to approach the predator to obtain prey.
Activity	Retreats, Advances	Total activity in the open-water area during the assay. Calculated by summing the distance traveled from retreats and advances.
Average Distance Traveled	Retreats	Mean distance at which an individual retreated. Represents the average distance an individual will venture towards the predator before turning back towards the refuge.
Time in Refuge	Emerge, Vegetation	Total time spent within the vegetation as a measure of willingness to explore novel/risky environments. High values indicate less time exploring.

Table 13. Correlation matrix for behavioral variables obtained from behavioral assays of 323 juvenile bluegill sunfish. ** indicate significant of Spearman Rank correlations at p<0.001. See Table 12 for definitions of behavioral variables.

Behavioral Variable	BC	BD	A	E
Latency to Consume Prey	1	-0.47**	-0.47**	0.47**
Average Distance Traveled		1	0.88**	-0.77**
Activity			1	-0.91**
Time in Refuge				1

Table 14. Directionality and weight of loadings from the Principal Component Analysis on the four behavioral variables (Table 12).

Behavioral Variable	PC1	PC2	PC3	PC4
Latency to Consume Prey	-0.47	-0.64	0.61	-0.06
Average Distance Traveled	0.46	-0.72	-0.36	0.37
Activity	0.55	-0.15	0.18	-0.80
Time in Refuge	-0.52	-0.23	-0.68	-0.46
Proportion Variance Explained	**0.69**	**0.14**	**0.11**	**0.05**

Table 15. Model selection results for logistic functions describing the proportion of *Daphnia* in the diets of 54 juvenile bluegill sunfish. K is the number of parameters, AIC is the Akaike's Information Criterion value, Δ AIC is the difference in AIC values of the specified model with the model with the lowest AIC value (FL+BS). Variables include: FL=Fish Length, BS= Boldness Score (PC1), and FL:BS is an interaction term.

Model	K	log (*L*)	AIC	Δ AIC
Null Model	2	75.3	-146.6	25.5
FL	3	87.6	-169.1	3.0
FL+BS	**4**	90.0	**-172.1**	**0.0**
FL+BS+FL:BS	5	90.0	-170.0	2.1

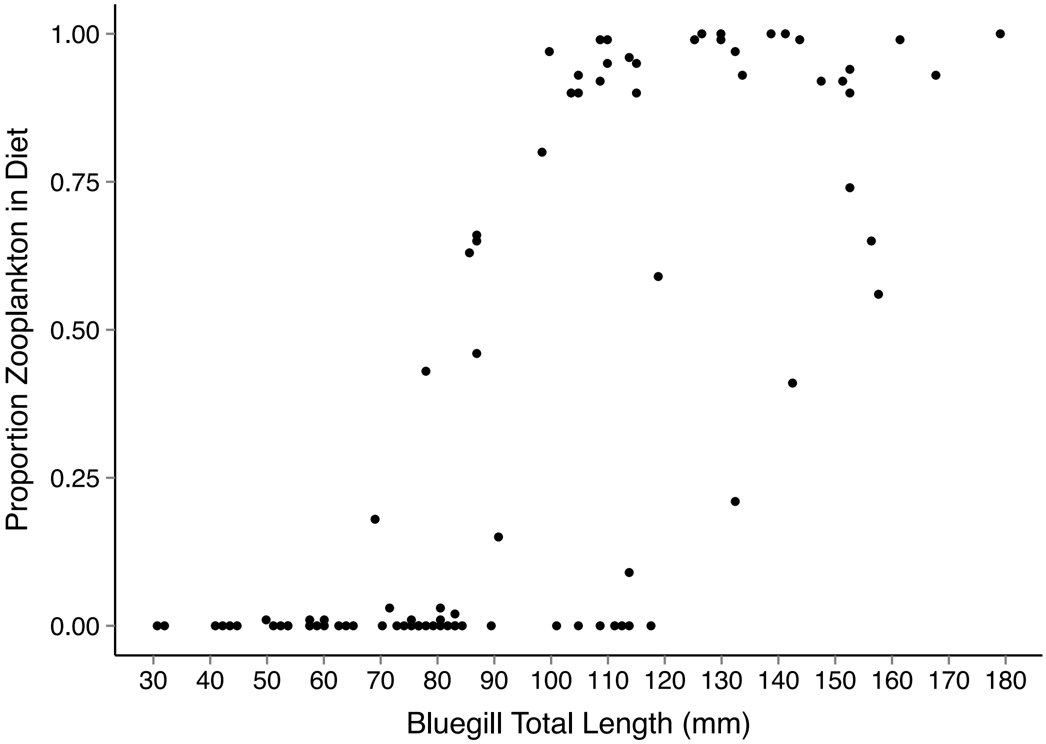

Figure 12. Percent plankton in the diets of bluegill collected from May-August from two small lakes in southwest Michigan. Use of pelagic habitats increases rapidly between 70-80 mm standard length, demonstrating an ontogenetic niche shift. Figure modified from Mittelbach 1984.

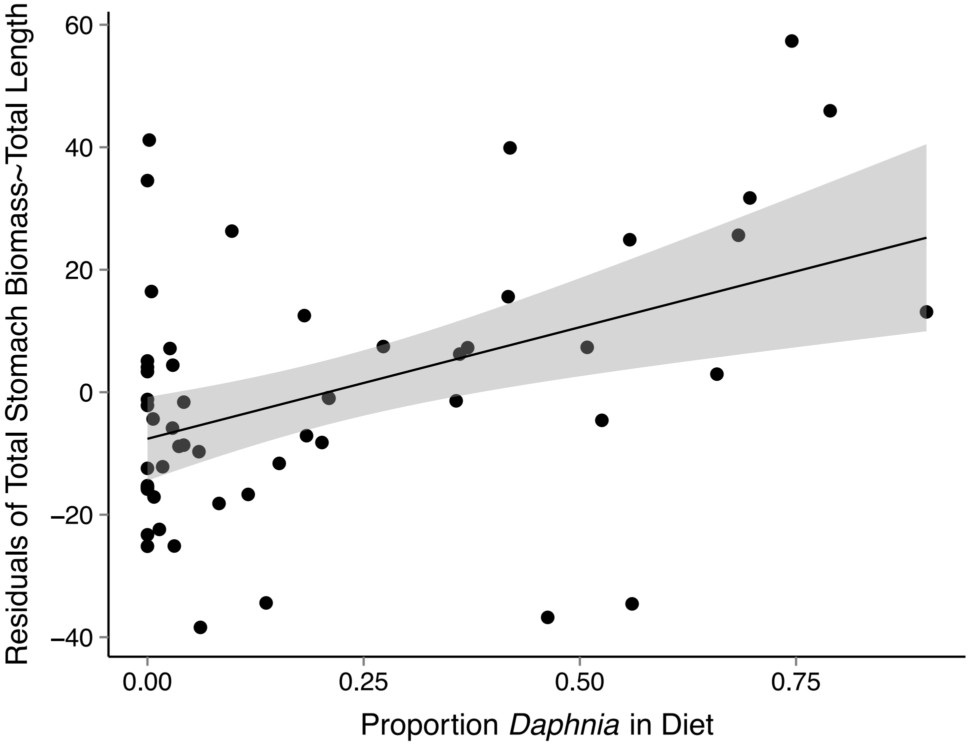

Figure 13. The residuals of a total stomach biomass regressed on total length are modeled as a function of proportion *Daphnia* in the diets of 54 pond bluegill. Individuals that have a higher proportion of *Daphnia* in their diets also have more total biomass in their stomachs. The shaded areas around the regression line indicate 95% confidence bands.

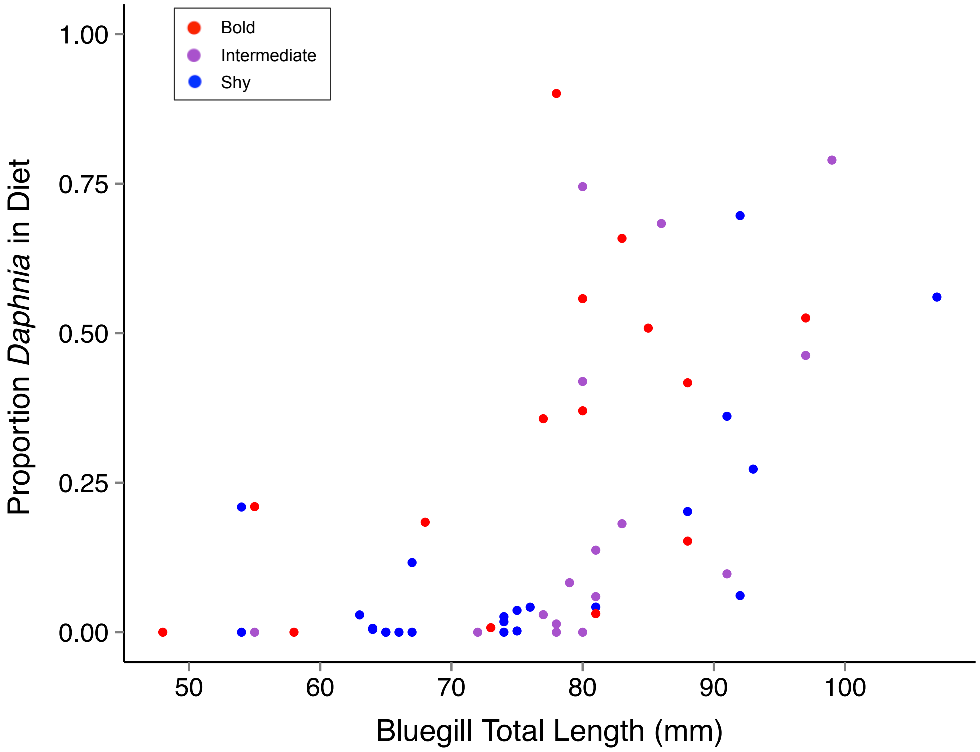

Figure 14. Proportion of *Daphnia* in the diets of 54 bluegill sampled from the pond experiment.

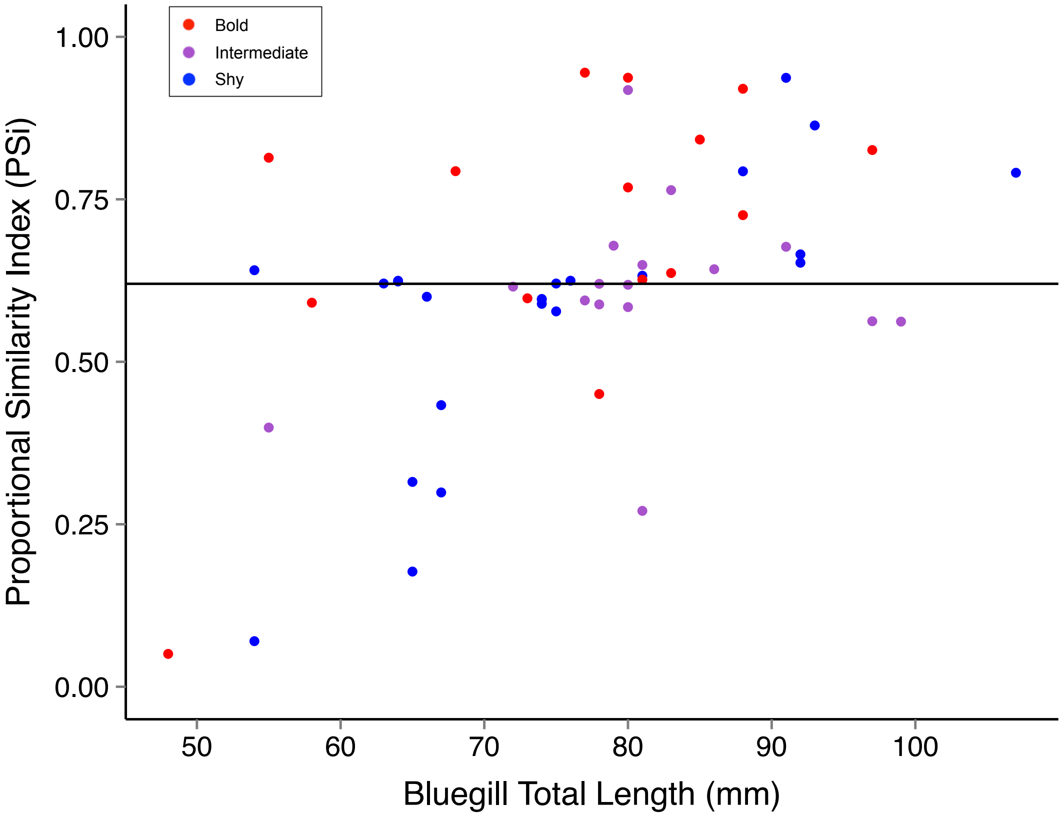

Figure 15. Proportional Similarity Index (PSi) of pond bluegill. Low PSi values indicate diet specialization, whereas diet generalists have higher PSi scores. The horizontal line represents the mean population PSi = 0.62.

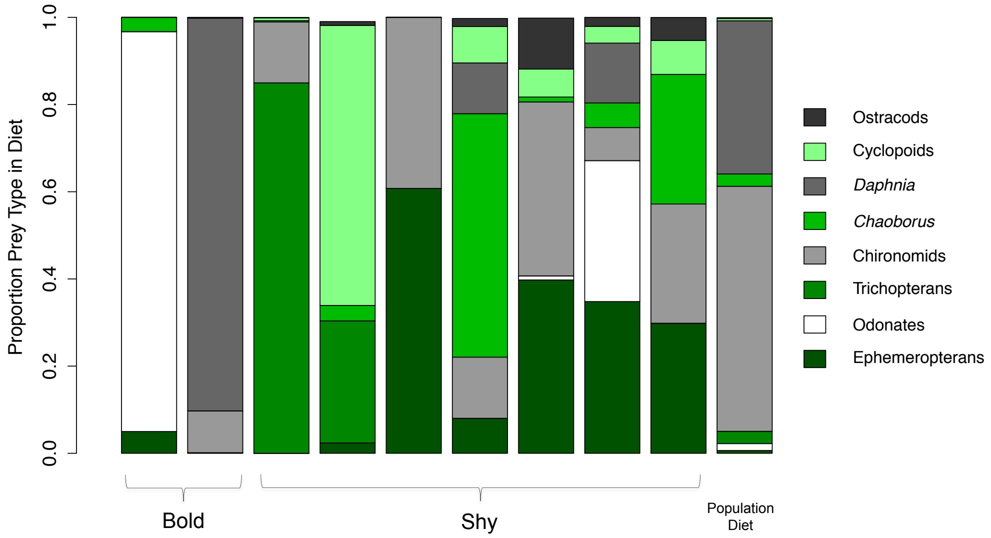

Figure 16. Diet composition of the nine individuals that had Proportional Similarity scores < 0.50, indicating a greater degree of specialization than the population on average (population mean PSi score= 0.62). Six prey types contributing <1% to the diets of these specialists are not shown. For comparison, the mean population diet is presented at the far right.

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CHAPTER FIVE

GUIDELINES FOR THE IMPLEMENTATION OF PASSIVE INTEGRATED TRANSPONDER TECHNOLOGY IN FIELD STUDIES OF FISH BEHAVIOR

Introduction

Background

Studying the behavior of organisms and associated fitness consequences in the field presents many challenges (Archard and Braithwaite 2010; Rehage et al. 2014). The disruption of natural processes and behaviors as a result of observer presence is a common concern for the interpretation of behavioral studies conducted in the field. In addition, aquatic systems are particularly difficult environments to minimize the effect of the observer on behavior. Behavioral ecologists interested in monitoring individual differences in the movement and habitat use of aquatic organisms without disruption would do well to consider various passive approaches used in fisheries management. There are several monitoring systems used to passively track fish movement within a body of water, including radio and acoustic telemetry and Passive Integrated Transponder (PIT) antenna systems. Here, I focus on PIT antenna systems because they are the most economical and thus allow for increased sample sizes for limited budgets (Zydlewski et al. 2006; Karp 2014). Additionally, PIT tag systems have short detection ranges (Zydlewski et al. 2006; Karp 2014), which may be advantageous when assessing fine-scale habitat use of individuals.

The majority of PIT systems currently in use were designed to track the migratory behavior of salmonids, determine the success of various hatchery programs, and examine efficacy of fish passageways (Fuller et al. 2008; Johnston et al. 2009). However, few studies have extended PIT technology from its current use into the field of behavioral ecology as a mechanism for collecting behavioral data on habitat use in species of varying sizes (Rehage et al. 2014). Tracking fish movement using PIT systems has great potential to uncover fine-scale movement patterns (see Armstrong et al. 1999; Rehage et al. 2014) and daily activity rates (see Brännäs et al. 1994 for use in artificial stream), and habitat use of individually marked fish (Rehage et al. 2014). Visual methods such as snorkel observations are limited in the number of fish that can be uniquely identified underwater, whereas PIT tags can be inserted into large quantities of fish without hindering data collection. Additionally, external tags necessary for visual observations are often unnaturally conspicuous, and may increase visibility to predators, whereas PIT tags are internal and unnoticeable (Gibbons and Andrews 2004). Visual observations are also time-limited and biased towards individuals encountered while underwater (e.g., shy fish may hide under perceived threat). Compared to the continuous-time collection of data from PIT tag antennas, snorkeling observations give a limited glimpse into the ecological consequences of behavioral variation.

The investment in hatchery systems throughout the United States has greatly advanced the development of technology behind the use of PIT antenna systems. Antenna systems have greatly increased in the flexibility of ecosystems and the size range of organisms that can be accommodated within the past decade (Meynecke et al. 2008; Bass et al. 2012). Even though technological development has made the use of PIT tag systems easier, there has been relatively little expansion in the usage of PIT technology outside the area of fish management and evaluation of stocking success (Rehage et al. 2014). This trend is largely due to the prohibitive costs to hire companies and the engineering necessary to develop PIT antenna systems specific to diverse project goals. As a result, there has been limited transfer to other fields, such as behavioral ecology, even though the ability to obtain data on habitat use and movement within ecosystems without interfering with the natural behaviors of study organisms is greatly needed.

Objectives

A few researchers have taken the initiative to create their own PIT antenna system rather than incurring the hire costs of commercially engineered systems (Zydlewski et al. 2006; Bond et al. 2007; Steinke et al. 2011). Along these lines, my objectives in this article are to introduce readers to PIT antenna systems and provide enough information for researchers to initiate the antenna design process. First, I offer items for consideration at the beginning of the design phase. Then, I offer instruction regarding the process of antenna design, development, and implementation paired with an example PIT antenna system designed to collect data on the habitat use of juvenile bluegill sunfish in a pond environment.

What are Passive Integrated Transponder (PIT) systems?

Passive Integrated Transponder (PIT) systems are a subset of radio frequency identification (RFID) tags that can be inserted inside an animal's body in order to record the presence of marked individuals at specific sites. Tracking movement of individuals is possible by incorporating multiple antenna sites within a system. When an individual moves from one site to the other, directionality can be obtained. PIT tag systems utilize electrical power sent from the transceiver to an antenna (wire loops) in order to produce a magnetic field that will receive the specified range of the PIT tags. When tags enter an antenna's read range, they are excited and send a unique identification code in the form of radio waves back to the transceiver. The transceiver (called reader from here on), will translate the code from the tag into a unique identification number. PIT tags therefore rely on the antenna to detect the tag and do not need a power source (i.e. battery) to be housed within the tag itself. PIT tag antennas can be used as an active way to scan individuals known to have a tag (e.g., pet microchips, mark-recapture studies) or as a passive way to collect data continuously at a given location (e.g., migratory salmonid stations). In the passive case, a data logger is also necessary to record and store information received from the antenna.

Initial Considerations of Antenna Design

Before beginning the design of a PIT antenna project, researchers should consider if there are alternatives that may be better fit to their project. Potential limitations to PIT tag technology include shorter read ranges than radio telemetry and acoustic methods (Gibbons and Andrews 2004) and the size limitations for small or juvenile organisms less than 45 mm. There is a low possibility of tag rejection in small fish, where the tag is expelled from the organism typically within 1-2 days after injection. It is recommended to monitor tag loss prior to the release of organisms, if possible. Despite these limitations, the most common constraint for the use of PIT technology in basic research is the cost associated with the commercial manufacture of PIT systems. Researchers that have developed their own systems find that costs are greatly reduced (see Bond et al. 2007). The total component costs for the antenna system described in this article were just under $1,000. Researchers should first begin assessing specific components that will work together to meet the objectives of their project. The most important parameters to determine prior to antenna design and implementation are: 1) half duplex or full duplex communication systems and tag selection 2) number and orientation of antennas that may be required, and 3) the available sources of power.

FDX vs HDX systems and tag selection

Identification of the communication system and tags that best fit project objectives is arguably the most important step and should be the first consideration when identifying appropriate components. Antenna systems and associated components are produced in either half duplex (HDX) or full duplex (FDX) (see Bond et al. 2007 for an integrated antenna design). Selection of transceiver (reader) units and tags, along with the design of the antenna, will be largely determined by their compatibility with FDX or HDX systems and their specifications (Table 16). The term duplex refers to the ability to both send and receive data between two units (in this case, antenna to reader). Half duplex systems can only communicate one direction at a time, allowing each unit to either send or receive at a given instant, whereas full duplex allows both units to send and receive simultaneously without interrupting the other unit (e.g., walkie-talkie radio transmission vs. telephone audio transmission). This difference in communication results in HDX systems having a lower read rate than FDX systems (about half as many reads per second; 14 reads/s vs 32 reads/s, Zydlewski et al. 2006). This difference may be important in habitats that have high water velocity, and thus need a system that can optimize tag read rates (Zydlewski et al. 2006).

When conducting research on juvenile fish or other small organisms (~45-90 mm), FDX systems will be necessary. FDX tags are available in smaller sizes (7+ mm) than HDX tags (12+ mm) and can be inserted with a needle, although healing and survival can be better when surgically implanted. HDX tags are larger because of an internal capacitor that stores energy, resulting in a stronger read range than FDX tags of similar size. It should be noted that regardless of whether a tag is HDX or FDX, tags of smaller sizes always have a smaller read range. Due to the shorter read range, FDX tag systems are best used in areas where the antenna can cover the entire stretch of interest (e.g., stream width, defined habitats, fish passageways). Alternatively, studies with objectives to monitor minute differences in specific locations of individuals may benefit from the smaller read ranges of FDX tags.

Additionally, antenna dimensions, tag orientation, interactions with other tags, and any nearby electronic noise can affect read range of tags. HDX systems can withstand more external noise without decreasing system performance. FDX antennas are more sensitive to interference and need to be in an airtight container (e.g., PVC tubing) to avoid the addition of noise from the antenna interacting with both air and water.

Number and orientation of antennas

The number of antennas required will depend on the project goals. Current use of PIT tag antenna systems is most commonly seen in systems where a single antenna can cover the entire passage way (e.g., small streams, fish ladders). These methods allow for detection of individual movement throughout water systems at a large-scale resolution. I suggest that PIT tags could be used to document and monitor patterns in individual differences in movement behavior, activity rates, and habitat use within a population. This fine-scale examination of habitat use will increase the number of antennas required. In order to detect the presence of individuals in areas of interest, paired antennas should be used such that the sequence of reads between the two antennas will determine which direction the fish is moving (see Brännäs et al. 1994). The use of many antennas in one area will require synchronization so that the antennas do not interfere with one another.

There are two antenna orientations that can be implemented: pass-over and pass-through. Pass-over antennas are used in shallow waters, where the read range of the antenna is comparable to water depth (shallow streams). Antennas are installed horizontally along the substrate at the bottom of the water body, and as the name implies, fish pass over the antenna. Pass-through antennas are placed vertically throughout the water column, increasing the maximum water depth at which tags will be detected. There are advantages and disadvantages of each antenna orientation discussed elsewhere (see Steinke et al. 2011).

The number of internal loops (Figure 17) is an additional consideration for the antenna design . An antenna will need to have sufficient read range for 1) the tags selected and 2) the antenna height. As noted before, the read range decreases when using smaller tags (such as FDX). The strongest tag detection occurs along the edges of the antenna. As the size of an antenna increases, the distance from the perimeter to the center of the antenna also increases, which will reduce tag read efficiency. Adding internal loops to the antenna allows for increased antenna size while ensuring tags will be read when passed through all areas of the antenna.

Power sources

The environmental conditions and available infrastructure surrounding the site of installment will dictate the type of power supply to be used. Typically, assessment of available sunlight will determine whether battery or solar powered systems are used when site locations are remote. However, if there is AC power nearby, it is the easiest and most reliable power source. Specific amperage and voltage required is dependent on the type of reader that is purchased (two models compared in Steinke et al. 2011).

As a final consideration for the design and implementation of PIT antenna systems for research on individual-level movement behavior, it is important to designate adequate time for testing the antenna throughout the design process. There are several components that need to be tested independently and as a part of the system to determine whether the antenna design is sufficient for reading tags. Additionally, testing the antenna design will require an appropriate amount of space to mimic the design as it would be in the field, and reduce the potential for issues as the antenna is transferred to the installation site.

Designing the Antenna

In this section I will describe the antenna design process using the developmental process I used as an example. My goal was to develop a pilot antenna to monitor the habitat use and movement behaviors of juvenile bluegill sunfish (50-75 mm) within an experimental pond at the Kellogg Biological Station. Several of the ponds had recently undergone renovation and natural vegetation had not yet colonized, creating an opportunity to manipulate the location and density of artificial refuge habitats available for juvenile bluegill within the pond. When juvenile bluegill are small, they are restricted to the vegetated littoral zone for protection against piscivorous predators, such as largemouth bass (*Micropterus salmoides,* Werner et al. 1983). Therefore, I designed refuge stations to include a pass-through antenna as an opening with artificial vegetative structure enclosed to the shore on both sides with nylon mesh seine material (Figure 18).

FDX vs. HDX systems and tag selection

The juvenile bluegill I used were quite small (50-75 mm), so I was limited to the FDX 7 mm tags, setting the communication system to full-duplex. One potential drawback of FDX is the interference with surrounding electrical noise. However, the location of the experimental pond facility is relatively remote, so this was not a concern. Additionally, FDX systems are more sensitive to the interchange between air and water, so enclosing the wires within a container (PVC) was an additional requirement.

Number and orientation of antennas

To test the efficacy of using PIT tag antenna systems as previously described, I aimed to create one antenna to pilot the system for use in the ponds. The pilot antenna was to be placed at the opening of vegetative refuge structures, with dimensions around 3.5 ft tall and 3 ft wide. I selected a pass-through antenna orientation due to the nature of the design of the vegetative refuge structures (Figure 18) along with the limits in water depth used with pass-over antennas. The small FDX tags used for this study required several internal loops into the antenna design (Figure 18) to ensure tags would be read at any location within the antenna structure. If I were to scale up to a full design to track the movement and habitat use of juvenile bluegill, I would need to incorporate two antennas in series at the entrance of each refuge station to ensure that directionality of fish movement could be determined (e.g., moving in or out of the refuge).

Reader selection and power sources

I used an Allflex RM 310 reader to decode the PIT tags as it can read FDX tags and was within the limits of my budget. The Allflex RM 310 can power one antenna and costs approximately \$495. Another model commonly used for larger studies, the Destron Fearing FS1001, can power up to 6 antennas, but costs approximately \$6K (see Steinke et al. 2011 for more details.). The RM310 reader requires 6-12 V DC and 2 A output for proper usage. The experimental pond lab facility has power sources located within short reach of the ponds. Therefore, I was able to utilize an AC power source for my pilot antenna. I selected an AC/DC converter with a linear 12 V DC, 2 A adjustable output.

Antenna dimensions and tuning

My antenna design underwent multiple iterations with varying dimensions of the antenna structure and the number of internal loops. During the design phase, it was easiest to set up boards to string wire around nails to secure the wires and provide a platform to test multiple designs (Figure 19). The first test to determine efficacy of the design is to ensure that the inductance is within 220-280 µH (optimal tag reading range). To do this, attach an LCR meter (on the 1kHz setting) meter to both ends of the wire loop. I used CAT6 ethernet cable (four pairs of 24 gauge solid copper wire) with each of the partner wires soldered to one another (e.g., solid yellow to striped yellow, Figure 20). The four pairs of wire within the CAT6 cable allow for a convenient way to test inductance of the antenna with 1-4 turns (loops of wire) during the design and testing phase. For example, start with one turn by using the ends of one set of wires (e.g., green), then test two turns by soldering one end of the initial loop to an end from another color (e.g., one green end connected to one yellow end). This would result in the output from the antenna being two different colors (e.g., green and yellow). The inductance of the antenna can be tested with up to four turns by connecting the ends of the remaining colors (Figure 20).

I piloted a design that was 32 inches wide and 36 inches tall. In order to increase the tag read efficiency within the center of the antenna, I added six internal loops (6 inches each, Figure 17). This design consistently had an inductance in the optimal range when the antenna had four turns (235-240 μH). Next, I attached an Allflex RM 310 reader module by soldering the two wire ends from the antenna loop to the incoming and outgoing wires provided from the reader. The RM310 also supplies wires for attaching the power supply. After attaching the power supply, optimize the current being relayed to the antenna by placing a multimeter capable of reading up to 2-3 amps in series with the circuit. The RM 310 supplies jumpers on the reader board to add or remove when optimizing amperage. Connect enough wire from the antenna to the reader to resemble what will be needed in the field. Adding this wire should not affect the inductance of the antenna, but it is best to test for changes in performance throughout the process.

Tag detection and read range

As soon as the antenna system is producing the correct inductance and has optimal amperage, it is time to test whether a tag can be detected. To ensure optimal performance, set up the antenna system in an area that will have minimal electrical noise (e.g., no fluorescent lights). Connect the power supply directly to the reader and set up the system to resemble the positioning it will have in the field. At this time it is important that slack in the cables is minimized. Any loops resulting from cable slack will interfere with the inductance and detection of tags. Once the pilot system is set up, secure the PIT tag into or onto an item that will allow easy determination of its orientation (e.g., parallel or perpendicular to the antenna). For example, I taped a PIT tag to a piece of paper large enough to minimize movement within a plastic vial. Tag detection is tested by slowly moving the secured tag (parallel to the ground) through the antenna. The green light on the RM310 reader will flash if the tag is detected. Determining tag detection at various points within and along the antenna is crucial for the success of the project. The point(s) furthest away from the corners will be least likely to read a tag (center of open area). If there are internal loops, check that the tag can be read in the center and corners of each loop. Assess whether the read distance is suitable for the project objectives by documenting the distance away from the antenna at which the tag is first read. For my project objectives, I wanted a very small read range to reduce the incidence of tags being read as a fish is swimming near, but not into the vegetative refuge structures. To test that the tag identification code is accurate, connect the reader to a data logging/computer device. The RM 310 reader has pre-installed output with a serial baud RS232 port connection. The RS232 connection can be used with an USB-RS232 adapter to accommodate various models of computers or data logging devices. Check the reader manual to determine which of the available programs will communicate with the reader.

Field testing

After the pilot antenna was proven to detect tags at appropriate distances and at all areas within the antenna structure, I tested the performance of the entire antenna system in an experimental pond. I set up the antenna in the water and stretched the connecting cable to the shore. I ran a tag secured in an airtight plastic vial through the antenna and found the antenna performance was maintained. Additionally, I informally tested the performance of the antenna in a large outdoor holding tank that contained bluegill with PIT tags. I found that the antenna detected tags when fish traveled through the loops.

Discussion

The advantages of Passive Integrated Transponder provide a compelling case for the implementation of the technique to monitor individual differences in behaviors such as movement, activity, and habitat use at a time when field research on the ecological consequences of behavioral variation is needed. There have been several calls for researchers to supplement existing data from laboratory experiments with studies conducted in the field (Bolnick et al. 2011; Dall et al. 2012; Sih et al. 2012; Wolf and Weissing 2012; Mittelbach et al. 2014). PIT systems have the potential to assist researchers with these questions by collecting spatial and temporal data on inter-individual behavioral differences in field environments (e.g., Brännäs et al. 1994; Armstrong et al. 1999; Sloman et al. 2002; Rehage et al. 2014). In addition, stationary antennas allow the data to be collected passively without disrupting the study organism and their natural behaviors. In contrast to several external marking techniques that can disappear or heal over time, PIT tags are permanent internal tags. The combination of continuous passive data collection along with permanent identification tags provides a potentially powerful mechanism to observe the long-term dynamics of inter-individual behavioral differences.

There have been few accounts of the use of PIT technology to quantify individual differences in behavior in the field (but see Armstrong et al. 2005; Rehage et al. 2014), likely because of prohibitive purchasing costs and a lack of guidance to assist researchers in developing their own antenna systems. In this article I shared the developmental process as I designed an antenna for monitoring juvenile bluegill habitat use in an experimental pond. The cumulative cost of the components described here were just under $1,000, providing a reasonable alternative to purchase costs from engineering companies. I show that PIT antenna systems can be made for a variety of contexts, but the full implementation of a research program examining individual differences behavior has yet to be piloted.

APPENDIX

Table 16. Comparison of specifications for use of full duplex and half duplex antenna systems

Specifications	**FDX**	**HDX**
Tag size	Smaller: 7-23 mm	Larger: 12, 23 or 32 mm
Fish Size Ranges	Smaller: 45+ mm	Larger: 100+ mm
Read Range	Smaller: 20-360 mm with 12 mm tags, up to 900 mm with 23 mm tags	Larger: 1000 mm, reduced with 12 mm tags
Read Rate	Faster: 32 reads/second	Slower: 14 reads/second
Size of Antenna	Smaller	Larger
Power Needed	More	Less
Habitat Limitations	Limitations in antenna size: Dependent on design and objective of projects	High water velocity

Figure 17. Antenna design for monitoring habitat use of juvenile bluegill sunfish in an experimental pond. The wire loops are made with CAT 6 ethernet cable contained inside PVC tubing. The incorporation of several internal loops was required to accommodate the limited read range for small (7 mm FDX) PIT tags. Each internal loop is 6 inches in height, with total antenna dimensions of 36 in (height) x 32 inches (width).

Figure 18. Artificial vegetation refuge structures were piloted in an experimental pond. The antenna described in this article was designed to be placed at the opening of the habitat structure to collect individual-level data on the habitat use of juvenile bluegill sunfish.

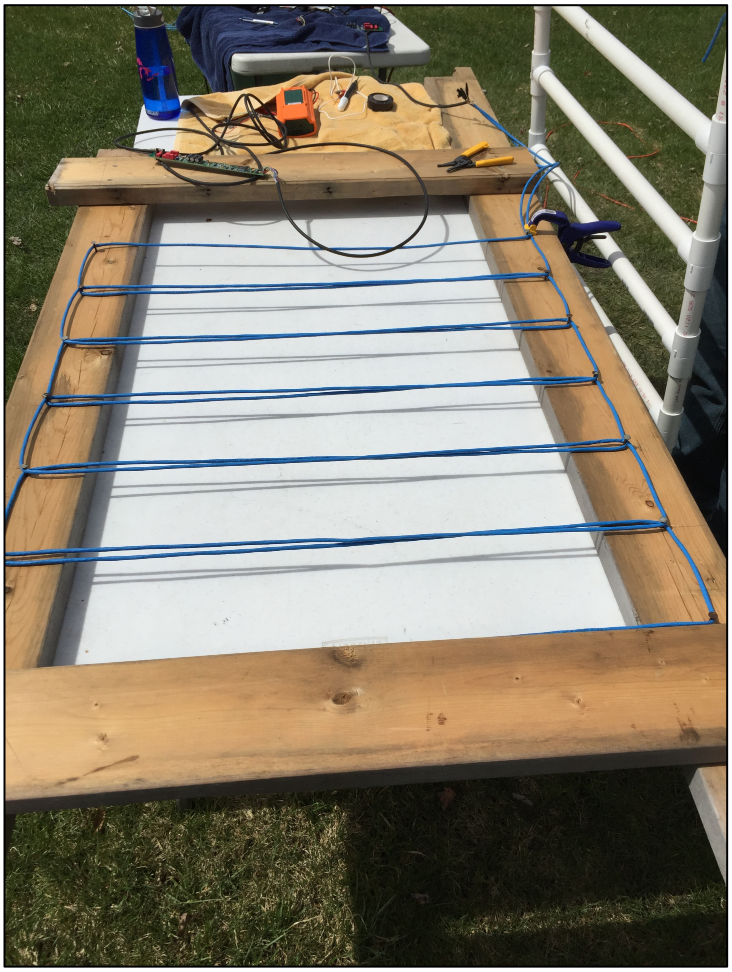

Figure 19. Example of CAT6 ethernet cable wrapped around nails to iteratively test the efficacy varying antenna designs.

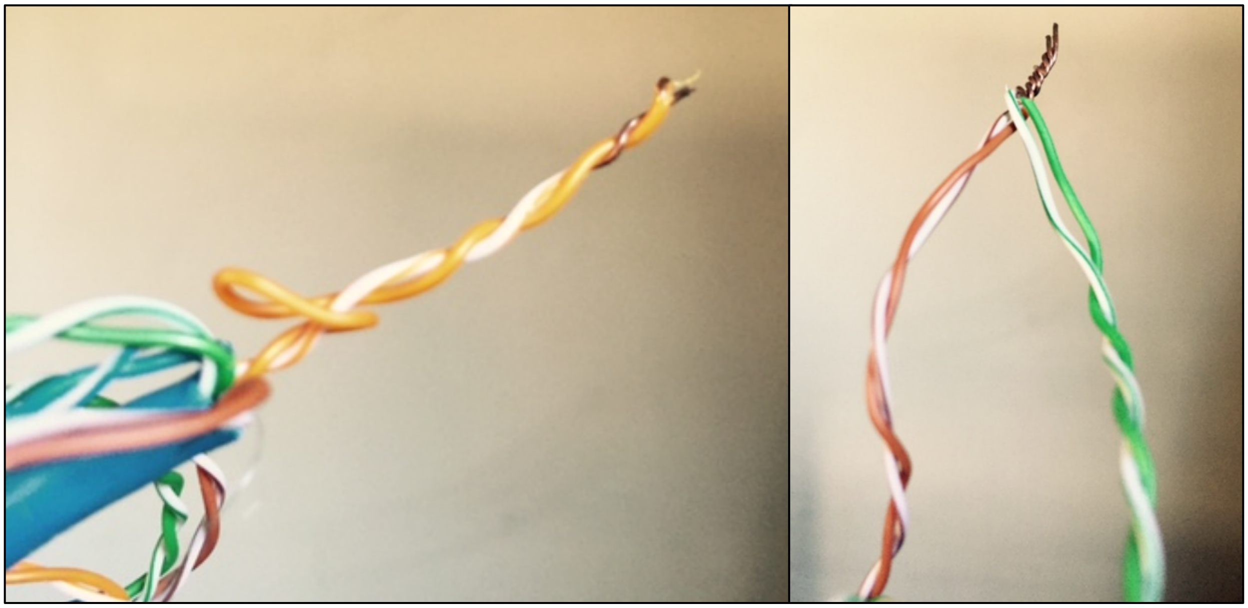

Figure 20. CAT6 Ethernet cable provides four pairs of wire that can be used to produce up to four turns within the antenna structure. The two strands of each color (e.g. solid and striped yellow, left image) should be connected prior to producing loops (shown pre-soldered here). After each end is soldered to its partner, loops can be produced by connecting the ends of two colors (right image).

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