

THE EFFECTS OF BOLDNESS ON THREAT-SENSITIVE DECISIONS IN FISHES

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Abstract

Personality describes the consistent, individual differences in the behaviour we observe among human and non-human models alike. One of the components of human and animal personality which has sparked interest, is the boldness trait. Boldness is the propensity to engage in risk taking behaviour, and as such, has important ecological impacts on the interactions between animals. By changing the amount of risk taking behaviour, boldness effectively alters the outcome of predator-prey interactions. Boldness may alter a prey's decision between an antipredator response or continuing other important behaviours (e.g. foraging or reproduction) when faced with a predator. The difference in response may be the result of a changed perception in the amount of local risk, or due to a differential amount of information gathered from the environment. In either case, more information about local predators increases the likelihood that a prey will appropriately respond to a threat. In aquatic systems, prey often receive chemical information from the scent of predators or chemical released by conspecifics. Specifically, alarm cues are released following damage to the skin tissue of many prey species and thus are reliable indicators of risk. These alarm cues are often the first line of prey defense, as they degrade slowly and are passed through the aquatic medium in currents. Secondary information regarding local risk often comes from the visual cues associated with the sight of a predator or frightened conspecifics. This provides prey with reliable information about risk since the transmission distance of visual cues is limited in water and further lessened by vegetation and turbidity. My experiment demonstrates that boldness in fathead minnows (*Pimephales promelas*) is stable over shorter temporal scales, and subsequently, affects how minnows acclimate to their environment and respond to visual and chemical information on local threats. In low risk environments, personality and turbidity strongly affect the threat-sensitive response of minnows. Shy minnows demonstrate no antipredator response when in turbid water and bold minnows elicit a strong fright response in turbid conditions. In clear conditions, the opposite occurs with large fright responses in shy minnows and no response in bold minnows. Conversely, when minnows were exposed to high amounts of environmental risk through the exposure to alarm cues, they all elicit strong fright responses regardless of personality or turbidity. This is the first experiment to investigate the complex interplay of personality and multiple cue types on the threat-sensitive response of prey fish.

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Chapter 1: Introduction

1.1. Human Personality

Many differences in human behavioural tendencies can be observed in our everyday interactions (Sih et al., 2004b). Personality is an individual's pattern of behaviour that is consistent over time and situation. One branch of personality theories attempts to define the basic dimensions of personality by focusing on each personality trait of humans. The aim is to denote the different ways in which humans will act based on personal characteristic or traits (Sneed et al., 1998). More specifically, psychologists developed a five trait model as a summation of the research on the most basic personality traits (McAdams, 1992; Norman, 1963). The five-factor model (FFM) has become the most widely accepted model to study personality traits and includes: (1) extraversion (2) openness, (3) neuroticism, (4) agreeableness, and (5) conscientiousness (Digman, 1990; McCrae and Costa, 1997). Since the FFM is hierarchical in nature, each of the categories can be further divided into "adjectives", with their respective measurable scale (McCrae and John, 1992). For example, an extraverted person would be defined by adjectives such as "active" and "assertive", and could be measured by total "warmth" or "activity" that they display (McCrae and John, 1992). Finally, the five factors are demonstrated to be repeatable over decades in adults such that they provide a reliable way to measure personality (McCrae and Costa, 1997; McCrae and John, 1992).

Application to Non-Human Models

Even though most scientists readily accept the similarity of animal physiology and anatomy to humans, they are reluctant to ascribe personality traits, cognition, and emotions to animals (Gosling and John, 1999). Nevertheless, our interactions with animals, especially domesticated pets, reinforce our belief that animals do possess some level of consistency among their behaviours. For this reason, pioneer studies such by Stevenson-Hinde were investigating the personality of primates by the 1970s (Stevenson-Hinde and Zunz, 1978). Additionally, scientists were observing analogous patterns of personality in laboratory rodents and domesticated animals (Gosling and John, 1999; Kilgour, 1975). Arising in multiple different fields of animal research, personality has been labelled using multiple terms including: coping styles (Koolhaas et al.,

1999), temperaments (Buss and Finn, 1987), strategies, behavioural tendencies, constructs, axes (Gosling, 2001), and behavioural syndromes (Sih et al., 2004b).

1.2. Personality Research in Animals

By definition, animal personality refers to consistent, within-individual, behavioural traits (Biro and Stamps, 2008; Dingemanse and Reale, 2005; Drent et al., 2003; Gosling, 2001). Similar to the study of personality in humans, temperament in animals can be observed at two levels: (1) context specific behaviours, such as the similarities in predator avoidance across different types of habitat, or (2) across context behaviours, such as the correlations between dispersal, mating, and aggressive behaviours (Sih et al., 2004b).

The importance of animal personality has been highlighted by recent studies that have revealed personality to be both heritable (Reale et al., 2007; van Oers et al., 2005) and affecting fitness (Dingemanse and Reale, 2005; Smith and Blumstein, 2008). For this reason, certain personality types may be subject to evolutionary pressures similar to those of physical traits (Gosling and John, 1999). More specifically, one personality type may convey benefits to an individual allowing them to survive longer or have higher reproductive success. Since personality may be inherited by offspring, we may expect to see one optimal personality type proliferating through generations. However, in natural ecosystems and among humans we see a variety of different personality types rather than one dominant type. As such, we may deduce that different personalities are adaptive or maladaptive, depending on the specific context in which the individual is in (Sih et al., 2004a).

Fitness Consequences of Personality

The exact fitness consequences associated with different personality traits often vary according to shifting environmental conditions including availability of food (Dingemanse et al., 2004), social conditions (Cote et al., 2008), and predation pressure (Reale and Festa-Bianchet, 2003). The first hypothesis that explains the stability of different personalities is the idea of frequency-dependent selection, where the fitness of the most frequent phenotype is lower than that of a less frequent phenotype (Dall et al., 2004; Gross, 1996). Thus, the personality type with higher fitness is a dynamic process dependent on its frequency within the population.

Alternatively, the context-specific hypothesis of personality explains that the fitness trade-offs

associated with personality traits drive differences in personality (Sinn et al., 2008). For example, a shy individual may spend less time actively foraging under the threat of a predator, but be exposed to less predation risk while under refuge. As such, a shier individual's increased survival comes at the cost of less time spent foraging or reproducing (Lima, 1998).

Results from a meta-analysis demonstrated a direct link between boldness and exploration, and fitness factors including reproductive success and survival (Smith and Blumstein, 2008). In particular, the boldness dimension had clear trade-offs between reproductive fitness and survival. Across bolder mammals and fish, there was an increase in reproductive success and a decrease in survival among captive populations, but no trend was observed within wild populations (Smith and Blumstein, 2008). The effect on males was more pronounced; bold males increased their reproductive fitness up to 4x more than bold females, at the cost of decreased survival. When looking at the meta-analysis for exploration behaviour, there was a weak increase in the survival of birds and mammals who explored more. However, this increased survival was not observed in males or in wild animals (Smith and Blumstein, 2008).

Framework for Animal Personality

Multiple terms have been proposed to define aspects of animal personality. Due to the recent debates about personality-related terminologies, I will refer to the theoretical framework composed of ideas from Mittelbach et al. (2014), Stamps and Groothuis (2010), and Sih and Bell (2008). In particular, I will use the following definitions: (1) behavioural response: an individual's context-dependent action or response; (2) personality trait: a behavioural pattern that characterizes consistent individual differences in behaviour in a given type of situation; (3) personality type: an individual's consistent response over a given period of time relative to other individuals for one or multiple traits; (4) animal personality: behavioural pattern that can describe multiple behavioural traits and the relationship between those traits over time (Mittelbach et al., 2014; Reale et al., 2007; Stamps and Groothuis, 2010). I chose this set of personality related terminology because the definitions are easy to understand and concise, and as such, they provide an excellent theoretical framework for my personality based research.

Five Axes of Animal Personality

Reale et al. (2007) proposed a theoretical framework for the study of behavioural syndromes in non-human models similar to the FFM for human personality traits. This contained five broad axes of animal personality including: (1) shyness-boldness, (2) exploration-avoidance, (3) activity, (4) aggressiveness, and (5) sociability. These were defined as the five major categories of personality research, which possessed distinct measurable differences, but also had convergent properties. They defined boldness as the measure of an individual's reaction to a perceived risk, which in most cases, is the presence of a predator (Reale et al., 2007). To separate boldness from other potentially risky behaviour such as exploration and activity, boldness would be measured in the presence of a predator rather than to a novel stimuli or environments. As such, the exploration axis would encompass individual behaviours that include the willingness to investigate novel environments, objects, or food items without any predation risk (Conrad et al., 2011). Conversely, the activity axis of personality would measure the frequency or quantity of movement in a safe and familiar environment (Conrad et al., 2011; Reale et al., 2007). However, this axis has come under scrutiny since the differences in activity may be due to intraspecific variation in organ size and resting metabolic rates (Careau et al., 2008; Nespolo and Franco, 2007). The fourth axis, aggression, may occur in a wide variety of contexts and developmental stages among organisms as the result of competition for territory, mates, or resources (Conrad et al., 2011). Most studies of aggression focus on the correlation between aggression and other personality traits rather than direct measures of aggression. Finally, the sociability axis is the summation of an individual's reactions to conspecifics or their cues, with asocial individuals avoiding others and social individuals being attracted to others (Reale et al., 2007). Although measures of sociability avoid aggressive interactions, they still form a part of the social interactions in most animals (Conrad et al., 2011). For this reason and others stated previously, measuring a sole personality axis is difficult in animals due to the interconnectivity of personality traits.

1.3. Predation

The act of predation is defined as the consumption of all or part of one living organism by another. It is an intense selective force acting on prey through the consequences of direct consumption (Lima and Dill, 1990; Volterra, 1926) or non-consumptive effects, including

reduced time spent foraging (Brown and Kotler, 2004; Lima, 1998; Lind and Cresswell, 2005). As such, the risk of predation often induces many phenotypically plastic responses in prey (DeWitt et al., 1998; Miner et al., 2005). In particular, prey may alter their life-history traits such as the timing or size of sexual maturation (Abrams and Rowe, 1996) or the timing of life-history switches in order to decrease their predation threat in a particular stage (Chivers et al., 2001a; Sih and Moore, 1993). Other prey may modify their morphology (e.g. body size or depth) to decrease the chance of predator attacks and to increase their survival following an attack (Relyea, 2001). However, prey species are often forced to alter their behaviour in order to deal with immediate threats. This is advantageous for prey because plastic behavioural changes are also energetically cheaper than the morphological and life-history alternatives (Lima and Dill, 1990). Nevertheless, displaying appropriate behavioural responses come at the cost of other fitness-enhancing behaviour such as foraging, defending territories, and/or reproduction (Lima and Dill, 1990). For this reason, it is critical for prey to optimize their antipredator response to match the amount of local threat (Chivers et al., 2001b). However, predation risk is often variable in space and time, such that prey must decide whether the cues used to gauge risk represent the actual risk of the environment (Dall and Johnstone, 2002). Often this leads prey species to adopt a “play it safe” strategy and display a hypersensitive response to predation threat (Bouskila and Blumstein, 1992).

1.4. Information about Local Predators

Prey are often forced to balance their time and energy spent between activities, such as foraging, reproduction, and predator avoidance (Lima and Dill, 1990). To optimize the trade-off between these behaviours, prey must be able to accurately assess the amount of local threat and respond appropriately (McNamara and Dall, 2010; Schmidt et al., 2010). This is known as the threat-sensitive predator avoidance hypothesis (Helfman, 1989). For example, if prey spend too much time remaining vigilant for predators, they cannot forage or actively look for mates. However, if they are not vigilant, their survival chance is dramatically decreased.

Chemical Cues

Within aquatic systems, chemosensory cues are commonly used by prey to assess local threats and respond accordingly (Brown, 2003; Ferrari et al., 2010b). These cues can be released

directly by a predator in their scent, known as a kairomone (Watson et al., 2004), or from their dietary cues (Brown et al., 2000). Responses to predatory cues can be innate, where prey will respond to specific predators upon their first detection (Ferrari et al., 2010b). This occurs in some taxa including freshwater snails (Dalesman et al., 2007; Turner, 1996), salmonoid fishes (Berejikian et al., 2003; Vilhunen and Hirvonen, 2003), and salamanders (Epp and Gabor, 2008; Mathis et al., 2003). However, many species must learn that the predator's scent represents a risk from their conspecifics through social learning (Ferrari et al., 2008b; Mathis et al., 1996) or conditioning with alarm cues (Crane and Ferrari, 2015b).

Alternatively, prey may receive chemical cues released by frightened conspecifics, known as disturbance cues, and through the chemicals released following damage in the skin of conspecifics (Chivers et al., 2012; Ferrari et al., 2010b). Alarm cues are chemicals located in the skin of the prey and released in the water column only via mechanical damage to the skin. Due to the nature of their release, alarm cues represent a reliable indicator of a local, actively foraging predator and are known to elicit strong antipredator responses when detected by nearby conspecifics (Chivers et al., 2012; Chivers and Smith, 1998). Numerous taxa rely on alarm cues including sea anemones, gastropods, echinoderms, larval amphibians, and fishes (Chivers and Smith, 1998) as an indicator of local risk. For this reason, prey who respond to alarm cues often increase their chance of survival (Mathis and Smith, 1993; Mirza and Chivers, 2003; Wisenden et al., 1999).

While such chemical information is always available in the aqueous medium, their reliability in space and time may be questioned (Ferrari et al., 2007). Chemical cues may persist in aquatic systems from minutes to days in laboratory and field settings (Ferrari et al., 2010b; Wisenden et al., 2009b) after their release, and currents may move these cues such that they are spatially and temporally inaccurate. However, alarm cues should be in higher concentration near a site of attack (or release), and hence, prey are known to respond to cues in a graded manner (Helfman, 1989), with high concentration of alarm cue eliciting a stronger antipredator response (Brown et al., 2013; Ferrari et al., 2005; Zhao and Chivers, 2005).

Visual Cues and Turbidity

As opposed to chemical cues, visual cues provide prey with more spatially and temporally reliable indicators of local predation risk (Lythgoe, 1979). These visual cues often come from the direct sight of a predator or through the reaction of other conspecifics to a threat. By their nature, visual cues are often risky to prey (Lythgoe, 1979) because their transmission distance through water may be limited and further degraded by vegetation (Ferrari et al., 2014; Mathis and Vincent, 2000) and turbidity (Abrahams and Kattenfeld, 1997).

Turbidity is defined as a measure of transparency of water and is affected by algal blooms, suspended solids, and dissolved organic carbon. It is most commonly measured through the degree to which particles within the water scatter light and is measured in nephelometric turbidity units (NTU). As the amount of NTUs of water increase, so does the opacity from clear water at around 5-10 NTU, up to 100 NTU in turbid rivers. As such, turbidity effectively reduces the amount of visual cues available to prey and has been demonstrated to alter the outcomes of predator-prey interactions in freshwater fishes (Bonner and Wilde, 2002; Gregory, 1993; Zamor and Grossman, 2007). In the case of prey, turbidity may reduce predation risk in northern pike (*Esox Lucius*; Lehtiniemi et al., 2005), white sturgeon (*Acipenser transmontanus*; Gadomski and Parsley, 2005), delta smelt (*Hypomesus transpacificus*; Ferrari et al., 2014), and allow fathead minnows to use dangerous habitats (Abrahams and Kattenfeld, 1997). In addition, turbidity may also benefit prey species by reducing the efficacy of visual predators. For example, turbidity lowered the foraging efficiency and prey selectivity in northern pike (Jonsson et al., 2013). In contrast, high levels of turbidity may negatively affect prey species by reducing predator recognition and generalization (Ferrari et al., 2010a), and prey's reaction distance to a predator (Quesenberry et al., 2007; Vogel and Beauchamp, 1999). Together, these findings highlight the importance of turbidity on the ability of prey to obtain accurate information and make optimal decisions when faced with a threat.

Information Complementation

Prey that can detect multiple cues should be able to show fine adjustments in response to a local threat (Brown and Magnavacca, 2003; Smith and Belk, 2001). According to the "sensory complementation hypothesis" (Lima and Streury, 2005), multiple cues should act in additive or

synergistic fashion. In particular, low concentrations of alarm cue may be ubiquitous in some systems due to the slow rate at which they deteriorate, such that they elicit a slight increase in vigilance or no antipredator response. However, with the complementary presence of visual cues, chemical cues often elicit very strong antipredator responses. For example, glowlight tetras (*Hemigrammus erythrozonus*) exposed to a low concentration of an alarm cue did not exhibit any antipredator behaviour in the absence of visual cues. In contrast, when they were exposed to a visually alarmed conspecific and alarm cue simultaneously, tetras significantly increased the intensity of their antipredator response (Brown et al., 2004). Alternatively, prey species may compensate for a lack of visual or chemical cues by increasing their antipredator response. When the visual cues of predators were degraded in turbid water, fathead minnows demonstrated a stronger antipredator response to chemical alarm cues (Hartman and Abrahams, 2000).

1.5. Thesis Objectives

In chapter 2, my first objective was to summarize our current knowledge of boldness and its measures in fishes. Particularly, my plan was to use emergence trials to measure the boldness of fathead minnows. However, emergence trials had never been conducted on fathead minnows and therefore, I needed to create and refine a methodology based on the emergence trials of other species (for review see Naslund et al., 2015). My goal was to have > 40% of minnows emerging during trials and a distribution of personality scores to compare individuals. In Chapter 3, my objective was to assess the effect of boldness on risk assessment in minnows. Specifically, I exposed minnows to alarm cues (high risk cue) or water (low risk cue) in either a clear or turbid environment, and recorded their antipredator responses. I hypothesized that if personality impacts the way minnows gauge threat, bold minnows should demonstrate less of an antipredator response than shy individuals when they are faced with similar amounts of chemical and visual cues. Finally, in Chapter 4, I discussed my results and the implications of my threat-sensitive experiment along with addressing some further questions.

Chapter 2: Methodological Development for Boldness

A relatively well-studied animal personality trait is the shy/bold continuum (Wilson et al., 1994). Boldness is defined as the propensity to engage in risk-taking and is often measured following a predation attempt or exposure to a novel habitat (Huntingford, 1976). Bolder individuals are more likely to inspect a predator (Dugatkin, 1992; Huntingford, 1976; Pitcher et al., 1986) and receive more accurate information regarding the predator (Brown and Magnavacca, 2003; Dugatkin and Godin, 1992). In addition, bold individuals are more likely to explore novel habitats and disperse further (Cote et al., 2010). However, individuals who inspect predators and novel habitats are at higher risk of damage and mortality (Dugatkin, 1992).

2.2. Boldness Across Taxa

The Shyness-Boldness axis spans multiple taxa including fishes, mammals, birds, amphibians, and invertebrates (Conrad et al., 2011). In mammals, some influential studies of boldness include: boldness of male bighorn sheep rams (*Ovis canadensis*) (Reale et al., 2009); boldness of captive-bred foxes (*Vulpes velox*) and their conservation (Bremner-Harrison et al., 2004); boldness and reproductive rates in grey mice (*Mus musculus*) (Dammhahn, 2012). Comparatively, avian studies include: boldness and dispersal in great tits (*Parus major*) (Dingemanse et al., 2003); boldness as a predictor for stress levels in urban dark-eyed juncos (*Junco hyemalis*) (Atwell et al., 2012). For the collective of invertebrates, amphibians and reptiles some influential studies include: boldness through ontogenetic shifts in lake frogs (*Rana ridibunda*) (Wilson and Krause, 2012); boldness and spatial learning of Eastern water skink (*Eulamprus quoyii*) (Carazo et al., 2014); boldness as a result of differential nutrition in hissing cockroaches (*Gromphadorhina portentosa*) (Mishra et al., 2011). These experiments demonstrate the breadth of boldness research across multiple taxon, which possess measurable differences in boldness.

Boldness and risk-taking tactics have been most extensively studied across a wide variety of fish including members of: *Centrarchidae*, *Fundulidae*, *Gasterosteidae*, *Percidae*, *Poeciliidae*, *Pomacentridae*, *Salmonidae*, and *Terapontidae* (Conrad et al., 2011). These include a mixture of laboratory (Wilson et al., 1993) and field based studies (Brown et al., 2005), which study the context-specificity of fish boldness (Coleman and Wilson, 1998), or behavioural syndromes

including boldness and its correlate behaviours (Ward et al., 2004). As such, this vast knowledge of fish boldness can be applied to important ecological consequences. More specifically, boldness is demonstrated to affect the fitness of fishes by altering: dispersal patterns (Fraser et al., 2001), social networks (Croft et al., 2009), growth (Hojesjo et al., 2011; Ward et al., 2004), survival (Dugatkin, 1992; Hojesjo et al., 2011), reproduction (Godin and Dugatkin, 1996), and habitat use (Kobler et al., 2011).

2.3. Measuring Boldness

There is a wealth of literature pertaining to the measure of boldness in fish including: latency to approach novel objects (Frost et al., 2007), latency to eat novel food (Sundstrom et al., 2004), latency to explore a novel environment (Fraser et al., 2001), latency to emerge in a familiar environment (Brown et al., 2005), predator inspection (Dugatkin et al., 2005), and response to predator stimuli (Wilson and Stevens, 2005). Although most of these experiments measure components of boldness, there is considerable debate on what truly constitutes boldness. From Reale et al. (2007) and Toms et al. (2010), a true measure of risk taking behaviour would be when a prey is subject to the presence of a predator, and not when they explore and forage within novel conditions. The reason for this argument is that bolder individuals have higher propensities to explore and as such, separating boldness from the exploration axis is a difficult task. Others believe that exploration into a novel environment is unrelated to boldness in response to predator. For example, the exploration tests of pumpkin seed sunfish (*Lepomis gibbosus*) did not predict their boldness (Coleman and Wilson, 1998).

2.4. Preliminary Emergence Trials

Since emergence trials had never been conducted in fathead minnows, no methodology existed. From the literature, I know that different species demonstrate different levels of activity and rates at which they acclimate (Naslund et al., 2015), such that I may not use methodology between species. For this reason, the first 4 months of my experimentation involved preliminary trials to determine: (1) emergence chamber design, (2) emergence tank layouts, (3) acclimation time and trial length, and (4) transportation and handling of minnows during trials. My goal during this time was to devise methodology that created a measurable distribution of boldness in fathead minnows and to have greater than 40% total emergence.

Isolation Chamber Design

Based on the review conducted by Naslund et al. (2015), the two chamber designs are: circular chamber made from PVC pipe (Beckmann and Biro, 2013; Brown and Braithwaite, 2004), and the most common, rectangular or square chambers made from plastic cardboard (Brydges and Braithwaite, 2009; Naslund et al., 2015).

For my experiment, I chose a circular design over a square design because minnows have high propensity to become stressed following any disturbance. As such, they may have been more likely to take refuge in the corners of an emergence chamber and thus, have lower chances to emerge. However, the primary concern with using a circular design is developing a door mechanism that can be easily closed without disrupting the fish (as opposed to the remote draw-bridge door of a square isolation chamber). In some emergence trials that use circular designs, there is a central pipe that may be lifted to allow minnows to escape (Brown and Braithwaite, 2004) while other chambers work on rotating an outer PVC pipe around a fixed inner tube (Beckmann and Biro, 2013). I chose the rotating outer tube so that I could cover the top of the emergence chamber and keep the inside dark while still being able to open it (see Figure 1). By delicately applying a smooth layer caulking to the inner tube, the outer tube could rotate without getting caught or causing significant mechanical disturbance. After conducting a few practice emergence trials, the rotation of the outer tube did not seem to stress minnows extensively and approximately 10% of minnows were emerging.

Isolation Chamber Size

From the literature, the width of the emergence chamber varies greatly from 5 – 40 cm (with an average of 18.3 cm) between square, rectangle, and circular shapes (Naslund et al., 2015). For my experiment, I approximated my chamber size based off of the chamber used in European minnows (*Phoxinus phoxinus*). For their emergence trials, the isolation chamber was 9 cm² and located at the far left side of the tank (Kekalainen et al., 2014).

To begin my preliminary tests on chamber size, I used an 8 cm diameter PVC tube with a rotating outer door, created from a 9 cm outer plastic pipe. After conducting a few practice trials with this size of PVC tube, only approximately 10% of minnows were emerging. From my observations, I believed that the problem was with the size of the minnows compared to the

diameter of the chamber. My unsexed adult minnows were a mean total length of 5.63 ± 0.61 (S.D) cm, with the largest minnows being approximately 7 cm. As a result, minnows were likely forced to maintain a narrow position in the water column or swim vertically which may have caused higher levels of stress. My solution to this was to increase the size of PVC tube to the next incremental size, with an inner tube at 10 cm diameter and outer tube at 11 cm in diameter (see Figure 1). The results were a two-fold increase in the amount of minnows emerging during the next set of emergence trials.

Door Size and Shape

Most doors used in emergence trials ranged from 2.5 - 40 cm wide, with an average of 9.3 cm, depending on the focal species (Naslund et al., 2015). In most cases, door size is a delicate balance. If the door is too large, there is a higher chance that fish will emerge because the chamber is perceived as unsafe, rather than emerging when they are prepared to explore the novel environment (Naslund et al., 2015). Conversely, if the door is too small, minnows may not be able to see predators or conspecifics in the environment.

For my isolation chamber door, my first plan was a 4 cm circular hole, drilled in the inner and outer PVC pipes. The hole was raised above the substrate in order to provide the minnows with a shelter point along the substrate. However, after 16 emergence trials I had no fish emerging within the allotted 20 minutes. In order to ensure that minnows were exiting based on a boldness decision, I did not alter the door size but instead the door shape. I performed 8 trials during which I noticed that minnows needed to swim up to the door and commonly maintained their position along the substrate. For this reason, I moved the hole down such that it touched the substrate and I also widened the circular exit along the substrate. The result was a more natural, cave-like shape which was approximately 5 cm at the base and narrowed to 2 cm at the top (Figure 1). Solely changing this aspect of the door increased my emergence success from 0% to approximately 15 – 20 % in the next set of emergence trials. I do not believe that the increase in emergence was due to the random chance of having a larger door, since the minnows that did emerge during these trials commonly inspected the cave-like exit before emerging.

Arena Layout

A significant amount of emergence trials have been conducted in barren tanks, equipped with small isolation chambers, and another refuge zone on the opposite side of the tanks (Beckmann and Biro, 2013; Bols, 1977; Budaev, 1997). Although variation in habitat complexity is understudied, a combination of a barren environment and a large door may lead fish to emerge prematurely (Naslund et al., 2015). Similarly, visible shelter or refuge areas may provide prey species areas to hide and thus, increase their likelihood to emerge.

For my emergence arena design, I did not make many adjustments to the arena design during my practice trials. My isolation chambers were placed at the back portion of the tank, such that the minnows emerged toward me. For the first batch of practice trials, I did not have a visible shelter for the minnows to take refuge under once they emerged. By placing a tile shelter directly in front of the isolation chamber exit (approximately 8-10 cm away), the percentage of minnows emerging increased by about 5-10%. This refuge seemingly created a bridge between the safe isolation chamber and the novel tank (Figure 1). Most minnows would emerge and swim directly under the tile shelter for 3 – 5 minutes, then continue exploring the tank from there. Nevertheless, I considered this emerging since minnows still have to risk swimming from the chamber to the refuge.

Acclimation Length and Emergence Limit

From the review conducted by Naslund et al. (2015), I saw a large variation in acclimation time from 0 seconds in the giant rivulus (*Rivulus hartii*) up to 1800 seconds in bluegill (*Lepomis macrochirus*), common roach (*Rutilus rutilus*), and northern pike (*Esox lucius*). Across the 29 studies that were analyzed, there was an average time of 429 seconds acclimation time (Naslund et al., 2015). When looking at the maximum trial length across the same studies, they ranged from 90 – 7200s with an average of 1228 seconds (Naslund et al., 2015). In addition, research suggests that longer acclimation times (from 3 to 5 minutes) produced more repeatable results in guppies (*Poecilia reticulata*) (Burns, 2008).

Over the course of my preliminary emergence trials, I experimented with acclimation time and maximal trial length. I began by testing 5 and 10 minute intervals for both acclimation and maximal trial length. Both of these times did not increase the amount of minnows emerging

from approximately 20% across practice trials. However, 10 minute trials seemed to capture a few individuals emerging at around 8-9 minutes such that increasing both acclimation time and trial time was yielding slight increases in minnow emergence. At 15 minutes of acclimation and maximal length, total emergence increased to approximately 25%. For this reason, I increased the time to 20 minutes for acclimation and maximal trial length. As a result, total emergence increased to approximately 30%, which was approaching my 40% acclimation goal. Despite the time constraints associated with the size of my experiment, I tried 30, 45, and 60 minute maximal trial lengths to observe if I could further increase my total emergence to 50%. Counterintuitively, minnows that did not emerge within 20 minutes also failed to emerge when provided with 30 or 45 minutes. Approximately 3% extra individuals emerged between 20 and 60 minutes, such that 20 minutes acclimation and maximal trial length would be the most efficient values.

Handling Techniques

Although many studies neglect to mention their handling methods, they were one of the most important factors in reducing stress for my experiment. A recent study of Panamanian bishops (*Brachyrhaphis episcopi*) found that using a water filled scoop instead of nets when transferring fish significantly decreased their stress levels but increased the latency to emerge (Brydges et al., 2009). Similarly, another study found that net-transferred sticklebacks spent less time in: (1) darker areas of the tank, (2) less time spent in the bottom third of the tank, and (3) further distanced themselves from safe areas (Thompson et al., 2016).

For my experiment, my original idea was to net fish from holding tanks into a transition bucket, and then net minnows directly into the emergence tanks. This involved using a net within R.J.F. Smith Center, putting minnows into the bucket and then using a separate net to take minnows out in the Aquatic Predation and Environmental Change (APEC) lab. However, this method created visibly stressed minnows that would dart or pace along the perimeter of the bucket. This corresponded to very low total emergence rates of under 10%. The first change I made to my handling technique was netting minnows in RJF and transporting the minnows with the net still in the bucket. This allowed them to acclimate with the net and use the net as a temporary shelter. This made the minnows visibly less stressed within the bucket, and decreased the time needed to catch the minnows by 50+%. Moreover, this increased the total emergence

from 0 – 10% to approximately 20 – 25% between emergence sets. Finally, I decided to let the bucket of minnows sit in a dark and quiet corner of the room for 10 minutes prior to beginning the acclimation times. This step yielded the least stressed minnows and increased my total emergence from ~20 to 35%. Although this process took longer to complete, it dramatically increased my total emergence and provided a measurable distribution of minnow behaviour.

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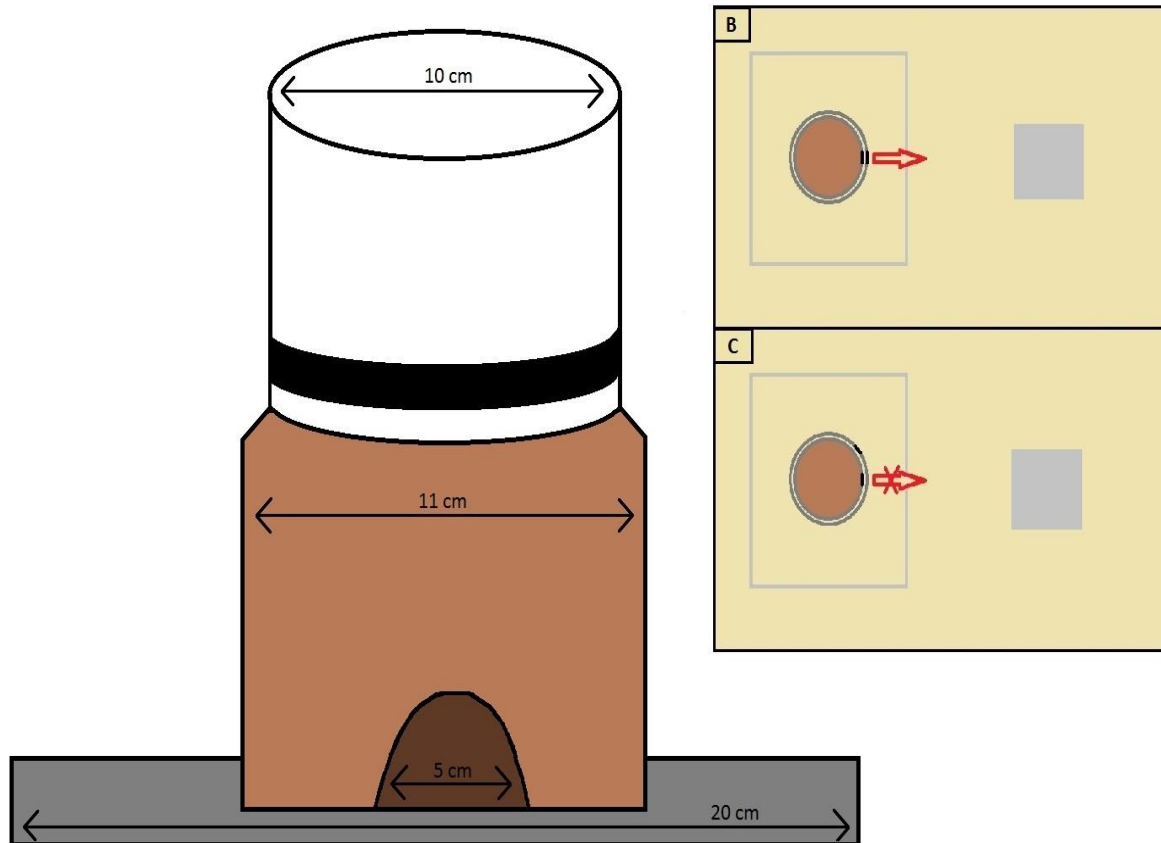


Figure 1: Finalized isolation chamber design (A) composed of: grey corrugated cardboard base, brown outer PVC pipe, inner white PVC pipe, and black rubber O-ring. The emergence tank layout (B and C) composed of the isolation chamber (left side; back of the tank) and 10 cm ceramic tile (right side; closest to observer). In the first panel (B), the holes in both pipes are aligned to form an exit; the second panel (C) has the outer pipe rotated to close the exit.

Chapter 3: Boldness and the Threat-Sensitive Response

3.1. Hypotheses and Predictions

The main objective of my experiment was to study the relationship between personality and the threat-sensitive decisions of prey after exposure to risk. More specifically, my goal was to manipulate the visual environment surrounding the fish (clear or turbid water) and provide them with a high-risk (alarm cues) or low-risk (water) chemical information. I hypothesized that if personality impacts the way minnows gauge threat, bold minnows should display a weaker antipredator response than shy individuals when they are faced with similar amounts of chemical and visual cues (Panel 1 of Figure 2). When minnows were maintained in a turbid environment, I predicted one of two outcomes. In the first prediction, shy and bold minnows would increase the intensity of their response to both alarm cue and water (Prediction 1). The reasoning is that shy and bold minnows may adopt a “play it safe” strategy (Bouskila et al., 1995) and compensate for a lack of visual cues (Hartman and Abrahams, 2000) in turbid water. Alternatively, I predicted that shy and bold minnows may decrease their antipredator response in turbid water, since turbidity may be perceived as a refuge for fathead minnows (Prediction 2; Abrahams and Kattenfeld, 1997).

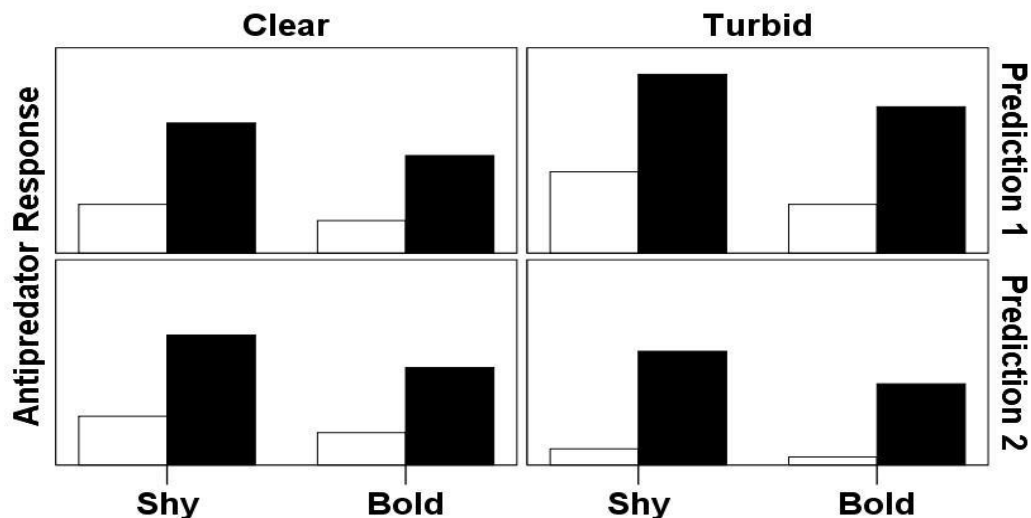


Figure 2: Prediction 1 and 2 of my threat-sensitive experiment on fathead minnows (*Pimephales promelas*). Personality, risk, and turbidity are manipulated.

3.2. Methodology

Study Species

Fathead minnows are a small, temperate fish that live in ponds, lakes, and rivers throughout North America. They are common prey of smaller piscivores, snakes, larger aquatic invertebrates, and birds (Warren and Burr, 2014). Fathead minnows typically grow from 5 – 8 cm long over their lifespan of 2 – 3 years in the wild (Wisenden et al., 2009a). They are a keystone species in smaller ponds across central North America since they regulate the ecosystem's energy flow and productivity (Zimmer et al., 2001), unlike other species that are more prone to winterkills from low dissolved oxygen (Divino and Tonn, 2007; Klinger et al., 1982). In addition, they are an extensively used lab species for animal behaviour studies (Crane and Ferrari, 2015b; Ferrari et al., 2008a) as well as toxicology (Ankley and Villeneuve, 2006; Parks et al., 1999) due to their tolerance for a variety of environmental conditions.

Minnow Collection

Unsexed adult minnows (total length: mean \pm SD = 5.63 \pm 0.61 cm) were caught in April 2015 at Feedlot Pond, on the University of Saskatchewan's campus grounds (52°09'21.4"N: 106°37'06.0"W). Extensive trapping over the last few decades has demonstrated that there are no fish predators found within the pond (Crane and Ferrari, 2015a). In particular, Gee's inverted minnow traps were set in the morning (from 8-10 am) and retrieved in early evening (4-8 pm) yielding approximately 25-300 minnows per trap. Minnows were removed from traps and transported to RJF Smith Aquatic center in 19 l buckets, filled with pond water and aerated with a portable aerator (Topfin™ DC-160). Once transported to the lab, minnows were allowed to slowly acclimatize to warmer temperatures in the respective buckets for 2-3 hours. During this time, the water temperature rose from ~16°C to ~20°C, at which point minnows were transferred into 1700 l flow-through tanks. These tanks were equipped with a carbon bubble filter and fed *ad libitum* with Nutrafin™ flake fish food. Over the 2-3 month acclimatization period, holding tanks were maintained under a 15:9 h light:dark cycle.

Alarm Cue Collection and Use

To prepare minnow alarm cue, I used a standard procedure (Crane and Ferrari, 2015a; Ferrari and Chivers, 2006) which involved sacrificing 5 minnows (total length: mean \pm SD = 5.61 ± 0.26 cm) with a blow to the head (in accordance with the Canadian Council on Animal Care). I then removed 12.3 cm^2 of skin from the minnows and placed it into a beaker with 20 ml of filtered water. Subsequently, I homogenized the solution using a Polytron PT-2500E and diluted the solution to a final concentration of $\sim 1 \text{ cm}^2/40 \text{ l}$, a concentration known to elicit overt antipredator behaviour in this species (Ferrari et al., 2005). Immediately after preparation, the final solution was frozen (-20°C) in 100 ml aliquots in order to avoid degradation of the alarm cues.

Alarm cue was removed from the freezer one hour before the start of trials to ensure that the alarm cue would be melted and of similar temperature to laboratory water ($\sim 18^\circ\text{C}$). The amount needed for each set of trials was removed from the freezer bags, and then the bags were returned to the freezer immediately to prevent degradation of the alarm cues.

Finalized Emergence Trial Methods

Minnows were kept in the RJF Smith Aquatic Center in two 1700 l plastic tanks. When they were needed for trials, minnows were netted and moved from the Smith Center in a plastic bucket with 12-15 cm of water, and 1 cm layer of gravel substrate to reduce stress. During the moving process from RJF Smith Centre to the APEC lab, I avoided shaking the bucket and noise to minimize stress. In the APEC lab, the bucket was placed into a dark corner and left undisturbed for 10 minutes to allow the minnows to acclimate as a group. The net used to transfer the minnows to and from the bucket was left within the bucket to provide shelter during acclimation.

Once 10 minutes had elapsed, minnows were individually netted and placed directly into one of four random isolation chambers. Once added into the emergence chamber, I covered the top (where the minnow was placed) with a 10 cm ceramic tile to prevent minnows from escaping and to darken the inside of the chamber. Once the minnows were placed into the isolation chamber, the entire room was kept in silence and without mechanical disturbance. After a 20

minute acclimation period had elapsed, I opened the isolation chamber manually by turning the outer PVC pipe slightly to align the holes.

During each set, all four trials were run concurrently while I observed the arenas from directly in front of the emergence tanks. I recorded minnows' latency to emerge as the time it took a minnow's entire body to exit the isolation chamber. Each minnow was assigned a score of boldness ranging from 1-1200s. Minnows who failed to emerge after 20 minutes following the chamber opening were given a maximum emergence score of 1200s. Since approximately 45% of all minnows failed to emerge from emergence trials (Figure 3), I arbitrarily split minnows into: (1) bold minnows – emerging before 600s, and (2) shy minnows – individuals emerging after 601s or failing to emerge. Most importantly, I conducted all measurements: (1) from 1-5 pm in the afternoon to avoid temporal biases for personality; (2) in silence and without mechanical disturbance in the lab; (3) behind window tint (20% light transmission) and in a darkened lab to prevent minnows from seeing me.

Extended Trials

With my original set of emergence trials, I had a total of 105 emergence scores for individual minnows. This was comprised of 62 (59%) shy individuals and 43 (41%) bold individuals (Figure 3). This original distribution represents my closest approximation of fathead minnow personality in wild populations. However, for my experiment I artificially selected more individuals that were bold to balance the sample sizes between treatments. As such, the resulting distribution of boldness scores was 65 shy individuals and 66 bold individuals (see Figure 3).

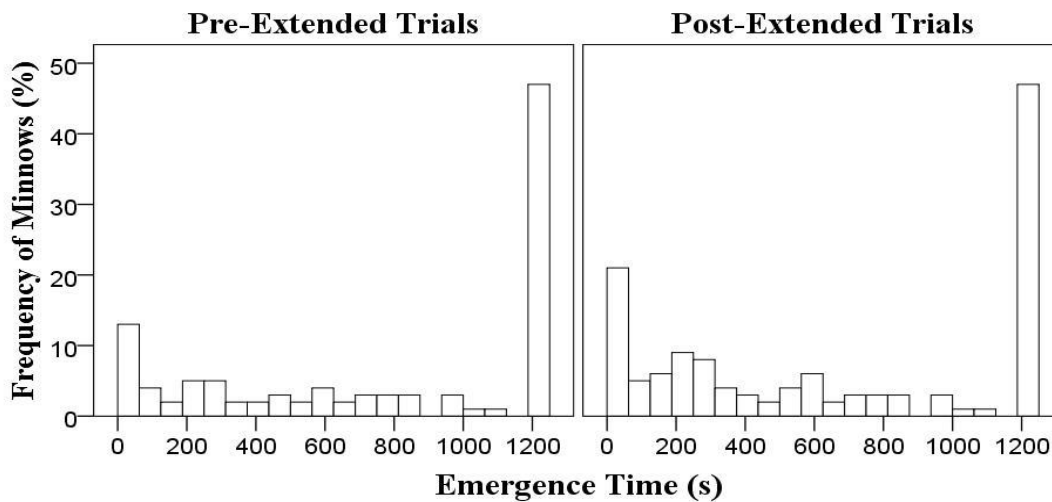


Figure 3: Total distribution before extended trials (n= 105) and post-extended trials (n= 131) of emergence scores in fathead minnows (*Pimephales promelas*) as proxy for boldness.

Interlude

Following the completion of emergence trials, minnows were allowed to rest in the emergence tanks for 30 minutes before being moved into a second set of tanks. During this period, the second set of tanks was prepared by: (1) redistributing the gravel to ensure the bottom be covered with at least 2 cm of gravel, (2) repositioning a 10 cm ceramic tile shelter to the center of the tank, (3) measuring and adding 1 gram of inert bentonite clay to turbid treatments; (4) repositioning of the injection hose close to the air stone. For my turbid tanks, I increased the turbidity to 18-20 NTUs before the minnows were added. The water within each tank was mixed to ensure that the turbidity had been distributed and that control tanks experienced equal levels of disturbance. Once the 30 minutes had elapsed, minnows were netted from their emergence tanks and randomly placed into antipredator tanks. Minnows were allowed to acclimate from 2-5 days, depending on the point at which minnows resumed normal activity. I defined this as the point at which a minnow was spending less than 80% of the time under shelter and actively exploring. The time it took to fully resume activity was measured at 8 am, 12 pm, and 6 pm and recorded as a “acclimation time” variable.

Antipredator Trials

My antipredator trials consisted of a 2x2 design: (1) clear water and alarm cue addition; (2) clear water and distilled water; (3) turbid water and alarm cue; (4) turbid water and distilled water. Since minnows were netted from the emergence trials and randomly distributed to antipredator tanks, bold and shy minnows were randomly distributed between all four treatments (with 30-40 minnows per treatment). As such, my experiment followed a fully factorial 2x2x2 design, with personality, risk, and turbidity as fixed factors. The clear water and distilled water treatments served as controls for turbidity and risk levels (respectively).

Once my alarm cue had thawed, I removed the appropriate amount for my high risk trials. A separate 50 ml syringe and beaker were used for my alarm cue in order to avoid contamination with low risk controls. Similarly, another specific 50 ml syringe and beaker were used for my distilled water controls.

Each trial was comprised of an 8 minute pre observation period, where time spent moving (activity variable) and time spent under shelter (shelter use variable) were simultaneously measured. My activity variable was defined as the time during which a minnow spent in motion (greater than a half body length/second) and foraging. Moving half a body length included: (1) darting, (2) pacing under the shelter, and (3) exploration outside the shelter, and other foraging behaviours such as pecking at the substrate, pecking at the top or bottom of the ceramic shelter, and swimming to the surface in search for food. Conversely, time spent under shelter was defined as any time the minnow spent with at least 75% of their body under the provided ceramic shelter. As such, both activity and shelter use were not mutually exclusive in all cases.

Once the 8 minutes of pre-stimulus observation had elapsed, either alarm cue or water was added to tanks via the injection hose (50 cm pieces of air tubing). In both cases, 5 ml of stimulus solution was injected into the hose and then flushed into the system very slowly (approximately 30-45 s total injection time) using 10 ml of distilled water. After 15 seconds, I began my 8 minutes of post-stimulus observation where activity and shelter use were recorded. Once antipredator trials for the entire rack (12 tanks) were completed, minnows were allowed to

rest for another 30 minutes to avoid further stress. After this period, I remeasured the current turbidity to ensure that it remained higher than 14 NTU during the time of recording.

Statistical Analysis

Emergence scores

Rather than using emergence scores as a continuous variable, I split boldness scores into two categories since 30% of minnows failed to emerge (scoring 1200+ sec) making a correlation analysis inappropriate. I arbitrarily chose a cutting point of 600 s (the half point of the observation period), since it represented a boundary splitting our dataset in half (n= 66 for emergence time of <600 sec, n=65 for emergence time of > 600 sec). For clarity, I categorized the fast-emerging fish as ‘bold’, while the slow-emerging fish were categorized as ‘shy’. A Mann-Whitney U test confirmed that the two groups differed in their emergence latencies (U=2211, $Z_{130} = -10.08$, $P < 0.001$).

Repeatability

Emergence trials are sometimes critiqued for the lack of inter-measure repeatability. As such, I compared emergence scores to acclimation period to see if there was intra-measure consistency of my boldness measures. Since acclimation time did not follow a normal distribution (one-sample KS test: $Z_{131} = 2.202$, $P < 0.001$), I compared both continuous variables with Spearman’s correlation and also with emergence scores as categorical variable (personality), with a Mann-Whitney U test.

Acclimation bias

After emergence trials, minnows were allowed to fully acclimate to the new tanks for 2-5 days before antipredator behaviour was recorded. To determine if minnows were acclimating faster under different treatments, I performed a three-way ANOVA with personality (shy or bold), turbidity (clear or turbid), and risk (water or alarm cue) as our fixed factors and time between both sets of trials as the response variable. Minnows were not exposed to any risk treatments during acclimation, but I still included risk as a fixed factor to ensure there were no unintentional biases on risk measurements.

Antipredator responses

To compare if baseline behaviour was different in shy and bold individuals, I performed 2 x 2 ANOVAs with personality and turbidity as our fixed factors, and baseline behaviours (activity or shelter use) as our response variables. Due to pre-stimulus differences in baseline behaviour between bold and shy individuals, I computed proportional changes for movement and shelter use $((\text{post} - \text{pre})/\text{pre})$ to compare the response patterns across treatment groups. Since the proportional change in shelter use varied in orders of magnitude, I used the absolute, logged value of shelter use. I analyzed movement and shelter use separately using two, 3-way ANOVAs. This 2 x 2 x 2 design included personality (shy or bold), turbidity (clear or turbid), and risk (water or alarm cue) as our fixed factors. Two-way ANOVAs and t-tests with Tukey corrections were used for post hoc analysis. All statistical analyses were run using IBM SPSS™ Statistics 23.

3.3. Results

Consistency of boldness

Emergence score of minnows measured during emergence trials is positively correlated to the rate at which fathead minnows acclimate during the interlude period (Spearman's $r = 0.232$, $P = 0.008$, $n = 131$). As minnows increased in shyness (time to emerge), they also increased in the time needed to fully acclimate to the test tanks. When I analyzed personality as a categorical variable, bolder minnows acclimated approximately 10 hours faster than their shy counterparts ($U = 1521$, $Z_{131} = -2.9$, $P = 0.004$; Figure 4).

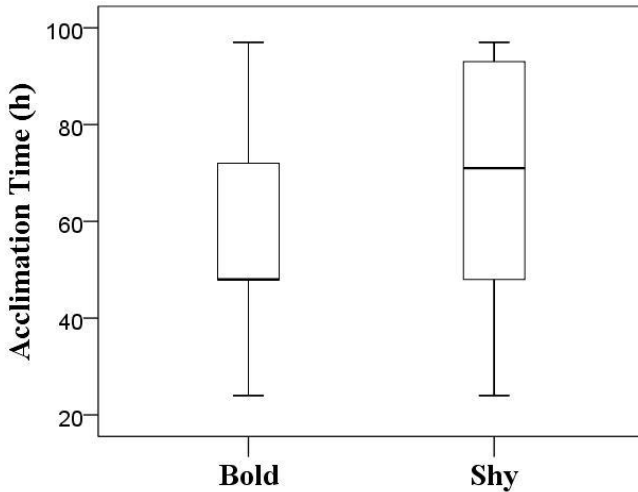


Figure 4: Boxplot showing the acclimation time prior to the behavioural assay, for bold and shy minnows, as defined by the emergence trial.

Acclimation bias

The acclimation time of minnows during the interlude was not affected by the three-way interaction of personality, risk, and turbidity ($F_{1,123} < 0.001$, $P = 0.99$) or any two way interactions (personality-turbidity: $F_{1,123} = 0.86$, $P = 0.77$; personality-risk: $F_{1,123} = 0.38$, $P = 0.54$; turbidity-risk: $F_{1,123} = 0.41$, $P = 0.53$). Similarly, acclimation rates were not related to baseline behaviour for shelter use (Spearman's $r = 0.091$, $P = 0.301$) and baseline activity (Spearman's $r = -0.171$, $P = 0.051$).

Baseline Behaviour

Before the addition of risk, minnows' activity was affected by their personality tendencies, with bolder minnows spending more time moving ($F_{1, 127} = 22.3$, $P < 0.001$; Figure 5a) and less time under shelter ($F_{1, 127} = 10.4$, $P = 0.002$; Figure 5b) than shier ones. However, turbidity did not affect the behaviour of the fish (movement: $F_{1, 127} = 0.03$, $P = 0.9$; shelter use: $F_{1, 127} = 0.1$, $P = 0.8$) and there was no subsequent interaction between the two factors (movement: $F_{1, 127} = 0.4$, $P = 0.5$; shelter use: $F_{1, 127} = 0.3$, $P = 0.6$).

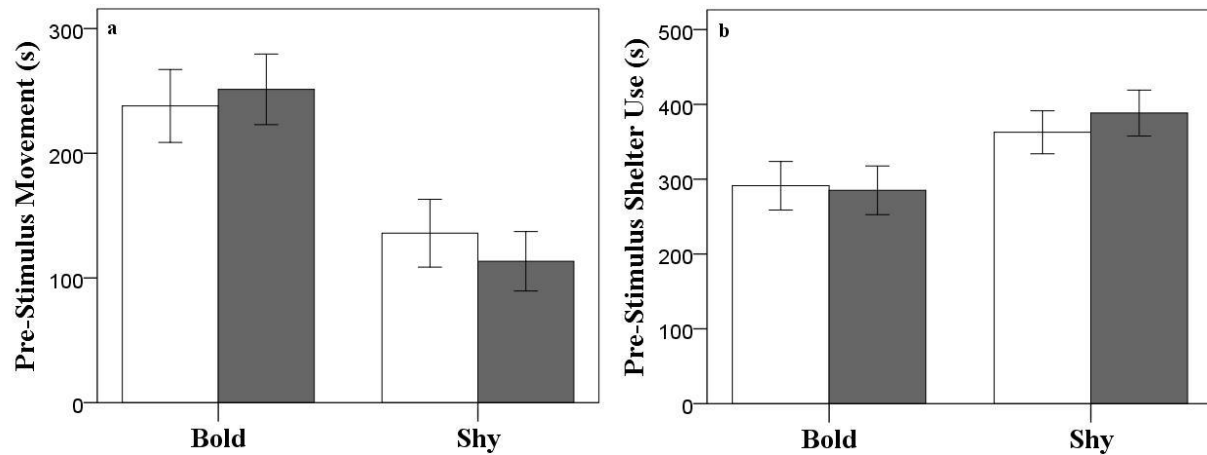


Figure 5: Mean (\pm SE) pre-stimulus movement (a), and shelter use (b) between personality (shy/bold) and turbidity (clear/turbid). White bars represent clear tanks, grey bars represent turbid conditions.

Antipredator Responses

Minnow behaviour was affected by a three-way interaction between turbidity, personality, and risk (activity: $F_{1, 122} = 4.48$, $P = 0.036$, shelter: $F_{1, 123} = 5.00$, $P = 0.027$). When fish were exposed to alarm cue, their personality (activity: $F_{1, 62} = 0.14$, $P = 0.7$; shelter $F_{1, 62} = 0.53$, $P = 0.5$) and turbidity treatment (activity: $F_{1, 62} = 0.012$, $P = 0.9$; shelter: $F_{1, 62} = 0.019$, $P = 0.9$) did not affect the intensity of their antipredator response. No interaction between personality and turbidity was found (activity: $F_{1, 62} = 0.14$, $P = 0.7$; shelter: $F_{1, 62} = 1.12$, $P = 0.3$, figure 6). All minnows responded to the alarm cue with the same intensity of antipredator response, regardless of their personality or their visual environment.

In contrast, when fish were given a low-risk water injection, their behaviour was affected by an interaction between turbidity and personality (activity: $F_{1, 60} = 6.16$, $P = 0.016$; shelter: $F_{1, 60} = 4.04$, $P = 0.049$). Specifically, bold minnows maintained in a turbid environment showed a similar fright response to water and alarm cue injections (activity: $t_{31} = -1.52$, $P = 0.14$, figure 6b; shelter: $t_{31} = 0.62$, $P = 0.54$, figure 6d). In contrast, when placed in clear tanks, their response to alarm cues was significantly stronger than that to water (activity: $t_{31} = -3.56$, $P = 0.001$; shelter: $t_{30} = -2.14$, $P = 0.041$). Conversely, shy minnows maintained in turbid tanks showed a stronger response to alarm cues than water (activity: $t_{28} = -2.48$, $P = 0.019$, figure 6a; shelter: $t_{29} = -2.89$, $P =$

0.007, figure 6c) but displayed a similar fright response to both water and alarm cues in clear tanks (activity: $t_{32} = -1.83$, $P = 0.077$; shelter: $t_{32} = -1.20$, $P = 0.24$).

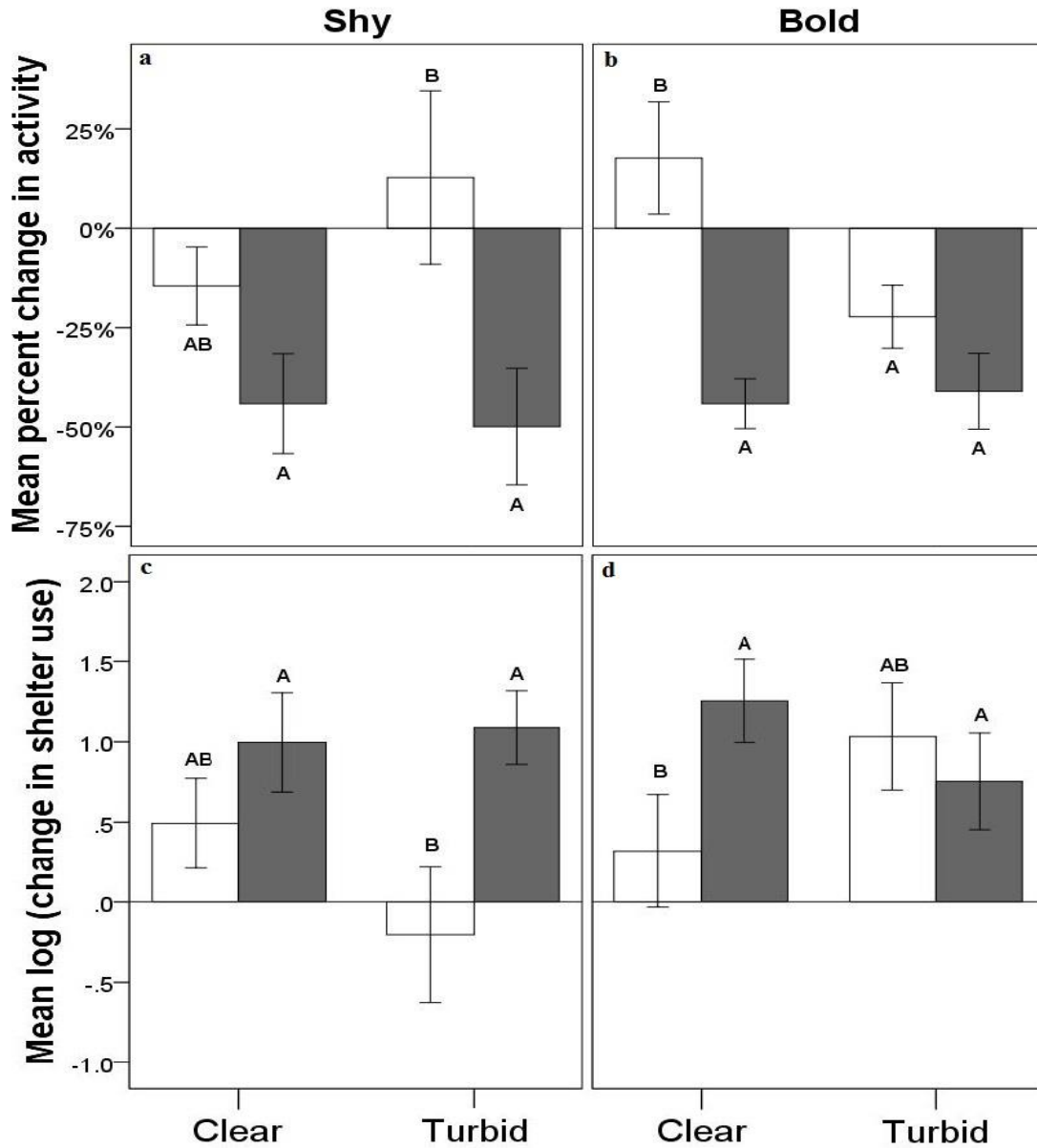


Figure 6: Mean (\pm SE) change in proportion of movement in shy (a) and bold (b) minnows exposed to: water/clear, water/turbidity, alarm cue/clear, alarm cue/turbidity treatments. Change in logged shelter use of in shy (c) and bold (d) minnows. White bars represent the water control, dark grey bars represent alarm cue.

Chapter 4: Discussion

4.1. Environmental Risk

Under high risk, personality and turbidity have no effect on the amount of antipredator behaviour shown by minnows. Since minnows respond to alarm cues in a graded manner, my high concentration of alarm cue may have masked the effects of personality or turbidity. At this concentration of alarm cue, a conspecific was likely attacked very close to the focal individual. For this reason, it may be too costly for minnows to underreact to the situation, regardless of their propensity to engage in risk. These findings parallel an experiment in mosquitofish, where personality-based dispersal was negated under higher predation risk (Cote et al., 2013). Without the presence of a predator, there were large differences in the sociability and boldness scores of resident and disperser mosquitofish, but when exposed to predation risk all the effects of personality dissipated. However, the effect of personality on prey fish under high predation risk seems to be species specific. For example, personality affected antipredator behaviour of Trinidad guppies (*Poecilia reticulata*) under high risk concentrations of alarm cue, but not in low risk or control treatments (Brown et al., 2014).

At lower levels of risk, shy and bold individuals demonstrate antagonistic based responses to the presence or absence of turbidity. When comparing this to current evidence within the literature, there is once again species specific difference on the effect of turbidity. In three-spined sticklebacks (*Gasterosteus aculeatus*), turbidity impairs risk assessment leading to weaker antipredator responses (Sohel and Lindstrom, 2015). However, we can observe the opposite response to turbidity in guppies, where they demonstrated larger antipredator responses and took longer to recover from predation threat in turbid water (Kimbell and Morrell, 2015). As opposed to this, I found both higher and lower antipredator reactions depending on turbidity and personality. In the case of shy minnows, they showed no antipredator response (activity or shelter use change) in turbid water. As such, they may perceive risk as less threatening when they can take refuge in the turbidity. As the antagonistic response implies, bold minnows showed a strong antipredator response when exposed to risk in turbid water. One possible mechanism behind this phenomenon is that bolder individuals prefer to explore risky environments and are

much more reliant on visual cues than shy individuals. For this reason, removing visual cues about local predators likely increases their perceived risk.

4.2. Baseline Behaviour

Before the addition of risk, bolder minnows were spending more time actively exploring the tank and spending less time under refuge than shier minnows. Since bolder individuals have a higher propensity to engage in risk taking behaviour, it could be expected that they would acclimate to the newer tanks faster and thus, have higher levels of activity. Shier individuals would be more like to adopt a “play it safe” strategy and wait longer to assess the local cues and resume foraging. In an attempt to avoid this, I allowed all minnows to resume normal behaviour before testing, such that I could separate their acclimation rates (proxy for boldness) from baseline activity (closer proxy for exploration). More specifically, acclimation to a novel tank involves risk taking behaviour in order to initially gather information about local predators. Once a minnow has fully explored a tank in the absence of predators, there will be less risk associated with any exploration such that a minnow’s activity should be relatively independent of their boldness. However, baseline movement was still slightly related to acclimation time, whereas shelter use was not related (see Results). For this reason, I cannot ascribe differences in baseline activity solely to a minnow’s propensity to explore, but rather a correlate of boldness and exploration.

Another interesting result of baseline behaviour was that there no significant differences whether minnows were placed in turbid or clear tanks. Most literature examining prey fish in turbid water demonstrates a significant reduction or increase in antipredator responses. For example, fathead minnows have been shown to reduce their response to risk and increase their use of dangerous habitats when in turbid waters (Abrahams and Kattenfeld, 1997). However, an experiment conducted on three-spined sticklebacks found similar results, where the presence of turbidity did not explain differences in exploration (Langenhof et al., 2016). I hypothesize that the lack of differences in turbidity treatments are due to minnows being exposed to the turbidity for 2-5 days until they fully acclimate. Without any visual or chemical cues about local predators, they may habituate to the turbidity and likely perceive it as too low of a risk to alter their behaviour. More specifically, the time that they would lose in foraging, exploring, or trying to shoal with conspecifics likely outweighs the benefits of the “play it safe” strategy.

Alternatively, another way to explain this behaviour is that there may be enough visual cues available in order for minnows to assess their surroundings as non-threatening. That is, with a turbidity of approximately 15 – 20 NTU, the distance at which they can perceive threat may extend to the perimeter of the tank such that they can accurately determine there are no local predators.

4.3. Habitat Preference

In my experiment, shy minnows demonstrate less of an antipredator response in turbid water, while bold individuals have lower responses in clear water. If we assume that all other conditions are similar, including predation risk, minnows will reduce their stress levels by avoiding habitats where the perceived threat is much higher. For this reason, they may preferentially choose habitats which minimize their perceived threat and maximize other fitness enhancing behaviours. The results of this could be a differences in distribution of shy and bold individuals throughout an environment based on turbidity. This link between personality based habitat selection and turbidity has not been directly explored in the current literature. However, one study found that habitat specific variation in boldness existed in three-spine sticklebacks when measuring foraging rates. When they compared personality between sticklebacks from four different habitats, boldness was not predicted by turbidity alone (Webster et al., 2007).

If boldness does predict the habitats in which we find prey fishes, this may have important impacts in the study of fish behaviour and conservation of endangered species. Assuming that this occurs, collecting individuals from one location may skew our observations and alter our results. Due to the implication of this idea, more research should further explore this phenomenon.

4.4. Consistency of Boldness

A large amount of scrutiny has been placed on the repeatability within emergence trials and between other methods of testing boldness (Beckmann and Biro, 2013). In particular, they found that there was no repeatability of boldness scores in two species of damselfish (*Pomacentrus wardi*; *Pomacentrus amboinensis*) exposed to novel environments. However, in my experiment I found that measures of boldness were highly correlated. Specifically, when I measured emergence in a novel tank as a proxy for boldness, I was measuring the rate at which

minnows acclimated to the isolation chamber. As such, this measure is similar to the rate at which minnows acclimate to the antipredator tanks during the interlude portion of my experiment. In fact, I found that minnows that took longer to emerge (more shy) were also the individuals that took the longest to acclimate during interlude period. For this reason, my boldness measures were consistent between different novel environments and over shorter temporal scales of 2 – 5 days.

4.5. Further Questions and Discussion

How does personality affect minnows subject to climate change?

One of the most important components of global climate change is the increasing risk of aquatic eutrophication in marine and freshwater systems. Created by an increase in anthropogenic nutrient inputs and localized warming of water bodies (Smith et al., 1999), eutrophication effectively increases the turbidity of aquatic systems due to the increase productivity. Along with the lethal effects of hypoxia and the sublethal effects including development, eutrophication causes important behavioural changes including reduced parental care (Jarvenpaa and Lindstrom, 2011) and increased energy expenditure for sexual displays in fish (Engstrom-Ost and Candolin, 2007). Similarly, eutrophication may affect the personality of prey species or differentially affect prey fish based on personality. In either case, the outcome of eutrophication is unknown, but it will most likely cause important individual or population level effects. For example, a bold prey fish may inhabit a lake where non-point source pollution from a nearby city has increased dramatically. The same individual is now forced into foraging and antipredator trade-offs in water that is substantially more turbid. Even under low risk scenarios, they demonstrate a maladaptive response and overreact to any mechanical or chemical disturbance by local piscivores. As such, they spend less time actively foraging, exploring, and finding viable mates. In turn, the individual may grow slower, worsen in body condition, and/or have difficulty finding viable mates which ultimately decreases their survival and reproductive fitness.

Were the minnows caught by trapping only the boldest individuals of a population?

Due to the risk-taking nature of bold minnows and other prey species, the most commonly caught minnows via fish traps are the boldest individuals of a population. As such,

the personality distribution of the minnows in Feedlot Pond is likely much more shy than my estimates and other experiments of prey fish who used similar trapping techniques (Pellegrini et al., 2010). In most cases, equally sampling all personalities within a population becomes non-feasible or too destructive. For this reason, underestimation of shy individuals in personality research may be an important issue which should be accounted for or discussed in more research.

Did I measure boldness according to the theoretical framework of Reale (2007)?

When I previously discuss the theoretical framework from Reale et al. (2007), my experiment would be considered a measure of exploration rather than a “true” measure of boldness. According to him, emergence trials represent a measure of exploration since minnows emerge into a novel environment in the absence of a predator. Nevertheless, my measures of boldness from my emergence trials still predicted the response of minnows to an immediate threat. Instead, I think my emergence trials capture aspects of exploration and boldness together as a form of behavioural syndrome.

Are personality differences in low risk scenarios not documented due to an antagonistic response?

The antipredator responses to water injections in shy and bold minnows were antagonistic, such that if I did not separate minnows based on personality, I would get a zero net change (see Figure 6). This is particularly interesting since this response may be completely overlooked in experiments and studies that do not separate individuals based on personality. Instead, this antagonistic response would likely create more statistical “noise”. It would be interesting if further studies examine the differences among bold and shy individuals, and found similar antagonistic responses in other contexts or in other axes.

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