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COGNITIVE ECOLOGY: HOW THE THREAT OF PREDATION INFLUENCES
INFORMATION PROCESSING AND DECISION MAKING

A Thesis in
Ecology

by

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ABSTRACT

Predation is arguably one of the most important selective pressures in evolution. As a result, many prey species are likely to assess predation risk through information processing (i.e. learning), thus cognition can play a crucial role in survival. Cognition and an animal’s ability to process, store and retrieve information also affects the way animals behave in other contexts. Experiments comparing the cognitive abilities of animals coming from populations exposed to contrasting ecological environments have provided insights into the factors that contribute to variation in learning and memory ability. To investigate this further, I selected natural populations of tropical freshwater fish, the Panamanian bishop (*Brachyrhaphis episcopi*) that are naturally exposed to contrasting levels of predation. The research presented in this thesis compares the spatial learning abilities and the memory duration of an associatively learned task in *B. episcopi* from these different populations.

First, I compared spatial learning performance to determine whether fish varied in their ability to solve a maze task, and in the information that they paid attention to as they moved through the maze. Fish from high predation sites were quicker to make decisions about which route to take, but were also more apt to make mistakes. In contrast, fish from low predation areas made slower, more accurate decisions. The relative exposure to predators did not predict which kinds of spatial information the fish would use, but differences were found between rivers.

My second experiment examined the ability of the fish to learn a conditioned response (associate a light cue with the delivery of a food reward) and then compared the rate of extinction of this learned association once the light cue no longer predicted the arrival of food. While fish with different experiences of predation showed no differences in the rate at which they formed the conditioned association between the light and the food, we did find that fish from low
predation sites were quicker to show an extinction response compared to high predation conspecifics.

Together, the results presented in this thesis indicate that ecological variables, specifically predation, play a substantial role in shaping learning, memory and other behavioral traits in fish.
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“We do not inherit the earth from our ancestors; we borrow it from our children”
Chief Seattle - Native American Proverb

“It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.”
Charles Darwin

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Chapter 1. General Introduction

Cognitive Ecology: An Historical Background

A key component of behavior that has a direct effect on information processing and decision making is cognition. Cognition refers to the mental processes that organisms use such as attention, memory, communication and problem solving skills. These important processes are fundamental to many aspects of performance and in particular they influence learning and decision making (Réale et al., 2007; Titulaer, 2012).

The ideas that make up the field of cognitive ecology have been established for more than a century. Charles Darwin was one of its earlier proponents; he sought to understand how natural selection operates when ecology and behavior come together to shape an animal’s phenotype (Dukas, 1998). In his book, The Expression of the Emotions in Animals and Man, Darwin proposed that many animals share similar emotional states, such as pain, fear, pleasure, anger and affection (Darwin, 1872; Fraser, 2009). Though Darwin was one of the initial figures to study behavior, he is rarely given credit for the various fields that grew as a result of his research (Eshleman, 2002). Decades after Darwin’s 1872 book, the fields of biology and psychology diverged and focused on separate processes, such as functional or mechanistic approaches to animal behavior (Dukas, 1998).

In the early 20th century, Auguste Comte pioneered the term positivism which influenced the direction of psychology by studying mechanistic processes in animal behavior and placing the highest value on observable phenomena (Lenzer, 1975; Dukas, 1998; Fraser, 2009). Positivism had distinctive methodological components compared to those used in biology and its subfields. The first difference was the emphasis positivism strictly gave to observable phenomena, and the
fact that we should ignore unobservable phenomena. Under the definition of “strictly observable” phenomena, emotions, feelings and mental states were considered outside the scope of the field (Fraser, 2009). Secondly, positivism proclaimed that unobservable processes fall beyond the capacity of science, in doing so positivism isolated the evolution of life and the origin of the universe. Finally, this new field held that all the sciences were built in a hierarchical manner. For example, biology came from chemistry which itself arose from physics. Thus, according to positivism, all behavior should be explained based on an animal’s physiology (Fraser, 2009).

Over time, the psychological view of positivism evolved, just as the many fields of science did. During the 1920’s and 30’s an experimental psychologist B.F. Skinner pioneered a newer term “behaviorism”. This reinforced postulates of positivism such that animal behavior “should only be explained in terms of empirically observed variables” (Dukas, 1998). Thus, behaviorism focused on the observed responses to variance in behavior, ruling out cognitive concepts (Bateson & Kacelnik, 1998).

Despite the obstacles that psychology may have placed on science, animal behavior continued to grow as a field. During the 1950’ and 60’s the emphasis on the evolution of behavioral traits gained greater acceptance with pioneering ethologists such as Tinbergen, von Frisch and Lorenz. In the last few decades, more scientists have further redefined the field of ethology and cognition. Researchers such as Jane Goodall and Dian Fossey demonstrated the value of observing animals in their natural environment. In 1993, Leslie Real coined the term “cognitive ecology”, this new field brought cognitive science and behavioral ecology together (Healy and Braithwaite, 2000). As a result, scientists now understand that subjects such as cognition, ecology, and evolution can, and indeed should, be studied side-by-side as they each contribute to produce and influence an animal’s behavioral phenotype and as such, have implications for fitness (Dukas, 1998; Healy, 1998; Titulaer, 2012). In the last two decades,
animal orientation, mate choice, foraging and song learning have all contributed to our understanding of cognitive ecology (Healy & Braithwaite, 2000).

A problem with having cognitive ecology branded as a “separate field” is that it may in effect detach itself from the other fields, limiting cross-disciplinary synthesis (Shettleworth, 2000, 2001). Despite such hurdles being in place, there is still promise for this field of research. Researchers prepared to bridge the gap between mechanistic processes (generally within the field of psychology) and processes with functional explanations (predominately behaviorists) are likely to yield significant advances to our understanding of cognition and ecology (Healy & Braithwaite, 2000).

**Cognitive Learning**

Cognition has been defined in short as the information animals take in from their environment and how they then use this (Shettleworth, 2001). So, when should an animal learn, and how long should the information gathered be stored? These questions arise, particularly when stability in an environment cannot be guaranteed. Research has shown that environments can be a key factor in learning (Kerr & Feldman, 2003). If we consider an extreme example of an animal living within a completely stable environment, without variability, then behavior would be expected to vary very little and we might expect animal responses to be genetically controlled (sometimes called “hard wired”), and so there would be little flexibility and no need for learning (Shettleworth, 1998; Braithwaite & Girvan 2003; Brydges *et al*., 2008 Brydges, 2008). On the other hand, unpredictably variable environments would also have little use for learning, as there would be no value in learning about different events or associations that change randomly (Shettleworth, 1998). Thus a “Goldilocks Principle” arises, where environments must be “just right” for learning to be beneficial (Kerr & Feldman, 2003). Some organisms do experience
variation in their environment that appears to be “just right”. This environmental variation is the prerequisite for animals learning how to respond and adapt to change.

Several steps are associated with how animals respond to ecological change. First the animal gathers incoming environmental stimuli and then processes relevant information. Second, the animal must compute accurately and rapidly those variables necessary for immediate response while remembering information for later use. Finally, the animal retrieves specific stored information when relevant (Dukas, 1998). Animal brains may not carry out all of the aforementioned steps, and even when brains do conform to these steps they may not happen quickly enough to produce the best decision. Thus costs, constraints, and trade-offs shape the capacity of the animal to learn. Speed accuracy trade-offs (Fig. 1-1) are the focus of my second chapter. In summary, an animal’s behavior and how it relates to its environment is highly linked to its cognitive abilities.

![Figure 1-1](attachment)

**Figure 1-1.** Relationship between speed and accuracy (SAT) in discrimination tasks. Accuracy can be improved by investing more time in the decision process or, conversely, decisions can be made more quickly but at the cost of making a higher number of errors. If the discrimination being tested is too easy (far left), then potential SATs will not be evident, because the subject will be performing with high accuracy over any range of reaction times. (From Chittka et al., 2009).
Fish as models for cognition studies

Until much of the last decade, it was all too common to hear how a fish's ability was driven by instinct, and generally these creatures were considered to have a “three second memory” (Laland et al., 2003). New research, however, has now shown how misconceived this image was, and fish are currently recognized as having many forms of intelligence, such as social intelligence (Bshary et al., 2002, 2006), capable of using cooperative strategies to inspect predators and catch food (Godin & Davis 1995, Dugatkin, 1997). Fish have been shown to distinguish social ranks of others and track relationships of third-parties (Bshary et al., 2002). They have even been documented using tools, building complex nests or bowers (Paxton & Eschmeyer, 1998) and have been shown to have considerable long term memory (Brown, 2001; Warburton, 2003).

Furthermore, experiments have examined the impact of cognitive ability on behaviors related to mate selection and mimicry, and how different factors influence the decisions animals make (Godin & Dugatkin, 1996). Dugatkin (1992) found evidence of copying behavior in female guppies (Poecilia reticulata) who “observed” a model female that had to choose between two males. After the model female was removed, the focal female was found to spend a greater proportion of her time next to the male preferred by the “model,” demonstrating that focal females could remember the identity of the males “selected” by the model. In an extension of this study, young females were found to be more likely to imitate the preferences of older females compared to younger females possibly recognizing that the older fish were better informed (Dugatkin, 1992; Dugatkin & Godin, 1993).
Biological and Ecological Applications

Cognitive ecology has many practical applications to the numerous subfields in biology. Various biological properties regarding cognitive function can be individually or collectively studied (Healy, 1998; Healy & Braithwaite, 2000). For example, scientists have studied how cognition has facilitated animal foraging. The different strategies animals use to search for food varies when they are distributed in different ways (Shettleworth, 2003). For instance, the noisy minor bird (*Manorina melanocephala*) uses spatial encoding of point locations when searching for non-moving sources of food such as nectar. However, when foraging for cryptic invertebrates or fast moving prey *M. melanocephala* switches to a strategy that focuses on spotting movement (Sulikowski & Burke, 2011).

Research has also examined the impact of cognitive ability of predator inspections (Godin & Davis, 1995; Godin & Dugatkin, 1996; Dukas, 1998) and detecting alarm cues (Brown & Cowan, 2000). Specifically, how differences within the environment influence the choices animals make. These two areas are sometimes thought to coincide, as detection, assessment and recognition, and attack avoidance are integral parts of predator avoidance (Kelley & Magurran, 2003). For example, experiments has shown that three-spined sticklebacks (*Gasterosteus aculeatus*) from predator rich environments are more vigilant, display enhanced fright reactions and have faster escape speeds than conspecifics from low predation areas (Huntingford et al., 1994). Similarly eastern minnows (*Phoxinus phoxinus*) that live sympatrically with pike (*Esox lucius*) tend to approach potential predators in larger groups and are more likely to school than populations with fewer predators (Magurran, 1986). Furthermore, there has been a great deal of research demonstrating the cognitive processes of gathering and using chemosensory cues (Mirza & Chivers 2001, 2002). Studies using *P. phoxinus* have shown that these minnows acquire the recognition of chemical and or visual cues of a predator after a single exposure to the predator cue while paired with conspecific alarm cue (Mathis & Smith, 1993; Chivers & Smith, 1994).
Other research has focused on spatial memory navigation. Studies show that learning navigational cues vary across different populations of the same species where there are different levels of ecological stability (Girvan & Braithwaite, 2000; Odling-Smee et al., 2008). Where the environment is inherently more stable, fish pay more attention to and remember landmark cues compared with populations from comparatively unstable sites which show a preference for ego-centric orientation cues, such as swim upstream, or turn left into the river bank (Girvan & Braithwaite, 2000).

Investigations using the hermit crab, *Pagurus bernhardus* have shown aggression and negotiations between rival crabs use shell rapping in an attempt to steal or sway the adversary into trading its shell (Dowds & Elwood, 1983; Briffa & Elwood, 2001). These bouts of aggression where the crabs grip hold of one another and then vigorously bang their shells together are interesting by themselves, yet they give a glimpse of the complex behavior that these small arthropods use to inspect, size-up and lay claim to desirable shells. The crabs use the bouts of shell rapping to learn about and remember the different qualities of their opponent’s shell (Briffa & Elwood, 2001). Learning clearly has its place even for the invertebrates.

Cognition and learning also has considerable importance for most vertebrates. For example, the fear of predators has significant implications regarding properly recognizing appropriate cues and is widely used as a case in point for social learning (Pearce, 1997). In an example by Mineka & Cooke (1988) wild monkeys that encountered a snake would react fearfully (using facial expressions, alarm calls & avoidance), whereas control lab reared naïve monkeys showed no reaction. Next, they showed that a naïve monkey whom “observed” a wild money display fear towards a snake. When this observation occurred the naïve animal would react similarly (fearful) immediately. Future encounters with snakes also resulted in displays of fear in these naïve lab reared monkeys. This type of social learning has been termed observational conditioning (Pearce, 1997).
Behavior vs. Temperament

The range of traits that can affect an animal’s personality has been referred to in a variety of ways ranging from coping styles (Koolhaas et al., 1999), behavioral syndromes (Sih et al., 2004), temperament (Réale et al., 2007) and more recently, behavioral types (Edenbrow & Croft, 2011). These different approaches to addressing animal personality have built upon the foundation of cognitive ecology. In this thesis I will be referring to these traits hereafter as temperament. Measures of temperament can include neophobia (fear of new places/things), boldness, aggression, risk taking and exploration (Brydges, 2008; Edenbrow & Croft, 2011). In chapter two, I use the concept of "boldness" and test how this correlates to cognitive performance.

Temperament traits are often misinterpreted as bimodal variables, in which two extremes are expected to have total representation (Koolhaas et al., 1999; Réale et al., 2007), for example shy-bold. In truth various degrees of boldness ranging from very shy to extremely bold are the temperament phenotypes observed for boldness in natural populations (Réale, 2007).

Exploratory behavior has traditional been used as a proxy for many temperaments because of the ease at which it is observable (Verbeek et al., 1994; Titulaer, 2012). Fast explorers move and inspect quickly however, they tend to inspect superficially, and they quickly lose their attention for the environment (Verbeek et al., 1994). These animals are more resistant to environmental change as the animals tend to be less flexible and are prone to developing routines. On the opposite end, slow explorers are thought to explore more thoroughly and to be guided by external stimuli. These external cues allow the animal to respond better and to be more aware of changes in the environment (Verbeek et al., 1994). These ideas have been supported from experiments using reversal learning. In reversal learning, animals first learn a task or to discriminate between stimuli in which one stimulus is rewarded. Subsequently in the reversal phase the reward is shifted to the previously unrewarded stimulus whereas the previously
rewarded stimulus now yields nothing (Titulaer, 2012). Slow explorers have an advantage in such a task as they are guided by external environmental cues, whereas the opposite is true for fast explorers (Verbeek et al., 1994).

Previous work by Bell (2005) with three-spined sticklebacks (*Gasterosteus aculeatus*) has shown that high predation populations of three-spined sticklebacks had positive correlations with boldness and aggression. This was not the case with fish from low predation populations where no such correlations were observed. Although some caution should be attached to these observations as the fish were only sampled from one high and one low predation river, the results suggest that an animal’s temperament does not always act as an evolutionary constraint (Bell, 2005). As such temperament can play an important role when investigating cognitive variation in fish.

The study species I use in my two data chapters is the Panamanian bishop fish (*Brachyrhaphis episcopi*). Previous research has shown in several different assays that high predation populations are bolder than low predation conspecifics (Brown & Braithwaite, 2004b; Brown et al., 2005; Archard & Braithwaite, 2011b). So data from *G. aculeatus* and *B. episcopi* report a positive correlation between boldness and high levels of predation. This may seem counterintuitive in a predator rich environment, but inspecting predators may give information about the predator’s intent, such as preparing to attack or move on (Lima & Dill, 1990). Furthermore predators that have been inspected have been documented as being more likely to abandon an approach towards prey that is aware of their location (Godin & Davis, 1995). Inspecting predators by the prey fish can thus be seen as an adaptive strategy and may have been selected in high predation areas to maximize time budgets and feeding rates (Sih et al., 2004).
**Navigation systems**

Spatial memory allows animals to relocate important areas such as a safe home or shelter, a profitable foraging patch or previously hidden food caches (Healy & Jozet-Alves, 2010). Dyer (1998) noted three navigational tasks that differ in the amounts of information that they require. Specifically, animals can (1) head toward an object in plain view, (2) head for an unseen location within a familiar home range or (3) head to a goal from a distant, non-familiar location (Dyer, 1998). To navigate, animals must be able to differentiate among different directions using a compass or using their own body orientation. Secondly, the animal needs to understand its position in relation to its goal (Healy, 1998; Dukas, 1998; Brydges, 2008). Animals can use a number of senses to help them find their way to a goal; such as smell, electric fields, temperature, sound, visual landmarks, or magnetic fields (See Mouritsen, 2001 for review). These senses facilitate various navigation strategies such as dead reckoning, which is sometimes referred to as path integration (or PI), short to medium range landmark use, and long range homing (Dyer, 1998). Some animals learn to rely on multiple navigation strategies while others use very simple spatial behaviors to move between locations.

Path integration is the process some animals use to keep track of their position, in relation to a known position. This strategy is used in many insect species (Dyer, 1998). A well studied example of a path integrator is the desert ant (*Cataglyphis* sp.). These ants take outward circuitous paths in search for insect prey, but the circuitous path is in fact made up of multiple small stages. At the end of each small stage, the ants stop briefly to update their internal vector, i.e. the distance & direction they would need to take to reach their nest entrance. This is supplemented by the fact that these ants can be fast learners (Collett et al., 2003). A drawback to using PI, however, is that it is very prone to errors and an animal that is somehow displaced can completely misjudge where it needs to be and so end up a long way from its destination (Wehner, 1982) (Fig 1-2).
Figure 1-2. Path integration in the desert ant *Cataglyphis fortis*. A star marks the location of the nest. *C. fortis* is displaced after a foraging trip (dotted line) and walks towards its virtual nest. Once it travels the distance and direction required to its presumed nest location it searches, though unaware of the displacement (Collett & Zeil, 1998).

Animals that use landmarks learn to encode reliable, easily found objects, and use the learned position of these to reach a particular goal (Dyer, 1998). Despite differences with path integration, the two strategies can sometimes be used together and help to reinforce one another. There can be age dependent variation in landmark usage. In a study by Nesterova (2007) using Columbian ground squirrels (*Spermophilus columbianus*), older squirrels were found to outperform younger squirrels when global more distant cues were available. However when only local, nearer cues were available, younger squirrels outperformed their older conspecifics. These results show both variation in cue usage and the importance older more experienced *S. columbianus* may place on more reliable global cues.
Animals that use homing strategies may navigate long distances from an unfamiliar starting point to their known destination or goal (Dyer, 1998; Prior et al. 2004). It is believed that animals that rely on homing use an extrapolation based on multiple cues that form a navigational map (Mouritsen, 2001).

In aquatic environments, some species have been selected to show a strong response to return to specific, successful, spawning grounds (Dittman & Quinn, 1996; Odling-Smee & Braithwaite, 2003). These spawning grounds may require that the fish travel considerable distances; for example the Pacific salmon (Oncorhynchus sp.) navigate thousands of kilometers during its anadromous life cycle. Salmonids can stay at sea for several years before navigating back to their freshwater spawning grounds where they complete their life cycle (Dittman & Quinn, 1996). Imprinting, where the fish learn about specific olfactory and gustatory cues associated with their spawning grounds and the route between these and the coastal area are the mechanism by which smolting fish lay down precise memories that aid their journey years later (Odling-Smee & Braithwaite, 2003).

Spatial behavior can therefore range between simple to complex strategies. There is a growing interest in determining what affects the different spatial behaviors adopted by animals. If we are to better manage wild populations we need to understand what governs or hinders their movements. In some cases, environmental effects appear to influence spatial cognition, for example some salmonid populations home more accurately to their natal streams than others and it is suggested that differences in the local streams may play a role in this (Odling-Smee & Braithwaite, 2003). The research I address in chapter 2 investigates a different kind of environmental effect, the influence of the presence or absence of predators, on the spatial learning of natural populations of fish.
Figure 1-3. Life cycle and migratory pattern of Pacific salmon populations in Ilamna Lake, Alaska. (A) Freshwater residence period during development (B) Ocean distribution and maturation cycle of salmon prior to homing migration (Diagram from Dittman & Quinn, 1996).

Conditioning and Memory

Memory has a variety of definitions. For this thesis I refer to memory as the capacity to store and retrieve information from the past (Dukas, 1998). Memory is an important process that has a role in many cognitive functions such as retrieving food, remembering safe locations, or
spawning grounds (Shettleworth, 1998). One simple form of memory that can be tested through conditioning is associative memory. Associative memory or classical conditioning is a form of learning whereby two events are paired together. These events are the conditioned stimulus (CS) and a biologically significant unconditioned stimulus (US) (Pearce, 1997). For example, in the famous experiments by Pavlov, his dogs learned to associate a metronome (CS) with the delivery of food (US). As a result of this conditioning the dogs would salivate when they heard the CS, this salivation has been described as a conditioned response (CR) (Pavlov, 1927).

There are a number of variations of associative learning or conditioning. The most basic forms of conditioning are trace and delay conditioning. In trace conditioning, the US is presented after the CS has stopped or been turned off. Therefore there is a gap in which no stimuli occur. The CS is absent a little while before the US reward is delivered, this gap is known as the trace interval (Pearce, 1997). As trace conditioning imposes additional task requirements since there is stimulus free time gap (trace interval), some telencephalic (forebrain) structures such as the hippocampus become engaged (Rodriguez et al., 2005; Kim et al., 1995). Additionally, trace conditioning is less effective when long trace intervals are used, thus short-term retention is a primary focus (Pearce, 1997).

In delay conditioning there is an overlap for both the CS and the US. This conditioning can be easier for animals to learn as the pairing easier to associate when there is no interval or gap between the CS and US (Rodriguez et al., 2005). In Chapter 3 delay conditioning is used to train fish to respond to a CS, to investigate whether this simple form of conditioning is affected by fish coming from high or low stress environments.

The cerebellum, or hind brain, is an important area of the vertebrate brain used in certain memory processes (Rodriguez et al., 2005). In a study by Salas et al., (2006) cerebellum ablation in goldfish (Carassius auratus) caused severe impairments in the classical conditioning of eye-retraction responses. Thus, the cerebellum plays an essential role in conditioning in delay
conditioning tasks. As previously mentioned, the hippocampus, which is additionally required for trace conditioning does not appear to be necessary for delay conditioning (Rodriguez et al., 2005).

Extinction is the process in which a conditioned response falls back to pre training levels. In order to investigate extinction an animal must first be conditioned through either trace or delay conditioning. After conditioning occurs then extinction training begins with the CS now given on its own, i.e. this is no longer given with a reward. This is repeated until the subject discontinues the CR when given the CS is given in the absence of a US. The research I address in Chapter 3 focuses on conditioning and the extinction response and the influence of the presence or absence of predators in natural populations of fish in terms of the speed with which they acquire and then extinguish a delay conditioned task.

**Study System**

Panama forms an isthmus in Central America that is approximately 70 km wide between the Gulf of Panama (Pacific Ocean) & the Caribbean Sea (Atlantic Ocean). Rainfall is highly seasonal with the dry season starting in December and lasting until April (Angermeier & Karr, 1983).

Waterfalls found in many streams that drain into the Panama canal create barriers that separate populations within a number of Panamanian rivers. Most notably these barrier waterfalls result in populations that experience very different levels of predation as predator species typically are not found above the waterfalls (Jennions & Telford, 2002; Brown & Braithwaite 2004a).

Panamanian bishops, hereafter referred to as *B. episcopi* are a small live-bearing Poeciliid fish endemic to freshwater streams and rivers in Central Panama. Their diet consists of mostly terrestrial and aquatic invertebrates (Angermeier & Karr, 1983). In the streams sampled for my
experiments, only two species are present above the waterfalls; *B. episcopi*, and the killifish *Rivulus brunneus*. Below the waterfalls, however, there are significantly more piscivorous predators including cichlids (*Geophagus* and *Aequidens* spp) and tigerfish (*Haplies* spp) (see Brown & Braithwaite 2004b for details on the range and density of fish fauna).

This system provides an opportunity to study how predation influences cognitive ability. The ease of maintaining *B. episcopi* in captivity and their longevity has made them ideal to investigate how ecological differences at the population level influence behavior. Previous studies on *B. episcopi* have addressed aggression (Archard & Braithwaite, 2011), boldness (Brown & Braithwaite, 2004b; Brown et al., 2005; Archard & Braithwaite, 2011b), cognitive function (Brown et al., 2007), stress response (Brydges et al., 2009), mate choice (Simcox et al., 2005) and exploration and activity (Archard & Braithwaite, 2011a).

*B. episcopi* were caught using seine and dip nets. They were collected in March 2010 before being transported back to Pennsylvania USA to research facilities at Penn State University. When necessary, fish were individually marked by elastomer dye (under MS222 anaesthesia), using color tags on both sides of their body behind their gill cover. Using *B. episcopi* provides a rare opportunity to explore the ecological implications of living with predators and the effects this has on animal behavior, and specific to my interest, how this affects their cognitive abilities.

**Aims/Goals of Thesis**

I have focused my research on cognitive ecology, particularly on predator prey interactions and their effect on learning. Cognitive ecology has received increasing attention over the last decade with more studies being published each year (Dukas & Ratcliffe, 2009). My research investigates differences among various populations of *B. episcopi* with the aim of determining how variation in environments influences cognition.
Structure of Thesis

My thesis consists of two data chapters and a final chapter that summarizes the thesis and proposes future directions for this area of research. Both chapters two and three have been prepared as draft manuscripts for submission to peer reviewed journals and thus there is a little bit of repetition in each in terms of the descriptions of cognitive ecology and aspects related to the field sites where the fish originated and so forth.

In Chapter two, I elaborate on the predator prey interaction and it’s affect on learning a spatial task. I test the hypothesis that fish from high predation sites are bolder than fish coming from low predation populations and how this influences performance in a maze task (Brown & Braithwaite 2004a; Brown et al. 2005; Archard & Braithwaite, 2011b). It was also hypothesized that fish from high predation sites would make rapid decisions about which route to take, and that this could lead to a speed/accuracy trade-off. Finally it was hypothesized that fish from different populations would vary in spatial information that they learned and remembered. In chapter two, I analyze how spatial information is acquired, stored, and how it is used when navigating.

The third chapter explores associative learning processes in *B. episcopi*, in particular, their capacity to stop responding to a learned association during an extinction process. It was hypothesized that fish from high predation zones would have longer extinction responses as previous studies have shown that they have lower behavioral flexibility than conspecifics from low predation environments (Brown & Braithwaite, 2004a; Brown et al., 2005). Thus, low predation fish are expected to adjust more quickly to the changed schedule and ignore the CS without food reward. In this experiment we conditioned fish to respond to a stimulus (light source) and then monitored their extinction rates.

The final chapter focuses on the ecological aspects of learning and how variations in environments can have significant implications on what is used from the environment. This chapter reviews previous research and explains the contribution of both my experiments in a
broader context. Finally I raise a number of implications that my research has for fisheries management, conservation and fish welfare.
Chapter 2.

Investigating the speed-accuracy paradigm in *Brachyrhaphis episcopi*: Do different experiences with predation pressure influence the way fish solve spatial tasks?

Summary

The way animals behave is influenced by their ability to process, store and retrieve information. The ability to perform these processes, known collectively as cognition, fluctuates between species and even between individuals within a species. Some of this variation is promoted by differences in ecological conditions, such as the relative stability of habitats or the number of predators that frequent an area. Experiments that compare the learning and memory abilities of animals coming from populations exposed to contrasting ecological environments can provide insights into factors that contribute to variation in cognitive ability. In this study we compared the tropical fish *Brachyrhaphis episcopi* from two rivers. Fish were sampled from 4 populations (2 populations per river), fish from sites downstream of waterfalls were exposed to large numbers of predators, while populations above waterfalls experienced few if any predators. Spatial learning ability was compared to determine whether fish varied in their ability to solve a maze, and in the cues that they paid attention to. Fish from high predation sites were quicker to make decisions about which route to take, but were also more apt to make mistakes. In contrast, fish from low predation areas made slower, more accurate decisions. In terms of which cues the fish learned to use, we found that relative exposure to predators did not predict cue use, but that fish from different rivers were using different spatial information.
Introduction

Experience with an environment can influence the way an animal processes information and can affect the decisions that it makes. In this way, ecology influences animal cognition (Dukas 1998; 2004). The processes underpinning animal cognition can be considered to be a series of stages. First the animal detects and internalizes environmental stimuli. Second, it processes variables that require an immediate response while also remembering information that it may need to use at a later point. Finally, the animal can retrieve specific stored information and compare this to the situation it currently experiences (Dukas, 1998). There is considerable variation in the ability and the speed with which animals make decisions (Burns & Dyer, 2008; Trimmer et al., 2008; Chittka et al., 2009; Stroeymeyt et al., 2010). Furthermore, certain temperament traits such as relative boldness or propensity to explore can affect other behaviors, including decision making and learning (Réale et al., 2007; Titulaer, 2012). As these different temperaments can affect the timing of decision making we sometimes find speed accuracy trade-offs whereby an animal may make fast but not very accurate choices (Burns & Rodd, 2008; Muller & Chittka, 2008 Chittka et al., 2009).

A variety of species and study systems have been used to explore different ways in which ecology has an impact on cognition and behavior. For example the different strategies animals use to search for food varies when these are distributed in different ways (Shettleworth, 2003). The noisy minor bird (Manorina melanocephala), for instance, uses spatial encoding of point locations when searching for non-moving food stores, such as nectar, but, when foraging for cryptic invertebrates, or fast moving prey, it relies on strategies that focus on spotting movement patterns (Smith, 1974; Sulikowski & Burke, 2011).

Other studies have found differences across populations sampled from contrasting habitats. For example, Metcalfe and colleagues (1987) found that juvenile salmon (Salmo salar)
from areas with different numbers of predators differed in their ability to distinguish between edible and inedible food (using pellet size to create these different categories.) They showed that salmon from low risk populations were more likely to orient to edible foods, to attack the food and made over all fewer mistakes. On the other hand, salmon from the higher risk environments were slower to respond to the food, less likely to attack it and more prone to making mistakes (Metcalfe et al., 1987). These results suggest that the ability of salmon to forage and discriminate between novel prey was influenced by the nature of their environments and, particularly, to variation in predation pressure.

More recently, learning was compared in the Panamanian bishop fish (B. episcopi) that also come from populations that naturally vary in the number of predators that they are exposed to (Brown & Braithwaite, 2004a). When different populations of fish were compared in an associative task that required the fish to learn a colored cue to locate a food reward, fish from low predation sites were able to learn this task significantly faster than those from high predation sites (Brown & Braithwaite, 2004a).

Following on from these results, we designed a study to compare the performance of fish as they navigate through different compartments of a maze, where they had to remember more than one piece of information to solve the task. We chose this design to investigate the kinds of spatial cues the fish use, to determine how well and how flexible B. episcopi are at updating their spatial understanding, as well as comparing the speed and accuracy of fish from different populations.

As earlier studies have repeatedly reported that B. episcopi from high predation sites are bolder than fish coming from low predation populations (Brown & Braithwaite, 2004a; Brown et al., 2005; Archard & Braithwaite, 2011b; Archard et al., 2012), we hypothesized that fish from high predation sites would make rapid decisions about which route to take. However, studies with other species have found that quick decisions are often less accurate (Lima, 1998; Burns & Dyer,
2008; Burns & Rodd, 2008), thus we further hypothesized that high predation fish would make more errors as they swam through the maze. As high predation environments are likely to have increased cognitive demands, we hypothesized that fish from high and low predation populations will vary in the cues that they learn to solve the spatial task (Girvan & Braithwaite, 1998). Finally, to examine spatial flexibility, once fish completed the original task we changed the design of the maze and tested how long fish took to learn the reconfigured maze.

Materials/Methods

*B. episcopi* are small freshwater poeciliid fish native to Panama that are found in streams, and feed primarily on a diet of small insects (Angermeier & Karr, 1983; Jennions & Telford, 2002). Fish were collected from four populations on two rivers using seine and dip nets. They were collected in March 2010 before being transported back to research facilities at Penn State University. Fish were collected from the River Macho and River Quebrada Juan Grande (QJG) (see Table 1 for details of the collection sites). Each river had an upstream population and a downstream population, with a waterfall acting as a geographical barrier separating them. Above the waterfalls only two species are present, *B. episcopi*, and the killifish *Rivulus brunneus*. Below the waterfalls, there are significantly more piscivorous predators including cichlids (*Geophagus* and *Aequidens* spp) and tigerfish (*Haplies* spp) (see Brown & Braithwaite, 2004b for details on the range and density of fish fauna). As previous studies have described several behavioral differences between sexes (Holder et al., 1991; Burns & Rodd, 2008; Archard & Braithwaite, 2011a), this study focused on adult females only.
Table 2-1. Populations used & GPS coordinates of capture sites.

<table>
<thead>
<tr>
<th>River Macho</th>
<th>River Quebrada Juan Grande (QJG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>9° 1' 03&quot;, 79° 45' 42&quot;</td>
</tr>
<tr>
<td></td>
<td>9° 08' 37&quot;, 79° 43' 00&quot;</td>
</tr>
<tr>
<td>Low</td>
<td>9° 11' 02&quot;, 79° 45' 36&quot;</td>
</tr>
<tr>
<td></td>
<td>9° 08' 37&quot;, 79° 42' 57&quot;</td>
</tr>
</tbody>
</table>

Fish were marked using elastomer dye (under MS222 anaesthesia), using color tags on both sides of their body behind their gill cover (IACUC protocol number: 36902). The color tag patterns were unique to allow individual fish to be identified. After marking, fish were transferred into a housing compartment of the test tank (see Fig. 2-1). The fish were given 6 days after the anaesthesia and tagging to allow them to acclimate to the test tank.

Initial group sizes were 15 fish per population. Individuals that tended to freeze and did not perform during the early training phase were removed which left four groups of 12 fish. Thus for each river we tested 12 fish from a high predation population and 12 from a low predation population.

**Experimental set-up**

Training and trials were performed daily between the hours of 1000-1600. We constructed two maze test tanks within glass aquaria (121.92cm L x 45.72cm W x 53.34cm H) with 16-18 cm depth of water. Each tank was divided into 6 chambers (see Fig. 2-1). When not performing in trials, the fish spent most of their time in the housing area. At the beginning of a trial, individual fish were moved into the start box. On leaving the start box, the fish could progress through the maze through chamber 1 (C1), into chamber 2 (C2) and then into the reward chamber (RC). To pass through each chamber fish had to find a route past a wall with two doorways, one door led to a dead-end but the other was open. Once the fish reached the reward
chamber, they had access to food as well as visual contact with a stimulus shoal in a compartment separated by a clear plexi-glass window. After the fish had consumed the food they were allowed to enter the social chamber (SC) via a small trap door (see Fig. 2-1).

**Figure 2-1.** Schematic view of both the maze layout, and the living area for fish during trials. The different areas were made accessible or sealed off by sliding doors operated by remote pulleys. Landmarks are positioned next to open doorways (location of open doorways, first right then left, or first left, and then right was counterbalanced across populations).

Opaque black plastic covered the four sides of the tank to minimize disturbance or distractions during trials. Small viewing slits were cut into one of the sides to allow the observer to monitor the movement of the fish. Trap doors between the living area, the start box, chamber 1, as well as the reward and social chamber were operated with a remote pulley system. This trap door system was used to prevent fish from back tracking and also allowed the separation of fish that had completed a trial from those that were still to be tested.
Throughout the experiment, *B. episcopi* were kept on a 12L: 12D photoperiod. Water temperatures were held at 76 ±1.5°F with the use of aquarium heaters. Fish were fed daily via the food reward and then *ad libitum* with flake food after all trials were conducted. Food rewards were freeze dried blood worms secured in a weighted plastic cup (1.2 cm deep and 2.8 cm diameter) filled with Vaseline.

Training and Testing

To provide the fish with the opportunity of learning the spatial task, the experiment was divided into distinct stages (i) acclimation, (ii) training (I & II), (iii) trials and (iv) reconfigured maze.

Acclimation

This provided all fish with the chance to explore the general layout of the test tank as a group. For this phase, the maze was left completely open, with no dead-ends or other obstructions so that the fish could swim freely through the entire tank. The goal of this phase, which lasted 3 days, was to encourage all fish to swim around and explore.

Training I

Fish were split into small groups consisting of 5-6 fish for daily training. A single group was guided (by dip net) from the home compartment into the start box. Once the entire group was in the start box the door to the housing area was closed behind them; the trap door leading to the maze was then raised and a stop-watch was started. This rapid start procedure, used to reduce potential stress from confining the group within the small area of the start box, was only done in this phase. Each group had 30 minutes to explore the maze layout. Now, each barrier wall had one open and one dead-end door. A small plant was positioned next to the open doorways to
provide a potential landmark. Reward food cups were placed next to open doorways to encourage the fish to approach and move through the maze. At the end of this stage fish that persistently froze were removed from the experiment.

**Training II**

Here, pairs of fish were allowed to swim through the maze. Each pair was isolated in the start box for 2 minutes before the door to the maze was raised giving fish access to the maze. Fish were allowed 10 minutes to complete the maze, whereas those that did not explore the maze were guided to back to their home chamber via dip net. This stage lasted until fish successfully reached the final reward chamber in 5 trials.

**Trials**

In this phase, single fish were isolated in the start box. After 2 minutes of settling time, the door into the maze compartment was opened. A stop-watch was started and the latency before fish left the start box was recorded and this was later used as a measure of relative boldness (see Brown *et al.*, 2005b). After this, the trial was considered to have started. The time to make a first choice (move through one of the doors in the first wall), time to make a first mistake and time taken to reach the reward chamber were noted as well as the total number of mistakes made en route (i.e. dead-end doorways entered). Fish continued single trials until they reached a criterion level of performance; successfully moving through the maze on three consecutive trials under 75 s making only 1 error.

**Determining the spatial cues used**

To find out whether fish solved the maze using the plant landmarks or whether they learned a sequence such as “take left door then right door,” the maze was reversed. Doors that
were previously dead-ends were now opened up and vice versa. The plants moved to the new open door location and thus continued to be reliable cues indicating open door position. Fish using landmarks were expected to follow these and so swim through the reversed maze relatively quickly making few errors. However, fish using the sequence of turns for open and closed doors would now presumably make errors as their strategy would cause them to keep entering dead-ends. Thus, their time to complete the maze and the numbers of errors made was expected to increase (see Girvan & Braithwaite, 1998).

Reconfigured-maze trials

After the first reconfigured maze test, trials continued to determine how long each population took to reach criterion in the altered maze. Trials with single fish continued until they successfully met the same criterion performance level as before (complete 3 consecutive trials under 75 s making only 1 error).

Statistical Analyses

Data were analyzed using Analyses of Variance (ANOVA). Prior to analysis data were tested for homogeneity of variance and when necessary \( \log_{10} \) transformations were applied to conform to the assumption of equal variance between groups. For each analysis the dependent variable was explored in terms of the level of predation, the river the fish came from and the interaction between these two factors. A probability value of 0.05 or less was used to determine the statistical significance of the comparisons.
Results

**Latency to leave start box**

There was a significant effect of predation with high predation fish being faster at leaving the start box compared to low predation fish ($F_{1,39} = 23.306, p < 0.001$; Fig. 2-2). There was also a significant river effect, Macho river fish were faster at leaving the start box than QJG fish ($F_{1,39} = 30.339, p < 0.001$). There was no interaction between river and predation regime ($F_{1,39} = 1.996, p = 0.166$).

![Figure 2-2. Boldness quantified as latency to leave the start box area and begin the maze. Bars represent mean values ± s.e.](image)

**Time to make first choice**

Using data from the last trial only, i.e. when the fish are assumed to understand the maze task, a comparison of the speed with which the fish entered their first doorway showed that fish from high predation areas entered a door sooner than fish from low predation sites ($F_{1,39} = $
14.422, \( p < 0.001 \); Fig. 2-3). There was no river effect \( (F_{1,39} = 0.792, \ p = 0.378) \) nor was there an interaction \( (F_{1,39} = 0.009, \ p = 0.927) \).

![Bar chart showing log time to make a decision by population](image)

**Figure 2-3.** Time to make first choice during the final criterion trial. Fish from high predation sites made quicker decisions to enter a chamber.

**Errors made**

During trials, fish from high predation sites made significantly more mistakes by entering dead-ends than fish from low predation sites \( (F_{1,39} = 9.612, \ p < 0.003) \); Fig. 2-4), and there were differences in the number of mistakes made by fish from the different rivers \( (F_{1,39} = 4.661, \ p = 0.037) \); fish from Macho river made more errors than fish from QJG river. The interaction was not significant \( (F_{1,39} = 2.438, \ p = 0.127) \).

![Bar chart showing number of mistakes by river](image)

**Figure 2-4.** Number of mistakes observed in the original maze (prior to reversal). Fish from high predation zones made more mistakes compared to fish from low predation zones.
**Time to make a first mistake**

When the time to make a first mistake was compared, there was a significant effect of predation ($F_{1,39} = 14.422$, $p < 0.001$). There was no difference in fish from different rivers ($F_{1,39} = 0.792$, $p = 0.379$) nor was there an interaction ($F_{1,39} = 0.009$, $p = 0.927$).

**Reaching criterion**

There were no differences in the number of trials taken to reach criterion during individual trials predation: ($F_{1,39} = 2.827$, $p = 0.101$); population: ($F_{1,39} = 2.711$, $p = 0.108$); interaction: ($F_{1,39} = 0.29$, $p = 0.866$).

**Reconfigured Maze**

**Spatial cues used (test on day 1 of reconfigured maze)**

The mean time fish took to complete the maze during their last 3 trials (i.e. the 3 trials used to achieve criterion) was compared with time they took to complete the first trial in the reconfigured, maze. There was a significant effect of river ($F_{1,39} = 4.01$, $p = 0.05$; Fig. 2-5A), with Macho fish reaching the end of the maze faster than QJG river fish. There was no effect of predation ($F_{1,39} = 0.932$, $p = 0.340$) and no interaction ($F_{1,39} = 0.158$, $p = 0.692$).
Comparing the number of mistakes made during the first reconfigured trial again found an effect of river with QJG fish making more mistakes ($F_{1,39} = 4.863, p = 0.033$; Fig. 2-5B), but there was no effect of predation ($F_{1,39} = 0.022, p = 0.882$) and no interaction ($F_{1,39} = 4.58E-4, p = 0.983$).

**Learning the reconfigured maze**

Comparing the number of trials fish took to make criterion in the reconfigured maze, there was no effect of predation ($F_{1,39} = 0.473, p = 0.496$) or population ($F_{1,39} = 0.044, p = 0.834$), however, analysis showed that there was an interaction ($F_{1,39} = 8.391, p = 0.006$; Fig. 2-6A). The number of mistakes made during these trial similarly showed no effect of predation ($F_{1,39} = 1.033, p = 0.315$) or river ($F_{1,39} = 0.172, p = 0.681$), but there was an interaction ($F_{1,39} = 8.655, p = 0.005$; Fig 2-6B).
Discussion

Fish from high predation populations were found to be faster at leaving the start box faster than low predation fish, which is consistent with previous measures of boldness in this species (Brown et al., 2005b). During trials high predation fish also made faster choices about which doors to enter, but they also made more mistakes than low predation fish. These results suggest a speed accuracy trade-off in which high predation fish made rapid, but less accurate decisions about the route to take. There were also river effects with Macho fish being bolder, making more mistakes, and completing the maze faster than fish from River QJG. Our hypothesis that fish from contrasting predation regimes would use different cues was not supported; contrary to that prediction, fish from different rivers varied in the cues they used to find their way through the maze, with fish from the QJG river preferring to use a turn based strategy & fish from the Macho river relying on the plant landmarks. The fish also responded to the reconfiguration of the maze in different ways. Fish from the Macho river high predation site learned the new maze
layout faster than those from the low predation site. In contrast, in the QJG populations, low predation fish learned the reversed maze faster than high predation QIG fish.

**Latency to leave start box**

There are many potential ways to both define and measure boldness, here, we considered fish that emerged more quickly as being “bolder” than those that took longer. There were both population and predation effects influencing boldness. In both river systems, high predation fish were bolder, supporting our initial hypothesis and also confirming previous observations (Brown *et al.*, 2005b; Archard & Braithwaite, 2011b). In addition, river Macho fish generally emerged faster than fish from river QJG. Considering the similarities of the river ecosystems (Brown *et al.*, 2005a), this result was not predicted, although surveys from 2004 reported that River Macho had overall fewer predators, and more conspecific competitors (Brown & Braithwaite, 2004). Macho fish may therefore be generally bolder as a result of increased competition (Archard & Braithwaite, 2011a).

Being bold & more active may seem counterintuitive in a predator rich environment, but being bolder in high predation areas may be necessary to allow the fish to find enough food, or have access to mating opportunities (Lima & Bednekoff, 1999) and it may help increase efficiency while feeding (Sih *et al.*, 2004). Furthermore, moving toward and inspecting predators can provide useful information about the predator’s intent to attack or move on (Lima & Dill, 1990), and predators that have been inspected often abandon their approach towards prey (Godin & Davis, 1995).

Our results suggest that similar to other populations of fish, *B. episcopi* exhibit “active” (fast explorers and bolder) and “passive” (slow explorers and shy) temperaments (Verbeek *et al.*, 1996). Fast explorers are typically guided by internal cues, tend to be less flexible and more prone to developing routines (Benus *et al.*, 1990; Titulaer *et al.*, 2012). While reactive fish are better
equipped at responding to environmental change, and can learn the location of new or safe feeding patches relatively quickly (Titulaer et al., 2012). Given the differences in boldness in fish from high and low predation sites we might expect the learning abilities of these fish to also vary.

**Errors made**

One way to measure learning ability is to look at accuracy during performance; here the data showed that fish from low predation sites made fewer overall mistakes. Fish with little or no experience of predation have previously been seen to learn to associate a colored cue with a reward faster than fish from high predation sites (Brown & Braithwaite, 2004a). These differences may be related to the temperament differences described above. If the bolder fish tend to be more ‘active’ in their behavior, then we would expect them to learn new tasks less well than more timid, reactive fish. In support of this, the boldness differences seen between the rivers also follow the same pattern, Macho fish are both bolder and made more mistakes during trials than river QIG fish.

**Time to make first choice**

Franks et al. (2003) define a speed accuracy trade-off to be where an animal exhibits more or fewer errors because of the speed with which it performs a task. In the current experiment, fish from high predation areas made faster choices about which doorway to enter, but these fish also made more errors, thus a speed accuracy trade-off was observed.

Our results are in contrast to those reported by Burns and Rodd (2008). Using a different Poeciliid species (*Poecilia reticulata*), they found that low predation fish made hasty, inaccurate decisions, and that high predation fish took longer to make decisions. They reasoned that their low predation guppies could afford to make faster, inaccurate decisions, while those from high
predation sites would need to be more accurate or risk predation. While there would seem to be some merit behind this theory, the authors acknowledge that “dithering in the presence of a predator could equally well be lethal” (Burns & Rodd, 2008). Clearly there are various strategies that could be beneficial, depending on habitat and ecological settings. Burns and Rodd compared their *P. reticulata* results to those of Brown and Braithwaite (2004a) in which low predation *B. episcopi* made fewer incorrect choices to reach a reward area. Burns and Rodd speculated that differences in protocols between the two experiments (maze design, species differences, female versus male subjects) could not allow for direct comparison. Therefore once again our results support previous studies using *B. episcopi* (Brown & Braithwaite, 2004a) and highlight the fact that low predation made fewer mistakes, but slower decisions.

**Reconfigured Maze**

**Spatial cues used**

Fish from the two rivers appear to have used different spatial strategies. This was demonstrated on the first day of the reconfigured maze, when fish from QJG made a greater proportion of mistakes and had slower times, compared to River Macho. We concluded from this that QJG fish used a turn based strategy whereas fish from River Macho followed landmark cues. Previous studies have found that cues used for spatial learning and landmark use varies across different populations of the same species where there are different levels of ecological stability (Girvan & Braithwaite, 1998; Odling-Smee et al., 2008). For example, experiments with the three-spine stickleback (*Gasterosteus aculeatus*), found variation in spatial learning across different populations. Fish from ponds showed a greater reliance on visual cues and landmarks, whereas river sticklebacks preferred to learn a series of turns and used the direction of water currents (Girvan & Braithwaite, 1998; Braithwaite & Girvan, 2003). In the current study,
differences in the preferred spatial strategy (landmark or sequence of turns) were found between two rivers. Why the fish from the different rivers vary in this way is unclear.

It could be that differences in the rivers contribute to the spatial information fish select to use. Given that fish from river Macho excelled with landmarks, perhaps they were more accustomed to using rocky outcrops, plants and other visual landmark/stimuli. Similarly fish from river QJG that we suggest do not use landmark cues may have a more spatially homogeneous environment with fewer, reliable visual landmarks. Certainly future measures or stream surveys would be helpful for the sampling sites to help interpret these river differences.

**Performance during trials in the reconfigured maze**

After the maze was reconfigured, low predation QJG fish made fewer mistakes than high predation QJG fish; this is similar to previous observations (Verbeek *et al*., 1994; Brown & Braithwaite, 2004a; Brydges *et al*., 2008; Archard & Braithwaite, 2011a). Previously, such results were explained by the fact that fish with little experience of predators, fish may have an advantage in learning ability as a result of decreased interspecific competition, less lateralization of eye usage (Brown & Braithwaite, 2004a) or lower needs for vigilance (Brydges *et al*., 2008).

River Macho high predation fish made the fewest errors during trials in the altered maze. Thus, high predation Macho fish took the fewest number of trials to reach criterion again. This outcome was not anticipated and contradicted what we saw in the first part of the experiment. However, understanding the differences in cue usage is critical to understanding why there was a significant interaction between rivers and predation level in the performance of the fish in the reversed maze.

River Macho fish used the reliable landmark cues. High predation Macho fish behaved and respond as “active copers” (Verbeek *et al*., 1994) with bolder & more exploratory behavior than their low predation counterparts. The superior performance of Macho high predation fish in
the reversed maze might be due to their exploring and learning the correct cues during training. Once these active copers learned that landmarks guide them to reward(s) they persisted with their “follow the landmark” strategy. Macho low predation fish, on the other hand, are less bold and may therefore be more aware of change and behaved more as “passive–copers” (Verbeek at al., 1994). Their performance in the reversed maze may have been compromised because even though they followed the landmarks, they were aware that the position of these had changed and this higher sensitivity to smaller changes may have confused the fish.

In comparison, the River QJG fish were more confused because their use of the turn-based strategy now led them into dead-ends. The superior performance of the passive low predation QJG fish here could reflect their better ability to relearn the task. Such a result is consistent with previous work that has found low predation site fish can learn more quickly (Brown & Braithwaite, 2004a; Brydges et al., 2007), and that passive-copers are better at adapting to change (Benus et al., 1990).

Summary

Considered together with previous work (Lima & Dill, 1990), and other studies with B. episcopi (Archard & Braithwaite, 2011b), the current results show that there is a speed accuracy trade-off affected by predation regime. High predation fish made faster decisions yet made more mistakes and navigated through the maze faster than their low predation counterparts. The results also support previous results showing that fish with increased exposure to predators tend to be bolder than conspecifics from low predation zones (Godin & Davis, 1995; Sih et al., 2004; Brown et al., 2005b).

Fish from high and low predation sites varied in their performance in a maze task. Interestingly we observed a difference in spatial performance across rivers. Fish from different rivers preferred to use different spatial information to solve the maze. This was unexpected and it
remains unclear as to why such a difference exists, though we speculate habitat differences such as substrate and available landmarks may contribute to this result. The differences in relative boldness across the high and low predation sites appears to underlie interesting differences in the way different populations adjusted to the reversed maze. Overall, this experiment demonstrates that there is variation in learning ability within different populations of the same species and that several different factors contribute to this.
Chapter 3.

How does Predation influence classical conditioning and extinction in the tropical fish *Brachyrhaphis episcopi*?

**Summary**

The way animals behave is often strongly influenced by the environment that they live in. One way animals can adjust their behavior to a changing environment is to monitor change through learning and memory. Learning and memory processes are often separated to distinguish between simple forms (e.g. associative conditioning) and more complex forms (such as spatial learning and memory). Brain lesion experiments in fish have recently shown that some kinds of associative learning are controlled by the cerebellum, whereas more complex cognitive tasks involve different regions within the telencephalon. Previous work with the Panamanian bishop (*B. episcopi*), a tropical, freshwater fish that either inhabits sites with many or very few predators have found that populations differ in spatial learning abilities, with fish from low predation populations performing more efficiently in mazes. To date, however, no studies have addressed whether living with many or only a few predators influences associative learning and how long such associations persist once they are no longer reinforced. To investigate this we compared fish, from 6 populations (3 from high and 3 from low predation sites). The ability to learn a conditioned response and the rate of extinction were compared. While there was no evidence of differences in conditioning rates based on predation regime or river, we did find that fish from low predation sites were quicker to show an extinction response compared to high predation conspecifics.
**Introduction**

Predation is arguably one of the most important selective pressures in evolution (Lima & Dill, 1990). Prey species are likely to assess predation risk through information processing (learning), thus cognition plays a critical role in survival (Bateson & Kacelnik, 1998; Dukas, 1998). Predator avoidance can be costly as vigilance and predator inspections can lead to a reduction in foraging opportunities (Lima, 1987; Godin & Davis, 1995; Brown, 2003; Brydges, 2007). However, risk of predation is not the only reason to learn. In a changing or flux environment, being able to learn and adapt is extremely advantageous, and may be essential for survival (Shettleworth, 1998; Brydges, 2007).

When animals learn a response to a previously neutral stimulus that predicts an important biological event, we call this classical conditioning, otherwise known as Pavlovian conditioning (Rescorla, 1988; Griffin, 2008). The neutral conditioned stimulus is often abbreviated to CS whereas the biologically significant unconditioned stimulus is known as, and hereafter referred to, as the US. When CS and US are paired together they often produce a conditioned response (CR), the amount of pairings and memory vary amongst animals. When species learn about the sequential structure of their environment, conditioning reveals its ecological importance (Pearce, 1997).

The cerebellum, which has been commonly associated with conditioning in fish (Gomez et al., 2005), has recently been studied in greater detail using the goldfish (*Carassius auratus*). Gomez et al. (2010) specifically showed that goldfish could be conditioned using the classical eye blink paradigm. To do this, a predictive light (CS) was paired with a mild electric shock (US) near the eye. Gomez et al. (2010) reported that goldfish with cerebellum ablations/lesions were not able to learn the association between the CS and the US. This procedure showed how the cerebellum was critical in the learning of a conditioned response in fish (Gomez et al., 2010).
When training animals in a conditioning task, learning is strongly influenced by the frequency, the intensity of reinforcement and the contiguity of events. This reinforcement is a key factor in influencing what is learned and the speed with which it is learned (Warburton, 2003). For example, when the delay between stimulus (visual cues) and reward (food) is short, the formation of learned associations occurs more efficiently (Breuning et al., 1981). Conditioning can have lasting effects in fish, in fact, the old adage that fish have a “three second memory” would seem to be a misnomer (Laland et al. 2003). For instance, studies with trout (Oncorhynchus mykiss) have shown that after learning a CR a fish’s memory lasted for weeks after training ceased (Moreira et al., 2004; Barreto et al., 2006).

The conditioned association can be lost, and when this occurs extinction is said to have occurred. An extinction response happens when a previously conditioned response (CR) ceases because the CS no longer elicits a response (US). Extinction has been typically studied in examples using fear conditioning, however in the current study we investigated extinction responses using an appetitive (food) reward system.

An important question regarding predator recognition is how individuals vary in the way that they learn and remember information while avoiding biologically or ecologically irrelevant stimuli. To address this, we compared conditioning and extinction responses using animals that have naturally been exposed to different numbers of predators. Specifically, we examined conditioning and extinction processes in different populations of the Panamanian bishop fish (Brachyrhaphis episcopi) that come from different sites, and are exposed to different degrees of predation.

We hypothesized that fish from high predation populations would have longer extinction responses as previous studies have shown that they have less behavioral flexibility than conspecifics from low predation environments (Brown & Braithwaite, 2004a). In contrast, low
predation fish are expected to adjust their conditioned response more quickly when the association is altered and the CS is delivered without a reward.

Materials and methods

Animals used

Panamanian bishops (*B. episcopi*), small freshwater, Poeciliid fish native to Panama, are found in streams, and feed primarily on a diet of small insects (Angermeier & Karr, 1983; Jennions & Telford, 2002). In our study, fish were collected from six sites on three rivers using seine and dip nets. Fish were collected in March 2010 before being transported back to research facilities at Penn State University. They were collected from the Macho river, Quebrada Juan Grande (QJG) and Limbo river (see Table 3-1 for details of the collection sites).

Each river has two distinct populations split by barrier waterfalls. Above the waterfalls only two species are present, *B. episcopi*, and the killifish *Rivulus brunneus*. Below the waterfalls, there are significantly more piscivorous predators including cichlids (*Geophagus* and *Aequidens* spp) and tigerfish (*Haplias* spp) (see Brown & Braithwaite 2004b for details of the fish fauna). As previous studies have described several behavioral differences between the sexes (Holder *et al.*, 1991; Archard & Braithwaite, 2011a), this study only used adult females.

We tested fish from each of the 6 populations for each replicate (See table 3-1). The numbers used were based on fish availability and where necessary we used extra fish from some of the populations to compensate for other underperforming populations (See table 3-2). This protocol was conducted using 3 sequential replicate sets of trials. Individuals that did not respond during the acclimation phase (see below) were removed leaving a total of 49 fish.
Table 3-1. Populations used & GPS coordinates of capture sites.

<table>
<thead>
<tr>
<th>River Macho</th>
<th>River Quebrada Juan Grande (QJG)</th>
<th>River Limbo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High</strong></td>
<td>9° 11′ 03″, 79° 45′ 42″</td>
<td>09°09′ 739″, 79°44′ 498″</td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td>9° 11′ 02″, 79° 45′ 36″</td>
<td>9° 09′ 837″, 79°44′ 487″</td>
</tr>
</tbody>
</table>

Table 3-2. Experimental replicate break down based on population. Numbers in the Total Fish column correspond to fish that performed over total fish used. Abbreviations for populations are as follows LQ - lower Que Juan Grande, UQ - upper Que Juan Grande, LM - Lower macho, UM - upper Macho, LL - Lower limbo, UL - Upper Limbo.

<table>
<thead>
<tr>
<th>Population</th>
<th>Rep. 1</th>
<th>Rep. 2</th>
<th>Rep. 3</th>
<th>Total Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQ</td>
<td>2/4</td>
<td>1/4</td>
<td>1/1</td>
<td>4/9</td>
</tr>
<tr>
<td>UQ</td>
<td>3/4</td>
<td>4/4</td>
<td>1/1</td>
<td>8/9</td>
</tr>
<tr>
<td>LM</td>
<td>3/4</td>
<td>3/4</td>
<td>4/5</td>
<td>10/13</td>
</tr>
<tr>
<td>UM</td>
<td>3/4</td>
<td>0/4</td>
<td>5/8</td>
<td>8/16</td>
</tr>
<tr>
<td>LL</td>
<td>2/4</td>
<td>4/4</td>
<td>4/5</td>
<td>10/13</td>
</tr>
<tr>
<td>UL</td>
<td>2/4</td>
<td>4/4</td>
<td>2/4</td>
<td>8/12</td>
</tr>
</tbody>
</table>

Experimental set-up

Trials were performed daily between the hours of 1000-1600. Throughout the experiment, *B. episcopi* were kept on a 12L: 12D photoperiod and dimmed to simulate dawn and dusk for 30 minutes at the start and end of the day. Fish were fed daily via the food reward.

Each replicate used 24 plastic aquaria tanks (38.1 cm x 20.3 cm x 27.9 cm [L x W x H]) for both conditioning and extinction response trials. The water was maintained at 24.0 cm depth (± 1.0). Each tank contained gravel substrate, a single plastic plant, an air driven bio-filter, and a heater (set to 76 ± 1 °F). Opaque black plastic covered three sides of each holding rack on which the tanks were positioned to minimize disturbance from activities elsewhere the room.

Furthermore, tanks had opaque barriers inserted 15 minutes before trials began to visually isolate the fish during testing. Each *B. episcopi* was placed individually in its own small aquarium. A floating feeding ring was secured to center of the front wall of the tank though not used until
conditioning started. Each tank was covered with a layer of netting that prevented the fish from escaping, but that had holes (covered by an additional, removable, layer of netting) so that food could be dropped into the food ring underneath.

The exterior of the tanks were marked with a solid line indicating an “Interest threshold” 15.3 cm from the bottom of the tank. Movement of the fish above this line was used to gauge when fish showed interest in the food ring. Fish that crossed the line almost always consumed food immediately.

**Training and testing for the experiment**

The experiment was divided into 3 distinct stages (i) acclimation, (ii) conditioning, and (iii) extinction.

**Phase 1 - Acclimation**

Fish lived in their individual tank and fed normally without the conditioned stimulus (CS) for 7 full days. The goal of this phase was to slowly acclimate the fish into their new surroundings.

**Phase 2- Conditioning/Acquisition**

During a trial, a flashlight was used as the CS. This was switched on and pointed down through the food ring. As the light was switched on a stopwatch was used to time the trial. After 5s with the CS light on, a few flakes of food (the US) were dropped into the food ring. These floated at the surface within the food ring. To feed the fish had to swim up and take the food from the water surface. We measured the response of the fish, noting the point at which it crossed the ‘Interest threshold’ line. Each fish was trained individually using care not to disturb the other fish as each trial proceeded.
The delay conditioning procedure was set out as follows; CS = 10 s, after 5s of the CS the US was delivered so that the CS and US overlapped for the final 5s of the CS being on. Time to cross the “interest threshold”, and whether it was food or the light that triggered the response was recorded. Fish that did not respond to the light/food after 45 seconds from the start of the trial were labeled as non-responsive and a 45 seconds ceiling value time was recorded.

Conditioning trials happened twice a day once in the morning (between 09.00-11.00) and once in the afternoon (between 13.00-15.00). After a fish moved towards the food ring within 5s of the light switching on for 3 consecutive trials it was considered “conditioned”. The number of trials to achieve the conditioned response was recorded. Ten minutes after all fish had completed their trials the visually isolating barriers between the tanks were removed to permit visual access to conspecifics outside the trial time.

Phase 3- Extinction

After the conditioning phase, fish were tested in their response to the CS (light) alone using the same light stimuli (10 seconds of flashlight) to measure their rate of extinction. Time to produce a CR was recorded. Trials were run twice a day as before. Flake food was administered into the feeding ring 60 minutes after the flashlight had been switched off.

Fish that did not respond to the CS without food (within 10 seconds of the light switching on) were recorded as having reached “extinction”, the number of trials to reach this stage was recorded. Once a fish had been in 3 consecutive trials demonstrating “extinction” they were considered to have unlearned their conditioned response and their trials were stopped.

Statistical Analyses

Data were analyzed using Analyses of Variance (ANOVA). Prior to analysis data were tested for homogeneity of variance and when necessary Log$_{10}$ transformations were applied to
conform to the assumption of equal variance between groups. For each analysis the dependent variable was explored in terms of the level of predation, the river the fish came from and the interaction between these two factors. A probability value of 0.05 or less was used to determine the statistical significance of the comparisons.

Results

Trials to achieve the conditioned response

The number of trials taken by fish to learn the conditioned response showed that there was no effect of predation ($F_{1,47} = 1.003, p = 0.321$; Fig. 3-1), population ($F_{1,47} = .350, p = 0.707$), nor was there an interaction ($F_{1,47} = 2.111, p = 0.136$). Thus all fish similarly acquired the association between the flashlight switching on and a delivery of food into the food ring 5 s later.

![Figure 3-1. Mean number of trials taken to reach conditioning criterion.](image)

Extinction response

The number of trials to reach extinction was compared and revealed that fish from low predation areas showed an extinction response more quickly than fish from high predation sites.
There was no river effect ($F_{1,47} = .819, p = .447$) nor was there an interaction ($F_{1,47} = .896, p = .416$).

**Figure 3-2.** Number of trials taken to reach an extinction response. Fish from low predation sites showed a quicker extinction rate over their high predation conspecifics.

**Discussion**

Fish from low predation populations took less time to extinguish the learned association between the light cue and the food reward than high predation fish. However, there were no differences in how quickly the fish learned the conditioned response regardless of the level of predation the fish experiences or the rivers they were sampled from. These results partially support the hypotheses we initially proposed regarding the effects of prior experience with predation and associative conditioning. While there was an effect of predation experience on the timing of the extinction response, we did not observe a difference in how quickly the fish learned the conditioned response. It seems likely that some of the differences seen in the extinction
response may reflect differences in fish temperament associated with the environments that they are sampled from.

Previous studies of cognition in this species have found that low predation fish were faster at learning to locate a food patch (Brown & Braithwaite, 2004a; Brydges et al., 2008). Thus we hypothesized that low predation fish would also be faster at learning a conditioned response. While we observed a tendency in this direction, we did not find a significant difference between fish from high and low predation sites. The results obtained for low predation fish by Brown & Braithwaite (2004a) were with a spatial task that is known to involve the telencephalon of fish, particularly lateral pallial regions that are functionally similar to the avian and mammalian hippocampus (Broglio et al., 2003). Delay conditioned responses, however, appear to be independent of the telencephalon in fish and instead rely on the cerebellum (Rodriguez et al., 2005). The different roles that these brain regions play in these different kinds of learning task may partially explain why we did not observe a significant different in the rate at which the different populations learned the conditioned response.

Divided attention may be a significant cause for the differences amongst predation regimes. Animals that are exposed to predation threat must balance out foraging and predator vigilance (Brydges et al., 2008). In our study high predation fish could have divided their attention while remaining vigilant, causing them to have longer extinction rates. Low predation fish without the added stressors from predation could therefore devote more attention to other tasks, such as locating food, learning disassociations with the US. These low predation fish appear to be adapted to uncoupling no longer relevant associations between stimuli.

Temperament has many varying definitions (reviewed in Reale et al., 2007), though we refer to it in terms of how it affects an animal’s behavior (i.e. personality). In chapter two, we found that *B. episcopi* exhibited traits consistent with “active” (fast explorers and bolder) and “passive” (slow explorers and shy) temperaments (Verbeek et al., 1996). The results from
Chapter two also showed that low predation fish were more timid yet more accurate when learning a spatial task. These coping styles correlate with both learning ability and the stress response (Koolhaas et al., 1999). Tonic immobility or freezing behaviors are associated with reactive coping styles, and corticosteroids can play a large role in this behavior (Koolhaas, 2007). Consistent with this, low predation (reactive) fish have been observed to freeze more when threatened (Beri, pers. obs.). High predation *B. episcopi* release lower levels of cortisol, and recover more quickly after a stressor has been applied (Archard et al., 2012). Archard et al. (2012) proposed that while *B. episcopi* have to cope with predation stress, they still need to forage and secure mating opportunities, and thus to cope in their high predation environment they down regulate their cortisol based stress responsiveness.

An individual’s stress responsiveness can have a direct effect on developing an extinction response. In a study by Moreira et al. (2004), it was shown that artificially selected lines of high response (HR) rainbow trout (*Oncorhynchus mykiss*), that is fish that produced higher levels of cortisol when stressed, showed a faster extinction response (taking fewer than 14 days) than fish from a low response strain (LR) which took up to 21 days to become extinguished. Low predation fish are more timid (Brown et al. 2004, 2005), have a passive or “reactive” coping style (as described in Koolhaas et al., 1999; Verbeek et al., 1996), and have high physiological reactions to stress (Archard et al., 2012), thus can be considered analogous to HR fish. Reactive individuals have been documented as being quicker to adapt to surroundings (Verbeek et al., 1994; Brown & Braithwaite, 2004a; Brydges et al., 2008; Ruiz-Gomez et al., 2011), while active individuals tend to work within fixed routines (Benus et al., 1990; Titulaer et al., 2012). Thus low predation reactive fish may have been more able to recognize that the CS no longer predicted food, and thus uncoupled the association and stopped their response to the light cue faster than the high predation “active” copers that continued to move to the light despite it being unrewarded. So when confronted with environmental change, reactive, HR individuals (Moreira et al., 2004;
Ruiz-Gomez et al., 2011) which in our assay were the low predation fish, have an advantage over conspecifics that deal with an increased predation threat. Recently, Reale et al. (2007) suggested that temperament traits and motivational states should be considered within an ecological context. Our results support this view.

**Summary**

The results in this study highlight that when confronted with environmental change, low predation fish were better able to adjust their behavior to new conditions than high predation fish. This coincides with *B. episcopi* displaying coping styles that vary with a reactive style seen in low predation fish, whereas a proactive style is more common in high predation fish. Such differences in coping style appear to have an effect on cognition, high predation fish showed a tendency to develop and follow routines, and were less likely to pick up on changes in their environment, whereas low predation fish were much faster at noticing a change when it occurred. There appeared to be a trend for low predation fish to learn the conditioned response more quickly than high predation fish, but this was not significant.
Chapter 4.

Discussion

In chapters two and three, I focused on behaviors that are linked with two distinct parts of the brain, yet in both cases, predation pressure appears to underlie the variation in cognitive abilities that were observed. Using wild caught Panamanian bishop fish from several different populations, I investigated how early life experience of living with many or just a few predators influenced two kinds of cognition. First, spatial learning was assessed to investigate how the fish collect and process information to help them complete a navigational problem (Chapter 2). Then I compared the conditioning and extinction responses of the fish in a simple associative learning task (Chapter 3). The results of these experiments demonstrate that ecology can play a role in what and how animals learn and remember.

Ecology and navigation

Animals use a variety of methods to navigate to both known and unknown goals (Dyer, 1998; Dukas 1998). In chapter 2, I discussed how predation affects decision making in relation to spatial tasks. The second chapter also revealed information about cue usage. Fish sampled from two different rivers used different strategies. This variation may arise owing to physical differences within the rivers themselves. For example the Macho river may have more features that lend themselves to being sued as landmarks, whereas these may not exist in the Quebrada Juan Grande river. This is something that should be quantified when researchers go back to these field sites.
Previous research has shown that fish were able to use landmarks to navigate a maze task (Girvan & Braithwaite, 1998; Odling-Smee & Braithwaite, 2003; Braithwaite & Girvan, 2003). Landmarks are relatively stable in many ecosystems. In these previous studies pond fish were more apt to use landmarks, this was explained by the fact that these pond systems had more stability. In contrast fish coming from more changeable river habitats ignored landmark cues and relied more on flow direction, or egocentric (turn-based) strategies (Braithwaite & Girvan, 2003).

Fish that did not use landmarks were assumed to have learned to rely on a turn based strategy. We based this assumption on the fact that fish did not have access to many other cues. Fish were isolated on their own and the maze was surrounded by opaque paper. However I need to mention that there is a growing evidence that fish can use geometry for orientation (Sovrano et al., 2002, 2003, 2007; Brydges et al. 2008b).

A suggested reason for the difference in navigational cues used by *B. episcopi* was the fact that rivers may have had different spatial structures, i.e. rocky vs. smooth benthic regions. The types of cues presented and the situation can affect how much or how little attention an animal devotes to it (Brydges, 2008). Thus again we are confronted with ecology and the variation and stability of habitats. The data in chapter 2 revealed differences in the spatial cues used based on river systems. This pooled data gives us a snap shot of how living with predation stress has shaped the way and what these fish learn.

**Comparative review of the brain**

The vertebrate brain is a highly complex organ. And, despite hundreds of published papers and articles, we still do not fully understand “how” brains work (Dukas, 1998). The intricacies of the animal brain are complex and changeable, making it a challenging organ to study. Regardless of our limited knowledge on the finer details of the brain, we have been able to gain an understanding with regard to certain brain functions.
Within the vertebrate brain lies a specialized region known as the hippocampus, this integral area belongs to the limbic system. The hippocampus, named in mammals for its similar appearance to a sea-horse (*Hippocampus hippocampus*), has long been identified as a critical component for the consolidation of short term memories into long term memories and also for spatial navigation. As such, its influence on navigational ability and spatial memory have been the focus of many experiments over the last three decades (reviewed by Shettleworth, 1998). A significant portion of this research has used mammalian and avian systems (See Dukas & Ratcliffe, 2009 for review). Several of these studies have acknowledged the role that spatial memory has on large scale processes such as food storing, migration, and homing. In addition, however, it is responsible for learning more local, regular spatial tasks such as learning and remembering the location of profitable food patches, safe foraging sites and the position of shelter or nests/ burrows.

It is worth mentioning that the fish brain has evolved earlier than other vertebrate brains. Therefore, while there are different terms such as the structure and name, there are similarities among the brain of fishes and other vertebrates, such as mammals (See Fig 4-1). Specifically there is a similarity among the hippocampus of mammals and birds, and the lateral pallium of teleost fish (Broglio *et al.*, 2003; Rodriguez *et al.*, 2002). In this thesis I will refer to the lateral pallium region as its analogous organ, the hippocampus.

Previous work using lesions to the avian hippocampus have resulted in spatial memory impairments (Sherry & Vaccarino, 1989; Hampton & Shettleworth, 1996). So we know that the hippocampus is important to spatial learning and navigation, but why? The principal neuronal cell types within the hippocampus are the pyramidal cells (Witter, 2012). The pyramidal cells have dendritic trees that extend to the hippocampal fissure. In addition to these specialized cells, there is a heterogeneous population of basket cells that have their cell bodies in the pyramidal cell layer (Witter, 2012). These specializations are excitation units that are likely to play a role in complex
object recognition and memory, which could be important for supporting navigation related behaviors. However, chronic stressors can lead to changes in the morphology of existing cells in the hippocampus, resulting in remodeling of the dendrites (Karatsoreos & McEwen, 2012).

Species that cache food have superior recruitment rates for hippocampal neurons (Hoshooley & Sherry, 2007), and while volumetric changes may not occur, the density of the neurons can be altered (Cristol et al., 2003). This indicates that comparative analyses of the hippocampus beyond simple volumetric measurements are needed (Dukas & Ratcliffe, 2009). Advances in our understanding of the role of the hippocampus and the mechanisms that support spatial learning and memory are likely to come from comparative studies that take into account multiple factors, rather than examining a single variable per study (Healy & Rowe, 2007).

Although several of the early studies of comparative brain anatomy, particularly in relation to spatial cognition, explored the relative size of the hippocampus, brains are a plastic organ with areas, including the hippocampus, that can continue to grow and change into adult life (Sherry & Healy, 1996). Many studies have used brains from adult animals, however, we may gain a better understanding of the developmental history in still foreign to us (See Healy & Rowe, 2007 for review). Hence we need to consider both relative brain size and development together.

Increasingly more studies have emphasized the importance of the cerebellum in mammalian conditioning as well as emotional learning (Brown et al. 2011). Interestingly, data shows that the corpus cerebelli of goldfish (analogous to mammalian cerebellum) is also involved in conditioning and emotional learning (Rodríguez et al. 2005, Gomez et al., 2010). In this thesis I will refer to this region as the cerebellum. Emotional learning is often studied through fear conditioning, though in our studies we used appetitive (food) reward system. We rationalized we could ask the question regarding extinction rates without stressing the subjects.

The cerebellum has been credited for its role conditioning in numerous conditioning experiments. To verify which locations of the brain were being used ablation studies have been
critical. Ablation studies have applied lesions to the cerebellum, through this research we have seen associations between conditioned stimuli and unconditioned stimuli that were not able to be paired. On the other hand fish that were part of the control, and did not receive lesions were able to make associations and showed a conditioned response (Gomez et al., 2010).

Understanding of the animal brain is a massive area of interest. Nonetheless it is an area in which we are still learning how the non-human animal brain works. To understand the brains workings we should focus on two perspectives of questions, this will facilitate understanding and bridge similar fields. In particular we must ask ecological & evolutionary questions from a neurobiological background. While at the same time we must seek answers to neurobiological questions whilst keeping ecological settings and evolution in perspective (Dukas, 1998).

**Figure 4-1.** Showing the involvement of the mammal hippocampus (H) amygdala (A), and the analogous organs in teleost fish. In fish these comparable structures are known as the lateral pallia (LP) and medial pallia (MP). Comparisons between different learning and memory processes. A “+” sign indicates the involvement in a given function, whereas the “−” sign denotes no involvement (Broglio, et al., 2005).
Ecology and how it affects temperament

Chapter two revealed a speed accuracy trade-off based on ecological settings. We witnessed that high predation fish were more likely to make fast inaccurate decisions whereas low predation fish made slower more accurate decisions. This study also looked at measures that could be used as a proxy to test boldness. In our study we considered the time to emerge from the start box as a measure of latency to start, or boldness to enter a new chamber. There are many ways boldness and timidity can be measured yet a common theme is the "propensity to take risks (Brown and Braithwaite, 2003). Thus we acknowledge that leaving a start box might not be a direct measurement of boldness. Nonetheless our "boldness" data supported results from previous experiments comparing populations of *B. episcopi* (Brown & Braithwaite, 2004a; Brown *et al*., 2005; Archard & Braithwaite, 2011b; Archard *et al*., 2012).

Temperament is not easily attributed to individuals because, as stated before, personalities are not intrinsically bimodal variables (Archard *et al*., 2012). In fact an individual's temperament can vary significantly within the spectrum of observable values. Fish are not simply either bold or timid, they can be any arbitrary value in the middle of these two extremes. For this reason it can be difficult to lump inconsistent personality into two distinct coping styles for the individual phenotype. However, when looking at populations, specifically our high and low predation fish, we find that coping style traits are similar with regard to predator exposure (Benus *et al*., 1990; Koolhaas, 2008).

We describe temperament as an animal's personality, and classify the sum of its personality traits as its coping style. Variation in coping styles appears to play a key role in the population dynamics and evolutionary fitness of a species (Koolhaas, 2007). These coping styles can help explain differences observed in the different populations. As such, temperament as well as coping styles can clearly be influenced by the environment, specifically through predation.
Research has shown that "proactive" animals work best in highly predictable environments as they thrive in stable conditions, whereas reactive animals have done better in unpredictable environmental conditions (Benus et al., 1987; Koolhaas, 2007). Similarly more reactive individuals learn more quickly (Brydges, 2008). We supported the idea of how temperament affects extinction rates in Chapter 3. In that I would argue that while our reactive low predation fish had faster extinction responses it was because they are quicker to adjust to a changing environment. Similarly, proactive individuals, whom are likely to form routines had a longer time to show extinction rates, showing their propensity to stick to routines.

The differences in behavioral flexibility and tendency to form routines can have implications in the performance of animals in the wild (Benus et al., 1991, Dingemanse & de Goede, 2004; Ruiz-Gomez et al., 2011). Specifically when responsive and unresponsive phenotypes have distinct advantages in diverse environmental conditions, survival will be affected. Benus et al., (1991) suggest that non-aggressive mice, which are more responsive to change can succeed in establishing territories in locations where environmental conditions vary, whereas routine-forming aggressive animals fail to do so.

Another example showing the implications of routine formation in a performance context has been documented in wild great tits (Parus major). Fast exploring individuals did better when the environment was optimal and stable, whereas slow exploring individuals did better in a varied, poorer environment (Dingemanse & de Goede, 2004). These two examples highlight how variation in an environment can illustrate varied foraging efficiencies in different environments.

Ecology and memory, learning and unlearning

The concept of a "Memory retrieval window" (Shettleworth and Plowright, 1992) has been described as a model often used in the context of foraging decisions (Mangel, 1990; Hirvonen et al. 1999). As previously mentioned in Chapter 1, in relatively constant
environmental conditions we would expect an animal to have a longer memory window, likewise when environmental conditions are highly variable, learning may be less beneficial and things are more likely to be forgotten (i.e. fall outside the memory retrieval window).

Ferrari et al. (2010) described a suite of traits' that impacted an animal's memory window. They suggested that extrinsic (predator encounter rates, probability of attack & success rate) and intrinsic (morphology, life history) factors would impact an individual's memory window. One example of intrinsic factor is growth rates. Increased growth during learning has been shown to affect memory in fish. A study by Brown et al., (2011) using rainbow trout (*Oncorhynchus mykiss*) showed that a high growth rate at the time of conditioning of a predator cue impacted how long anti predator behavior was remembered. Only low growth trout maintained a threat sensitive predator avoidance after eight days, whereas high growth trout did not show predator avoidance.

Acquired information should be retained as long as the information is relevant (Kraemer & Golding, 1997; Brown et al., 2011). Living with predators may increase the "cost of living" (Brown et al., 2011). Trade-offs associated with increased predation, specifically, vigilance, threat assessment and predator avoidance, could induce a higher "cost of living".

In chapter 3 the results showed that high predation *B. episcopi* took longer to show an extinction response. This result could be interpreted a number of different ways, for example, taking the cost of living model, we could expect these high predation fish to forget more quickly, however, in fact they remembered for longer. Thus we had to reassess the most practical way to interpret our results.

This brings us back to temperament and coping styles. If we take into consideration that our high predation fish had to learn a change in their environment, we could argue that these fish had a difficult time unlearning the conditioned response. We would attribute this set-back to the fact that bolder, more active animals are prone to developing routines (Benus et al., 1987;
Koolhaas, 2007). Specifically that these high predation fish learned a habit of moving to the light for food, yet once the CS and US were uncoupled they took longer to show extinction.

In chapter 3 we hypothesized that we would find differences in conditioning rates based on predation regimes. This hypothesis is based on a similar experiment by a fellow student (Grassie, per conversation). Grassie found that her low predation *B. episcopi* conditioned faster than fish from high predation environments. While we did not support this outcome in our study, we noted some key differences that may have attributed to the variation in the results.

The most significant was that our fish had been in the lab for 2 years before our experiment. Another compounding factor was that the fish used in this experiment may have been previously used by Grassie in her conditioning experiment (Grassie, in prep). Most, yet not all fish had been uniquely marked, so there is the chance that some fish had prior knowledge of this assay and that this may have biased the conditioning results. Another factor that may have caused fish to condition at different rates could have been our protocol differences. The overlapping interval during our conditioning was significantly shorter (5 seconds, compared to 12 seconds) than what was used by Grassie (Grassie, pers. comm.).

While I may have announced my disclaimer that the fish in my experiment had spent a significant portion of their lives in the lab, it is important to point out that these fish were from a wild strain. In my experiments, all fish were original stock that had been taken directly from the wild, thus all fish had spent the first few months of their lives in real rivers. The tests that I used to explore fish cognition tried to simulate learning tasks that are biologically relevant. Being able to find their way around the river, or learning associations about places that are profitable feeding patches are the kinds of task fish could face in the real world.
Final conclusion

Fish are truly amazing creatures; there are more than 27,000 known species (Hoare & Krause, 2003). This staggering number is nearly half of the species in the phylum chordata, and there is tremendous variation among different species. Fishes are an ancient taxa which have been found to exploit nearly every conceivable aquatic environment. Unfortunately their intelligence has been sorely underestimated (Laland et al., 2003). The idea that fish are dim-witted and driven by instinct is an outdated belief that scientists have been diligently working on in an effort to shed light on the truth. Specifically that fish are intelligent creatures that can think and respond to change.

The experiments I have conducted are but a small assay of what we can learn about fish learning and cognitive ecology. Understanding how and what information fish take from their environment may help conservation and fisheries programs worldwide.
References


