

The Pennsylvania State University

The Graduate School

College of Agricultural Sciences

**INVESTIGATING THE PHYSIOLOGY AND BEHAVIOR  
OF THE PANAMANIAN BISHOP FISH**

A Thesis in

Wildlife and Fisheries Science

by

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## ABSTRACT

Aquatic environments can present a number of challenges for the organisms that inhabit them. Some of these challenges are physiological, like the need to take up oxygen from the water through gills, and, as some animals have higher oxygen demands than others, a way to regulate the efficiency of gas exchange may be needed. Teleost fish use their gills for respiration and ion exchange, among other things. These adaptations allow them to inhabit many kinds of aquatic ecosystems, from freshwater to seawater, acidic to alkaline water, extreme high or low temperatures, and hypoxic or anoxic environments. One physiological adaptation that allows them to inhabit different environments is the ability to remodel their gills to change their respiratory surface area, and thus alter their ability to uptake oxygen and discard waste gases. One aim of this thesis was to examine the plasticity of the gills of Panamanian Bishop fish, *Brachyrhaphis episcopi*. Previous work has shown that these fish differ in temperament with some individuals being bolder than others. Such traits will demand different levels of oxygen thus degree of boldness may be related to gill morphology and specifically how much of the respiratory surface area is exposed to the environment. Here I show that there is no difference in respiratory surface area for bold and timid fish.

Other challenges faced by fish are more ecological in nature such as the threat of predation. Many fish have other fish that create a dangerous threat in terms of predation. Fish can adjust their behavior to decrease their risk of predation. One behavioral adaptation that can improve survival is the ability to learn from others, specifically conspecifics. To investigate the use of local enhancement, a type of social information used to locate food; I used *B. episcopi* sampled from high or low predation areas. Fish from high predation sites may rely more on local enhancement cues compared to low predation conspecifics, but more study is needed.

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# CHAPTER 1

## INTRODUCTION

Owing to their aquatic lifestyle, fish are a vertebrate group that faces several physiological challenges. The aquatic environment can be harsh; water is much denser and more viscous than air, and it holds less oxygen, which is also slower to diffuse through water than air. Fish can also withstand extreme environments, such as high or low temperatures, little to no oxygen, or high or low pH, which may affect their physiology and behavioral abilities. To cope, different species of fish have developed a number of adaptations both behavioral and physiological.

### **The Panamanian bishop fish (*Brachyrhaphis episcopi*)**

*Brachyrhaphis episcopi* is a tropical poeciliid fish that tends to live within dominance hierarchies; with larger females often adopting territorial behaviors and smaller females living in groups (Archard & Braithwaite, 2011a; 2011b). These different tendencies create a range of behaviors within the species. *B. episcopi* inhabit freshwater rivers from the Atlantic and Pacific slopes of central Panama (Mojica, 1998). These rivers contain natural barriers that separate the species into different environments within the same river. The individuals located above these barriers (i.e., waterfalls) are exposed to few predators, while individuals located below these barriers are exposed to multiple predators (Brown & Braithwaite, 2004). Jennions & Telford (2002) investigated the life history traits of different populations of *B. episcopi* from sites with high predation pressure and low predation pressure and found differences in multiple life history traits possibly indicating several adaptations to living alongside predators.

Brown & Braithwaite (2004) showed that smaller *B. episcopi* emerge from a darkened enclosure faster than larger *B. episcopi* which suggest that smaller *B. episcopi* are bolder. In a different study using a similar technique, they also reported that boldness is correlated with body mass, where bolder fish tend to be smaller, though competition and availability of resources may also be determining factors in an individual's boldness score (Brown et al., 2007). Brown et al. (2005a) also showed that high predation fish (which were smaller) were bolder than low predation fish (which were larger). They also found boldness difference between rivers, and that males tended to emerge from a shelter earlier than females (Brown et al., 2005a). Using a different kind of temperament assessment Archard & Braithwaite (2011a) found similar effects of predation. *B. episcopi* from high predation populations are more willing to explore novel areas and have a higher activity level during open field trials where a single fish is released into an enclosed arena and allowed to swim around unhindered for a fixed period of time (Archard & Braithwaite, 2011a).

Low predation populations have also been found to be more aggressive than high predation populations when viewing a reflected image of themselves in a mirror (Archard & Braithwaite, 2011b). The authors proposed that within species competition may be higher in these populations and that this may create situations where conflict arises (Archard & Braithwaite, 2011b). It was also reported that *B. episcopi* from low predation populations vary in the way that they investigate a novel object, with the fish from low predation populations examining the objects more frequently (Archard & Braithwaite, 2011b), a response that is interpreted as showing decreased anxiety levels.

In other experiments the learning and memory ability of *B. episcopi* have been assessed (Brown & Braithwaite, 2005b; Beri, 2012). Fish from low predation populations have a better cognitive ability than high predation populations when solving spatial tasks (Brown & Braithwaite, 2005b; Beri, 2012). To date these learning and memory assays have only investigated how individual fish use trial and error learning to solve a maze task.

Together these different studies of *B. episcopi* temperament and behavior show that several kinds of within species adaptation have arisen that help the fish cope with living alongside predators. A recent experiment found that *B. episcopi* also has a physiological adaptation, where bolder fish coming from high predation environments have a lower cortisol response when stressed compared to conspecifics from low predation environments (Archard et al., 2012). The lower cortisol response was suggested to be a physiological adaptation given the chronic exposure to stress from predators in the high predation sites (Archard et al., 2012). Given the differences in temperament, stress physiology and behavior across *B. episcopi* populations it seems likely that there may be different metabolic demands as well. I might expect the need for oxygen to vary across individuals for example.

### **Gill Plasticity**

Gills are an important organ for aquatic respiration, osmoregulation, nitrogen excretion, and acid-base regulation in fish. Gills consist of gill arches, gill filaments and lamellae; the gill arches are anchors for the filaments and these are where the lamellae attach. The lamellae are the primary site for gas exchange. Blood flows through the lamellae in the

opposite direction to the water flowing over the gills, maximizing oxygen uptake through diffusion across the membrane. Some fish have developed ways to deal with different kinds of respiratory challenges. For example, some fish regulate their circulatory system by altering their blood flow through their lamellae in order to adjust the rate of gas exchange to work with the oxygen levels that are available (rainbow trout (*Oncorhynchus mykiss*) Sundin & Nilsson, 1998; Atlantic cod (*Gadus morhua*) Stenslokken et al., 1999). Goldfish (*Carassius auratus*) have developed the ability to breathe at the air-water interface using a technique called “air-gulping”, which can increase blood oxygenation enough to avoid hypoxia (Burggren, 1982) allowing them to survive in low oxygen environments.

The uptake of oxygen from the environment is also dependent on the respiratory surface area. In the aquatic environment fish use their gills to uptake dissolved oxygen from the water, so fish that have a larger respiratory surface area can increase the amount of oxygen taken up by the gills. Some fish have evolved adaptations to increase or decrease their respiratory surface area when exposed to environmental challenges such as hypoxia or extreme temperatures (Sollid et al., 2003; 2005). By increasing the surface area of the lamellae (i.e., gill remodeling), the amount of oxygen available to the fish increases.

The plasticity of the gills varies across species; Gray (1954) found that active, fast-swimming marine fish have a larger respiratory surface area than slow, benthic marine fish. Crucian carp (*Carassius carassius*) have the ability to remodel their gills by changing the size of their interlamellar cell mass (ILCM) in a hypoxic environment (Sollid et al., 2003). In addition, crucian carp and goldfish change their ILCM due to

changes in temperature (Sollid et al., 2005). The amount of dissolved oxygen in the water is directly related to temperature; the higher the temperature, the lower the amount of dissolved oxygen in the water. A common factor between goldfish and crucian carp is that they are both highly tolerant of hypoxia and anoxia. During anoxia, goldfish and crucian carp adapt to the anaerobic environment using glycogen stores as fuel in the ethanol production pathway. Ethanol is then removed as waste from the body of the fish without the harmful effects of lactate acidosis which is the more common anaerobic respiratory pathway (goldfish, Shoubridge and Hochachka, 1980; crucian carp, Johnston & Bernard, 1983). But not all species have these adaptations. For example, Johnston & Bernard (1983) found that common carp (*Cyprinus carpio*) do not survive anoxia well as they do not produce ethanol and so under low oxygen conditions these fish experience lactate acidosis. Given the capacity for these two species of fish to remodel their gills in response to environmental challenges, it would be interesting to examine the respiratory surface area of fish with different oxygen demands to determine if this leads to some form of gill plasticity. Different temperament traits of individuals within the same species are likely to create different kinds of oxygen demand. Thus one of the aims of this research was to compare the gill structure of fish with contrasting temperaments. I predicted that bolder individuals would have a larger respiratory surface area than the more timid individuals because of a higher physiological demand.

## **Temperament**

An animal's temperament (also referred to as 'personality') describes an individual's consistent response over time and situations (Reale et al., 2007). Different personality

traits can be influenced by a range of factors such as resource availability, the social environment and predation pressure (Adriaenssens & Johnsson, 2009). Temperament exists on a continuum, known as the boldness-shyness continuum (Reale et al., 2007; Wilson et al., 1994), where the boldest individual is at one extreme, the most timid at the other extreme and other individuals falling in between.

Within-species comparisons of temperament between populations experiencing different environmental conditions have been widely studied in fish, in particular with regard to predation pressure (e.g. in threespined sticklebacks (*Gasterosteus aculeatus*): Dingemanse et al., 2007; in common carp: Huntingford et al., 2010; in guppies (*Poecilia reticulata*): Burns & Rodd, 2008, Harris et al., 2010; and in *B. episcopi*, Brown & Braithwaite, 2005b; Brown et al., 2007; Archard & Braithwaite, 2011a). Thus, there are well described temperament traits and validated methods for measuring these temperaments in fish.

### **Social Interaction and Learning**

Learning about available foraging resources using information gathered from observing others can be beneficial for animals (Kieffer & Colgan, 1992). The “Information Center Hypothesis” (ICH) describes the behavior of groups of animals in terms of their acting like a “superorganism”. According to the ICH, the individuals within the group observe each other and use the information gathered from resource patches to determine their own foraging behavior (Valone, 1989). However, foraging can be difficult and can be influenced by the environment and social interactions with conspecifics as well as predators (Moberg et al., 2011; Galef Jr. & Girdalneau, 2001; Piyapong et al., 2009). If

observers can use cues to locate good quality foraging patches, there is the potential for them to reduce predation risk, and search time.

Social learning refers to the way information is obtained (Valone & Templeton, 2002). An animal's attention, for example, may be drawn to an area where another animal is successfully foraging (sometimes referred to as "local enhancement"). Using social information is different from sampling, where the individual uses trial and error approaches to personally assess where it should forage. Sampling may have more costs associated with it however; costs from passing up a foraging opportunity at a patch where others are foraging, or from time and energy expended to locate and forage at a patch. However sampling does offer the ability to determine how profitable a patch is because the individual physically samples the resource for itself (Gotceitas & Colgan, 1991). Coolen et al. (2003) found that nine-spined sticklebacks (*Pungitius pungitius*) could assess the quality of a patch using public information (i.e. by watching the behavior of others in a group setting). Some fish may not use social information though, if they have reliable personal information from their own sampling experience, which can override social information that has been recently collected (Milinski, 1994; van Bergen et al., 2004).

However, social information use may be dependent on the social environment or specific habitats. Buckley (1996) found that black vultures (*Coragyps atratus*) had better success at foraging than turkey vultures (*Cathartes aura*) in southern Texas because they foraged in larger groups, and used local enhancement to find food. Buckley (1997) also found that when food is readily available, colonial breeding is the preferred strategy for seabirds (local enhancement), but when food is limited, dispersed nesting is preferred.

Social information use, such as local enhancement, could improve foraging success when individuals are exposed to high predation situations because it could reduce the risk of predation (Webster & Laland, 2008; 2011).

To investigate this, I tested the ability of *B. episcopi* from different predation backgrounds (high and low) to learn about the location of a foraging patch through social information (e.g. local enhancement). I expected that individuals from a high predation background would use local enhancement to identify a foraging patch, whereas individuals from low predation backgrounds that experience fewer risks may prefer to use their own experience to find good foraging sites.



## CHAPTER 2

### BOLDNESS IN FISH: IS THERE A PHYSIOLOGICAL COST?

#### ABSTRACT

Gills are an important organ for respiration, osmoregulation, nitrogen excretion and acid-base regulation in fish. The gills uptake dissolved oxygen from the water, so an increase in the respiratory surface area of the gills, increases the amount of oxygen available for the fish. Interestingly, fish have evolved adaptations to increase or decrease their respiratory surface area when exposed to environmental challenges such as hypoxia or extreme temperatures. Given this ability for fish to remodel their gills in response to their environment, my objectives were to determine if bolder individuals have a larger respiratory surface area as these individuals presumably have higher physiological demands. In the Panamanian bishop, *Brachyrhaphis episcopi*, some fish are bolder, meaning that they are more exploratory and have higher activity levels, than other more timid fish. In this study, this species of fish was tested, using an open field test, to determine individual temperament, bold or timid. Fish exhibiting extreme bold or timid traits were then euthanized and their gills were dissected. Scanning Electron Microscopy (SEM) was used to create high resolution images of the gills so that the size of the respiratory surface area could be measured. Although there was some variation in boldness and timid traits, there was no difference detected in the size of the respiratory surface area between bold fish and timid fish.

## INTRODUCTION

Fish are unique among vertebrates because of their multifunctional organ, the gills. The gills are the gateway to the internal and external environments of the fish and they use their gills to uptake dissolved oxygen from the water. Fish can adapt to a reduced dissolved oxygen environment by increasing water flow over the gills, changing the blood circulation in the gills, or by increasing the surface area of the gills (Sundin & Nilsson, 2002; Olson, 2002a; 2002b; Nilsson, 2007).

The main sites of gas exchange in the gills are the lamellae. The lamellae are attached to the gill filaments, which are attached to the gill arches (Figure 2.1). This structure enables gas exchange to quickly occur in the aquatic environment, facilitating oxygen uptake, carbon dioxide removal, and regulation of ion fluxes (Perry & Gilmour, 2002; Wilson & Laurent, 2002). However different fish species have differing gill adaptations, Gray (1954) found that of 31 marine teleosts, the active, fast swimming, schooling fish have larger respiratory surface areas than fish that are slow, and/or benthic. Crucian carp (*Carassius carassius*) and goldfish (*Carassius auratus*) have shown the ability to remodel their gills depending on the oxygen availability, or temperature of their surrounding environment (Sollid et al., 2003; 2005). While there are benefits to having a large respiratory surface area, there are also consequences such as increased exposure to toxins, pathogens, or increases in ion fluxes.

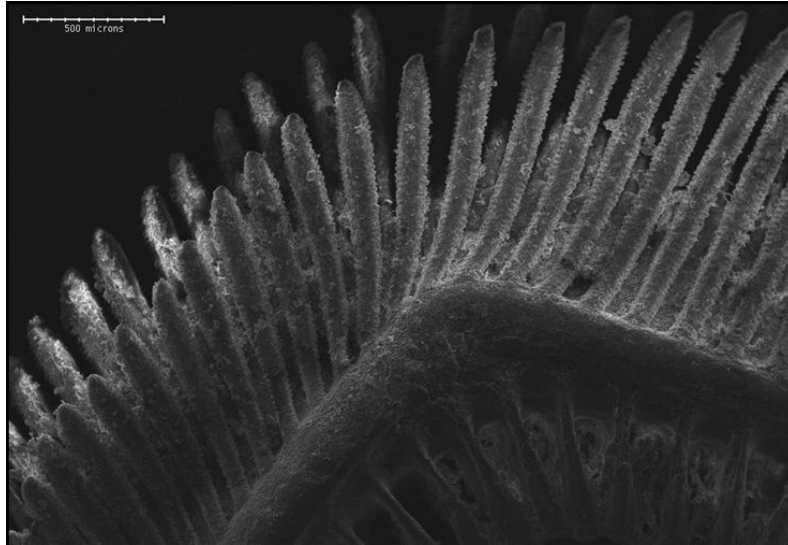


Figure 2.1: SEM image of a gill arch with gill filaments attached. The lamellae protrude from the gill filaments functioning as the primary site for gas exchange.

As the gills are the main organ for gas exchange it might be expected that fish with higher oxygen demands may alter the morphology of their gills to increase the capacity or efficiency with which oxygen can be taken up. One way to do this would be to increase the surface area of gill tissue exposed to the aquatic environment. Given the ability for fish to remodel their gills in response to environmental challenges, I might expect fish with higher energy demands to have different gill morphology compared to fish with lower energy demands. This hypothesis could be addressed by comparing fish within a species where different individuals are known to vary in their activity levels. The Panamanian Bishop (*Brachyrhaphis episcopi*) is an ideal species in which to investigate this as recent studies have demonstrated different activity and general temperament traits between individuals in this species (Brown et al., 2005a; 2007; Archard & Braithwaite, 2011a; 2011b).

*B. episcopi* are a small tropical poeciliid fish that exhibits a range of bold to timid temperament within the same species (Archard & Braithwaite, 2011a). These fish are

located in multiple freshwater rivers in the Republic of Panama and have populations that are separated within the river by waterfalls. The *B. episcopi* located above the waterfall are exposed to low predation pressure, while those below are exposed to high predation pressure. Previous experiments on this system have found high predation populations are bolder than the low predation populations, meaning they are more willing to explore novel areas, have higher activity levels, have a smaller body size, and tend to emerge sooner from a shelter (Archard & Braithwaite, 2011a; Brown & Braithwaite, 2004; Brown et al., 2005a; 2007). However, low predation populations have a slower pace of life, take more time to consider decisions, and tend to be more territorial (Brown & Braithwaite, 2005b; Archard & Braithwaite, 2011b; Beri, 2012).

One major temperament axis investigated in many animal species is the boldness-shyness continuum (Reale et al., 2007; Wilson et al., 1994). This continuum provides a range of behavioral phenotypes that can vary in number across population and/or species. I expect then within each population a range of bold individuals to more timid individuals, allowing me to relate temperament type with gill morphology.

## **MATERIALS AND METHODS**

### **Fish**

The *B. episcopi* used in this experiment were females obtained from a freshwater river in the Republic of Panama, Rio Macho (upper site 79°45'36"W, 9°11'02"N and lower site 79°45'42"W, 9°11'02"N). The populations were collected from their natural streams in

March 2010 before being transported to Pennsylvania State University where they were held in aquaria separated by population with each population spread across 3 tanks.

Each aquarium had filtered aeration, gravel, and enrichment (shelters and green plants). The laboratory had a 12L:12D cycle and a room temperature of  $25 \pm 3^{\circ}\text{C}$ . The fish were fed twice a day, with flake food in the morning and brine shrimp in the afternoon. Each fish was individually marked using a visible implant elastomer tag so that it could be identified. The fish ranged in size from 5.0-6.1cm long and 1.24-4.15g in weight. This experiment was conducted November-December 2012.

### **Open Field Test**

Open field tests were performed by placing a single fish into a novel open environment and recording behavior following the methods of Archard & Braithwaite (2011a). Fish were selected by netting each fish into an enclosed container for transfer into the open field test apparatus, to reduce transfer stress (Brydges et al., 2009). All fish held in the aquarium tanks for each population were tested. The open field test apparatus was a clear plastic container (30.5cm x 56cm x 39.5cm), covered on all sides with black plastic to reduce outside interference. When fish were being tested, the apparatus was filled with water at a depth of 10cm. The bottom of the container was marked with a grid pattern (6cm x 5cm), and had a line 3cm from the edge, to help assess the movement of the test fish during the trial. The fish were housed at the fisheries building located at Rock Springs, and trials were conducted in the same location. A black curtain separated the testing area and the observer area so the observer did not interfere with the behavior of

the fish. Each trial was recorded using a digital video camera for later analysis. The camera was set up to record from a bird's eye view above the tank.

Each trial started with the open field test apparatus filled to the water line (10cm). The video camera was set up above the center of the test apparatus to film behavior during the trial. A clear plastic cylinder (10.5 cm) placed into the center of the test apparatus acted as a start chamber. Recording began when the fish was added to the start chamber. The fish was allowed to acclimate to the new environment for 2 minutes, and then the cylinder was remotely lifted using string and pulley giving the fish free access to the open field test apparatus. The behavior of the fish was recorded for 5 minutes. After 5 minutes the video camera was stopped, the fish was removed, and the test apparatus was emptied with fresh water added for the next trial.

### **Video Analysis**

Videos were analyzed using QuickTime (Apple Inc, Version 7.69.80.9) to determine when the fish were moving or frozen, when the fish were in the middle or at the edge of the apparatus, and the movement rate of the fish during the test; all observations were recorded in seconds. This information was used to calculate the initial time to reach the edge, the time to first return to the middle, the amount of time spent in the middle, movement rate in the arena, the frequency of crosses between middle and edge, average duration of time in the middle, amount of time spent moving, and the longest consecutive time in the middle. The initial time to reach the edge is the number of seconds the fish took once released from the cylinder to cross the edge boundary of the apparatus including time spent frozen. The fish was determined to cross the boundary when its

head up to its gills was across the boundary line and the fish stayed on that side for at least 1 second. The time to first return to the middle is the number of seconds it took the fish to cross the boundary from the edge into the middle for the first time. The amount of time spent in the middle is the total number of seconds spent in the middle of the apparatus. Movement rate was measured as the number of times the fish moved between each quarter of the test apparatus (23.5cm x 15.5cm for each quarter). The frequency of crosses is the total number of times the fish crossed the boundary from middle to edge or edge to middle. The average duration of time in the middle is the average number of seconds the fish visited the middle of the apparatus after initially reaching the edge. The amount of time spent moving is the number of seconds during the trial that the fish spent moving. The longest consecutive time in the middle is the longest period of time spent in the middle after initially reaching the edge. These measures have previously been used to assess fish temperament and, in particular, boldness (Burns & Rodd, 2008; Archard & Braithwaite, 2011a). A bold fish will take longer to initially reach the edge, will first return to the middle sooner, will spend a larger amount of time in the middle, will have a larger movement rate, may have a high frequency of crosses between edge and the middle, will spend a longer time visiting the middle, will spend more time moving, and will stay in the middle for a longer period of time than a timid fish.

### **Gill Structure**

The 10 boldest and 10 most timid of 37 fish examined in the open field trial were euthanized and measured for length and weight. The first gill arch was removed unless it was damaged, then the second gill arch was removed. The gills were fixed in a 3%

glutaraldehyde in 0.1M cacodylate buffer for future scanning electron microscopy (SEM) work.

### **Scanning Electron Microscope (SEM)**

The gill arches were dehydrated using a sodium cacodylate buffer and increasing concentrations of ethanol, finishing in 100% ethanol. A critical point dryer (Bal-Tec CPD 030) was then used to dry out the gill arches further. Liquid CO<sub>2</sub> was used to wash the samples repeatedly until the critical point of CO<sub>2</sub> was reached. The dissections were mounted on double stick carbon sticky tape then coated with a layer of argon using the sputter coater (Bal-Tec SCD 050) for 180 seconds. After coating was completed, the samples were imaged using the SEM. Two images were taken for each fish, so take there were at least 2 gill filaments to take measurements from. The image taken was selected based on the view of the gill filament. I imaged the middle of the gill filament and took the image straight on so the ILCM measurements would be accurately measured from where the lamellae attach on either side. The lamellae measured were chosen based on the ability to accurately measure them using ImageJ. The lamellae had to have a crisp delineation so the area or surface length was not distorted based on the angle the image was taken. All measurements were made based on two-dimensional measurements. The image was used to measure the surface length of the lamellae, the lamellae area, and the interlamellar cell mass (ILCM) width, see Figure 2.2 for details (5 lamellae from 2 different gill filaments for each individual were measured). In only one case was there an individual where no ILCM width could be measured accurately.



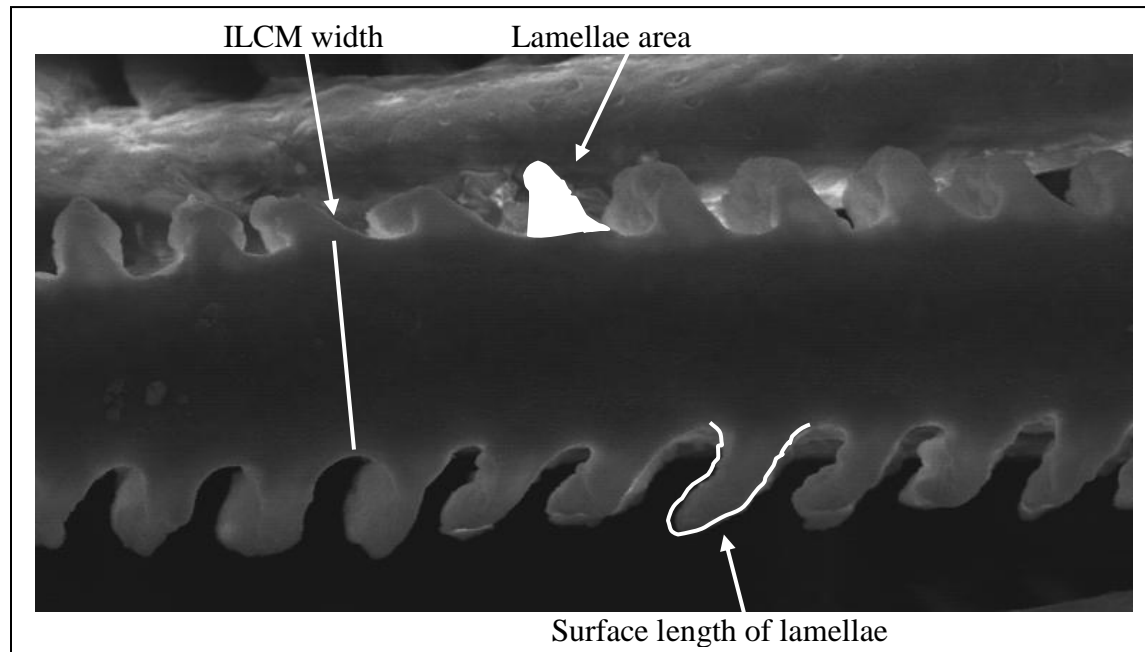


Figure 2.2: SEM image showing the 3 measurements taken: the surface length of the lamellae, the lamellae area, and the ILCM width.

### Statistical Analysis

There were 37 fish in total that were observed in the open field test. Principal component analysis (PCA) was used to examine the behavioral variables measured for each fish in the open field test to determine the boldest and most timid individuals. The 10 boldest and 10 most timid fish had their gills imaged and 3 measurements were taken using Image J: (i) the surface length of the lamellae, (ii) the lamellae area, and (iii) the ILCM width (Figure 2.2). Each measured variable was then compared to the temperament of the individual (based on the PC1 scores) using an ANCOVA with the length and weight of the fish as covariates; assumptions of normality were met. The variability of the measurements taken was examined for equality of variance. The surface length of the lamellae and the lamellae area had equality of variance for all 10 measurements, while the ILCM width proved to be highly variable with only 6 out of the 9 measurements

taken having equality of variance, showing that this measurement is not as consistent as the other 2 measurements.

## RESULTS

A PCA was performed on the eight behavioral variables assessed from the video recordings of each open field trial. This analysis supplied three PCs that explained 79.34% of the variance and had eigenvalues greater than one. For PC1 the eigenvalue was 2.94 and explained 36.69% of the variance. Here the influential coefficients are time to initially reach the edge, time to first return to the middle, amount of time spent in the middle, average duration of visits to the middle, and the longest consecutive time in the middle (Table 2.1). Based on these PC1 scores I examined the gills of the 10 boldest and 10 most timid individuals tested (Figure 2.3).

Table 2.1: Behavioral variables with principal component score coefficients from the principal component analysis (PC1, PC2, and PC3) of <i>Brachyrhaphis episcopi</i> using an open field test			
Behavioral variable	PC1	PC2	PC3
Time to initially reach the edge	<b>0.30</b>	-0.08	<b>0.66</b>
Movement rate	0.07	<b>0.58</b>	0.20
Time to first return to the middle	<b>-0.39</b>	<b>-0.47</b>	-0.05
Proportion of time in the middle	<b>0.54</b>	-0.19	0.01
Frequency of crosses	0.21	-0.05	<b>-0.65</b>
Average duration of visits to the middle	<b>0.48</b>	-0.11	-0.02
Longest consecutive time to the middle	<b>0.42</b>	-0.21	-0.16
Proportion of time spent moving	0.11	<b>0.58</b>	-0.27
Bolded coefficients are considered influential and are >0.30.			

Comparing the 3 measurements taken to the temperament of the individual there was no difference for the surface length of the lamellae,  $F_{3,16}=1.02$ ,  $p=0.41$ , the lamellae area,  $F_{3,16}=0.04$ ,  $p=0.99$ , or the ILCM width,  $F_{3,15}=1.38$ ,  $p=0.29$  (Figure 2.4).

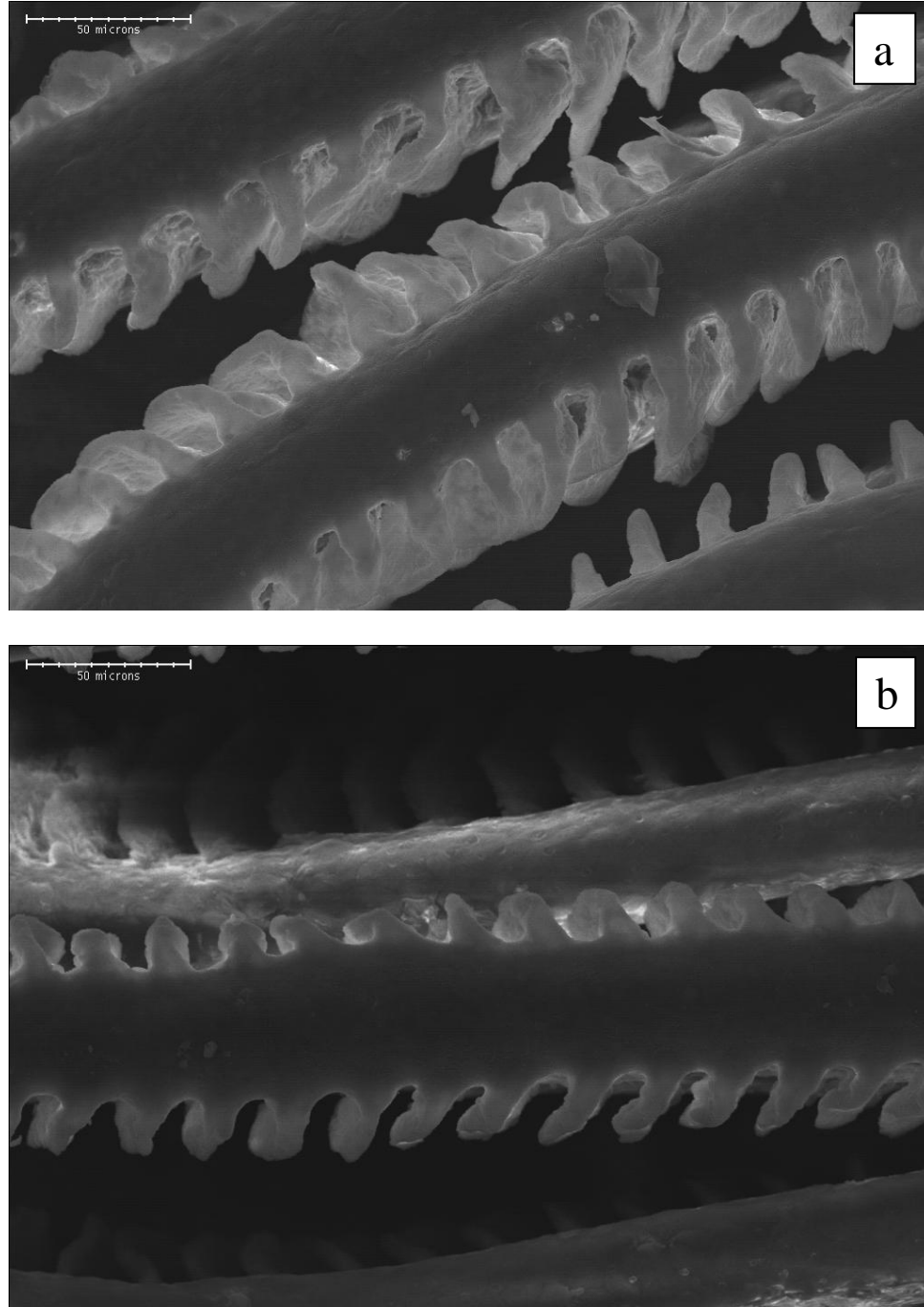


Figure 2.3: SEM images of gill filaments with protruding lamellae. *Brachyrhaphis episcopi* with a) a bold temperament, or b) a timid temperament.

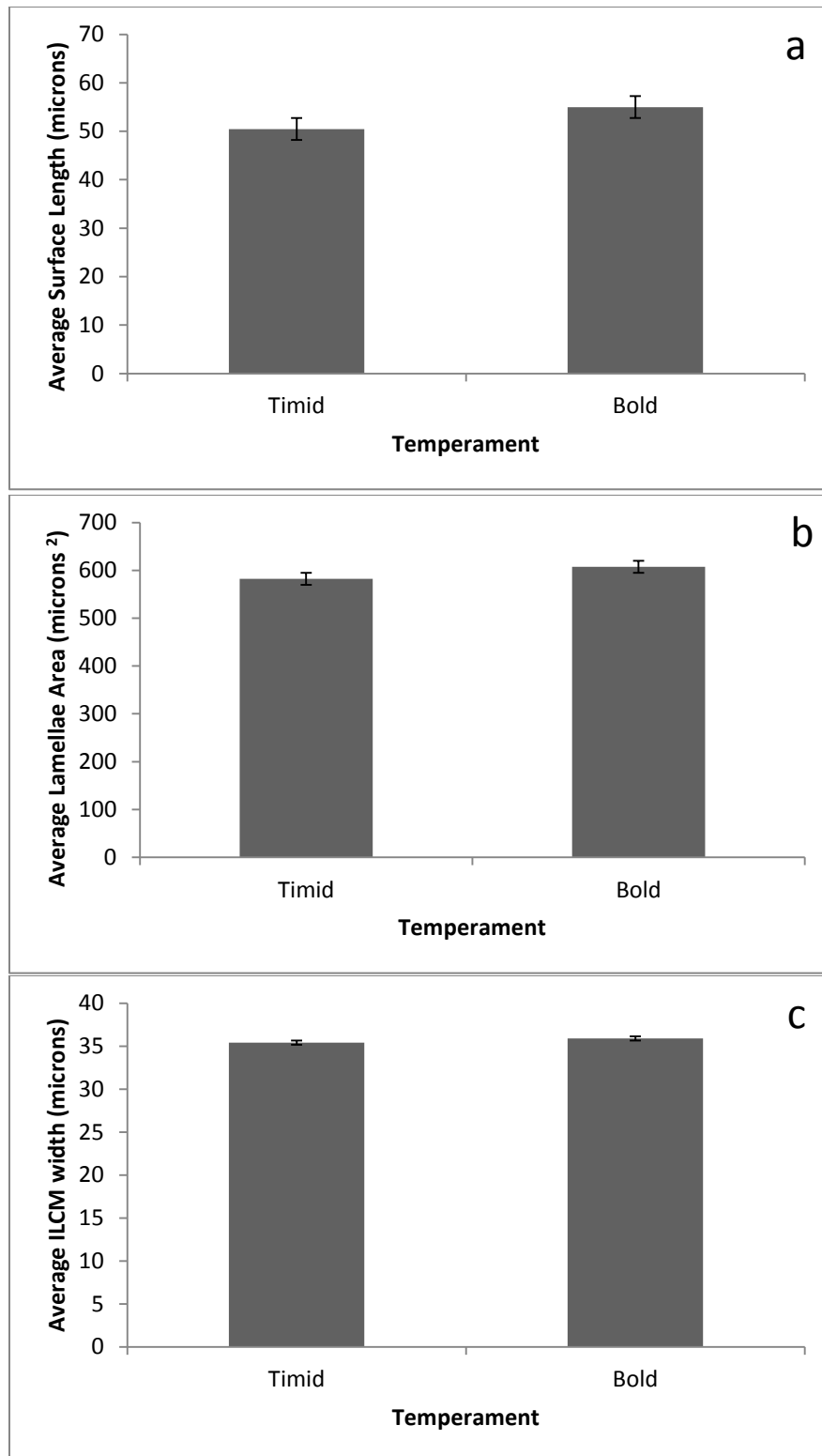


Figure 2.4: The size of the respiratory surface as measured by the a) surface length, b) lamellae area, and c) ILCM width by temperament. Bars show means  $\pm$  standard error.

## DISCUSSION

I found no difference in the surface length of the lamellae, the lamellae area, or the ILCM width when compared to the temperament of the individual. I expected that bolder fish would have a larger respiratory surface area for gas exchange. Boldness encompasses several aspects of behavior, and previous tests with *B. episcopi* have found that bolder fish are also more active (Archard & Braithwaite, 2011a), which presumably exerts a higher oxygen demand on the fish. One way to obtain more oxygen is to have a larger gill surface area. This increased area would also help increase the exchange of waste gases such as carbon dioxide. The increased area may also be due to bolder individuals having a higher physiological demand, because these bolder animals typically explore more and have high activity levels. Turko et al. (2011) found that mangrove rivulus (*Kryptolebias marmoratus*) had the ability to remodel their gills based on a behavioral phenotype. Fish that selected to expose air to their gills more frequently had a smaller ILCM height than fish that spent more time in water, similarly, ILCM height was smaller when gill exposure to air was prevented (Turko et al., 2011). Similar to our results, Turko et al. (2011) did not find changes in all the gill measures they took, and found no changes to overall lamellae length or thickness. It is possible that I could not determine differences in the respiratory surface area of the gills because I was using a two-dimensional measuring technique that could not accurately capture the three-dimensional image.

It is also possible that boldness measures are more variable, and that other temperament measures would be more relevant. For instance, aggression may be a better measure for comparing gill plasticity. Aggressive individuals may have a higher physiological demand due to protecting foraging or reproductive territories. Huntingford

et al. (2010) found a correlation between boldness and aggressiveness in individuals; they also found that the bolder, more aggressive individuals had a low responsiveness to stress associated with differences in metabolic and stress physiology. This low responsiveness to stress could be because the bolder, more aggressive individuals experience a stressful environment, whereas more timid and docile individuals do not naturally experience as much stress, so they have a higher response to a stressor when it is experienced. This is similar to the results reported by Archard et al. (2012) that found less bold, low predation exposed *B. episcopi* had a lower cortisol response than their bolder, high predation counterparts.

The PC1 scores were influenced by time to initially reach the edge of the arena, time to first return to the middle, amount of time spent in the middle, average duration of visits to the middle, and the longest consecutive time in the middle. The bold individuals took longer to reach the edge, returned to the middle sooner, spent more time in the middle overall, took longer visits to the middle and spent the longest consecutive amount of time in the middle.

Future studies that examine gill morphology and temperament would benefit from using a three-dimensional measuring technique which may be useful in obtaining more accurate data. Also, increasing the number and kind of behavioral test given to each individual such as including boldness and aggressiveness scores in the model along with the length and weight of the individual as covariates may increase the ability to detect differences in respiratory surface area. Another factor that would be interesting to explore would be stress responsivity, particularly in relation to a chronic stressor such as presence of predators. Individuals from different predation backgrounds or subjected to

chronic predation stress may have a larger respiratory surface area than individuals not exposed to predation.

### **ACKNOWLEDGEMENTS**

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**CHAPTER 3**  
**HIGH PREDATION PRESSURE STIMULATES LOCAL ENHANCEMENT IN**  
***BRACHYRHAPHIS EPISCOPI***

**ABSTRACT**

Learning is an important form of behavior for almost all animals. There are many different forms of learning from simple conditioning processes through to more complex tasks that require animals to integrate several kinds of information. One form of more complex learning that is often observed in group living animals is social learning. Social learning refers to the ability of an animal to collect information from another individual or from a group of individuals. The definition of social learning can be further refined to ‘local enhancement’ which describes the ability to locate a place with food due to observation or copying the behavior of others.

Individuals that live in risky environments, where increased time spent sampling their environment can be dangerous, could benefit from using local enhancement information. I measured whether Panamanian Bishop fish (*Brachyrhaphis episcopi*) use local enhancement. Observer fish were exposed to two demonstrator fish, only one of which had access to food. After observing the demonstrator’s behavior, the observer was allowed to make a choice about where to spend their time. Observer fish from high predation populations may have been more likely to use information gathered from demonstrators than observers from low predation populations. These results are inconclusive however because of a large river effect. Further study is needed to test this hypothesis, examining more rivers with different levels of predation.



## INTRODUCTION

Animals often rely on information that they have gathered to help them make informed decisions (Giraldeau, 1997). Thus the process of gathering facts is important for animals as it allows them to gauge and assess their environment. Animals can collect information individually or by observing other animals (Shettleworth, 2010). Information collected individually is generally more accurate but can be time and energy expensive (Hoppitt & Laland, 2008). Socially collected information can be more efficient and can reduce exposure to dangers such as predation risk, however, the reliability of the information obtained by watching others can be an issue (Koops, 2004). Over the last few years this field of research has received growing attention, and now know that information can be gathered to inform individuals about foraging availability (Buckley, 1996; Coolen et al., 2003; Reebs & Gallant, 1997), mate choice and/or reproductive success (Webster & Laland, 2011), predator aversion (Webster & Laland, 2008), and social recognition (Metcalf & Thompson, 1995; Utne-Palm & Hart, 2000; Ward et al., 2007; 2009).

Social information can be defined as using the location where there is some form of desired resource such as food, shelter or a potential mate (referred to as “local enhancement”) or it can describe the way information is obtained (referred to as “social learning”) (Valone & Templeton, 2002). Social information is collected when the individuals within the population observe each other and then use the information gathered about different resources to determine their own behavior (Valone, 1989). If observers can use social cues to locate suitable food patches, for example, then there is the potential for them to reduce predation risk, search time and energy expenditure. Coolen et al. (2003) found nine-spined sticklebacks (*Pungitius pungitius*) can assess patch resources using public information by assessing the quality of resources based on the success/failure of others.

Individuals can make a choice about which information they will use depending on their environment (Webster & Laland, 2008), their ability to learn from conspecifics and/or heterospecifics (Carlier & Lefebvre, 1997; Coolen et al., 2003), and/or social context (Webster et al., 2007; Webster & Laland, 2011; 2012). Some animals may not use social information if they have reliable personal information from their own sampling experience, which overrides what social information they may have recently collected (Milinski, 1994; van Bergen et al., 2004). According to Valone & Giraldeau (1993), social foragers may use three types of foraging information to determine patch quality: 1) sample information gathered during the individual use of the patch, 2) “pre-harvest” information gathered before exploiting the foraging patch, and 3) public information gathered from observing the success of other individuals in the patch. Individuals may use a combination of these methods to assess patch quality although different individuals may value one method more highly than another.

The Panamanian bishop is a poeciliid fish that has females which range in their behavior, from some that maintain territories to those that form small groups and remain in shoals, thus creating a range of temperaments within the same species. These fish inhabit freshwater rivers containing natural barriers that separate the species into different predation pressure environments within the same rivers. Previous work on this species found that the fish vary in terms of their learning and memory ability (Brown & Braithwaite, 2005b), but to date these assays have only looked at how the fish develop private information through trial and error learning. Here, I investigate whether the *B. episcopi* located in the different predation pressure environments use local enhancement to determine the position of a profitable foraging location. My hypothesis is that high predation fish will be more likely to use foraging information gathered from observing other fish because this information could help to reduce their exposure

to predation risk as well as reduce the amount of time spent looking for a profitable foraging patch.

## MATERIALS AND METHODS

### Fish

*B. episcopi*, are small tropical poeciliid fishes found in freshwater rivers in the Republic of Panama. The fish used in the experiment were females and obtained from three rivers: Rio Limbo (upper site 79°44'28"W, 9°09'54"N and lower site 79°44'25"W, 9°09'38"N), Rio Macho (upper site 79°45'36"W, 9°11'02"N and lower site 79°45'42"W, 9°11'02"N), and Rio Quebrada Juan Grade (lower site 79°43'00"W, 9°08'37"N). The populations within each river are separated by a waterfall which acts as a natural barrier blocking predator fish from moving further upstream. Above the waterfall the fish are exposed to very few predators, but below the waterfall there are several predator species (Brown & Braithwaite, 2004).

Fish were collected from their natural streams in March 2010 and were transported to Pennsylvania State University where they were held in aquaria separated by population with each population spread across 3 tanks. The fish were held in captivity for approximately 2 years with no predation exposure. Previous experiments conducted in the lab with these fish found differences in the fish exposed to different predation pressures (Berl, 2012). The fish range in size from 5.0-6.1cm long and 1.24-4.15g in weight. Each aquarium had filtered aeration, gravel, and enrichment (shelter and green plants). The laboratory had a 12L:12D cycle and a room temperature of  $22 \pm 2^{\circ}\text{C}$ . The experiment was conducted December 2011-June 2012. As there were insufficient numbers of fish from one of the sites on Rio Quebrada Juan Grande I decided to use a population from this river as demonstrator fish (high predation population), while the

fish from both high and low predation sites on Rio Limbo and Rio Macho were used as observer fish. By using two river systems that each had a low and high predation population I was able to compare the behavior of the observer fish to determine whether the level of predation pressure experienced in the wild prior to captivity influenced the social learning abilities of *B. episcopi*. Fish from both Rio Limbo and Rio Macho were tested as observers until 12 “good” trials were achieved for each predation population or there were no more fish in the population available to test. A “good” trial was when the demonstrator fish started feeding within 30 seconds of the trial starting and fed continuously until the 5 minute feeding observation period was complete. There were only 7 “good” trials from Rio Limbo low predation population and 10 “good” trials from Rio Macho low predation population.

## **Apparatus**

Six experimental tanks were set up so that multiple fish from the different populations could be tested each day. The experimental tanks (91.44cm x 30.48cm x 30.48cm) were divided into three sections: 25.4 cm in from each side leaving the center section 40.64cm long with one demonstrator fish on each end and the observer section in the middle (Figure 3.1a).

To decrease the stress of handling, the experimental tanks were designed so that the demonstrator fish could be housed in these tanks for the duration of each trial (i.e. several months). The observer fish could also be housed in the experimental tank and was placed in the tank 24 hours before the trial. All experimental tanks contained enrichment during the non-test periods; however, 10 minutes before testing began the enrichment was removed to allow the behaviors of the demonstrator fish to be seen by the observer fish and filmed.

To allow the demonstrator fish to become adept at displaying foraging behavior I trained them to take food from feeding rings that were attached to the front of the tank in each demonstrator section. These were initially provided with small amounts of flake food twice a day, once in the morning and once in the afternoon, so that the demonstrator fish would clearly approach the feeding ring on demand when the experimental trials were being run. I conditioned the demonstrator fish to use the feeding ring by training them to associate the onset of a light cue with the delivery of food. I used a delayed conditioning technique where a light signaling food delivery would be switched on 5 seconds before the food was delivered and remain on as the fish continued to feed from the feeding ring.

The observer fish could not see the food/feeding rings directly, but rather just the foraging behavior of the demonstrator fish. A visual barrier was placed on the tank dividers so the observer fish could not directly observe food being dropped into the feeding rings (Figure 3.1a). A transparent cylinder placed in the center section against the front wall served as the observer compartment during the feeding trials. The clear cylinder allowed the observer fish to watch the activity of the demonstrator fish on both the left and right sides of the tank while restricting the movements of the observer fish to a confined area (Figure 3.1a).

To limit visual access of each demonstrator fish so that their behavior was independent of one another, an opaque barrier was placed in the central part of the middle compartment and joined onto the observer compartment. A black, opaque covering was taped on all sides of the tank so that the human observer who was delivering the food could not be seen directly by the fish. A pair of white strings were pulled across the top of the tank and acted as the goal lines so that the camera which was positioned directly over the test tank central chamber clearly recorded

when a fish swam past a goal line into a ‘goal zone’ (Figure 3.1b). All fish were deprived of food for 24 hours before testing to ensure a high motivation for foraging behavior for each trial.

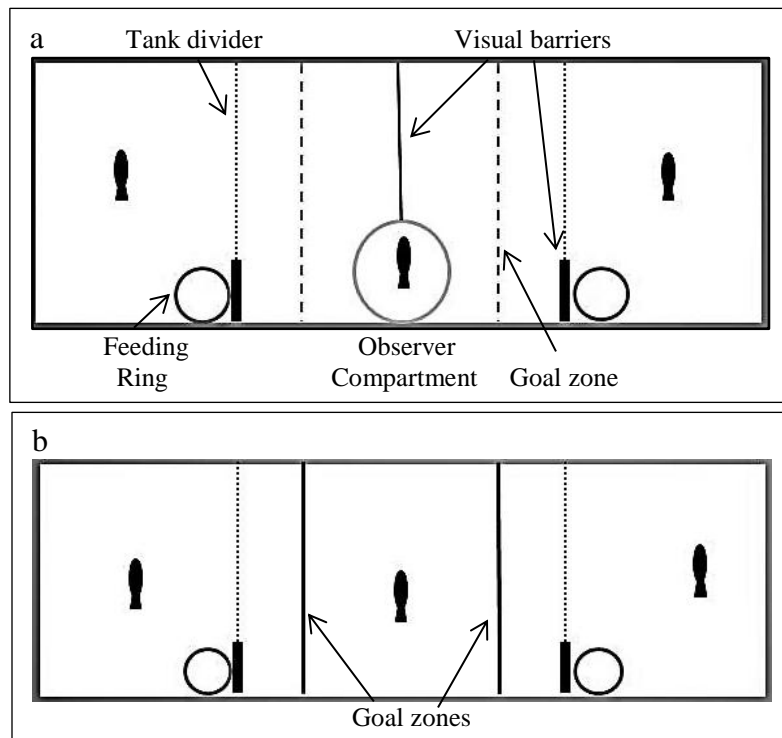


Figure 3.1: Schematic of the experimental apparatus from a bird's eye view. a) Apparatus for the foraging observation period. The large circle is the observer compartment, the small circles are the feeding rings, the dotted lines are the tank dividers, and the blocks next to the feeders act as visual barriers to prevent direct visual access to the food and feeding ring. The thin black line joining the observer compartment in the center is the visual barrier that stopped the demonstrator fish from seeing each other, and the dashed lines are the goal zones. b) Apparatus for the choice trial. After the foraging observation period and a short acclimation period the opaque barriers are lifted and the observer fish begins the foraging period. The black solid lines represent the goal zones.

## Methods

Before the start of each trial, a single observer fish was placed in the central compartment and was housed there for 24 hours before testing. During this time the fish could swim freely within the central area but was not fed. To begin a trial the observer fish would be placed in the observer compartment, the opaque central barrier would be positioned, and the observer fish was

allowed to acclimate for 5 minutes. After this period, the 5 minute foraging observation period started. One demonstrator was fed during each trial so the observer could see foraging occurring on one side of the tank but not the other. This allowed me to investigate whether the observer fish uses the foraging behavior of the demonstrator fish to determine where foraging is taking place. The ‘food’ side of the tank received food (flake food fed in a feeding ring) every minute once a minute during a 5 minute observation period (i.e. 5 times in 5 minutes). In contrast no flake food was delivered to the feeding ring on the ‘no food’ side of the tank. The side that was presented with food was counterbalanced across different observer fish so that the demonstrator fish were fed on the right side 20 times and on the left side 21 times over the experimental period. The demonstrator fish were used for multiple observers (Table 3.1). Once trained, demonstrator fish did not always stay trained and some did not continue to feed for the duration of the trial. I used at least 2 different “good” demonstrator fish so that the observers as a whole were exposed to different demonstrator fish and not the same one each time.

The observer fish could not see the food directly so that their foraging decisions would be based on the information they observed by watching the behaviors of the demonstrator fish. After the foraging observation period, opaque barriers were placed at each goal line (Figure 3.1b) and the observer compartment and opaque central barrier were removed. The observer fish was then allowed to swim freely for 3 minutes within the central zone with the barriers blocking the view of the demonstrator fish. After the acclimation period, the opaque barriers were removed and the camera started recording. The observer fish was able to swim freely in the central section into either of the goal zones. During this observer choice period I measured the amount of time the fish spent in each goal zone during the 30 second trial, and its initial choice to be in the ‘food’ zone, the ‘no food’ zone, or made ‘no choice.’ The fish crossed the goal zone when its

head up to its gills was across the goal line for at least 1 second, in either the ‘food’ or the ‘no food’ zone. The fish made ‘no choice’ when it entered neither goal zone during the 30 second observer choice period. During this phase the demonstrator fish were confined by opaque barriers within their section so as not to influence the observer during the choice period.

Table 3.1: The number of “good” trials each demonstrator performed during the experiment.		
Tank	Left Demonstrator	Right Demonstrator
1	0	1
2	13	2
3	1	0
4	0	7
5	0	0
6	7	10
Total	21	20

## Video Analysis

Videos were analyzed using QuickTime (Apple Inc, Version 7.69.80.9) to determine which goal zone the fish entered first and the amount of time spent in each goal zone in seconds.

## Statistical Analysis

Following the same methodology as Coolen et al., (2003), I used  $\chi^2$  tests to analyze the initial choices made by observer fish against the expectation of the fish having no preference for where they spent their time (i.e. 33.3% chance of choosing the ‘food’ zone, the ‘no food’ zone or making ‘no choice’). The proportion of time spent in each zone was also compared using ANOVA, the data were checked for normality, were arc sin square root transformed (as recommended for proportional data), and outliers were removed based on the Cook’s Distance threshold values. For the comparison between levels of predation 1 observer was removed from the low predation Rio Limbo population; so the sample size would be n=16 for low predation



and  $n=24$  for high predation. For the comparisons between the different rivers and the different populations 1 observer from the high predation Rio Limbo population was removed in addition to the individual that was removed for the predation level comparison; so the river sample sizes would be  $n=17$  for Rio Limbo, and  $n=22$  for Rio Macho and the population sample sizes would be  $n=11$  for high Limbo,  $n=12$  for high Macho,  $n=6$  for low Limbo, and  $n=10$  for low Macho. I used a Tukey post hoc test to examine the differences between populations. I also used an ANOVA to check for possible non-specific demonstrator fish effects (e.g. one demonstrator was much more effective than another at demonstrating foraging at the feeding ring). Assumptions of normality were met and no outliers were removed for the comparison of demonstrator fish.

## RESULTS

Observer fish were not randomly distributed across the tank during the choice phase. Most fish moved into either the ‘food’ or ‘no food’ zones (Figure 3.2). The low predation populations from both rivers and the high predation population from Rio Macho chose the ‘food’ zone initially more often than expected, meaning that these fish chose the ‘food’ zone initially in more than 33.3% of the trials (Table 3.2 & Figure 3.2a-c). However, observer fish from the high predation population from Rio Limbo chose the ‘food’ zone approximately 33.3% of the time indicating no clear preference for the ‘food’ zone because these fish chose it initially the same as if they had chosen randomly.

When comparing the proportion of time fish spent within the ‘food’ zones during the 30 second observer choice period, there was a main effect of predation,  $F_{1,38}=4.55$ ,  $p<0.05$  (Table 3.3, Figure 3.3a), with high predation fish spending more time on average in the ‘food’ zone. There was also a main effect of river,  $F_{1,37}=4.58$ ,  $p<0.05$  (Table 3.3, Figure 3.3b), with Rio

Macho fish spending more time than Rio Limbo fish in the ‘food’ zone. Looking more specifically at which populations spent the most time in the ‘food’ zone, it became clear that the high predation population from Rio Macho spent more time in the ‘food’ zone than the other three populations,  $F_{3,35}=4.61$ ,  $p<0.05$  (Table 3.3, Figure 3.3c).

When comparing the difference between each demonstrator used and the amount of time the observer fish spent in the ‘food’ zone, no difference was found,  $F_{6,34}=1.73$ ,  $p>0.05$ . There was also no difference between demonstrator fish and proportion of the time spent in the ‘no food’ zone,  $F_{6,34}=2.24$ ,  $p>0.05$ .

Table 3.2:  $\chi^2$  values comparing the choice made by the observer fish (compared to the expectation of no choice i.e. 33.3% of their time spent in ‘food zone’, no choice zone, or ‘no food’ zone).

		$\chi^2$	p-value
Predation:	Low	13.49	<0.05
	High	19.26	<0.05
River:	Limbo	8.88	<0.05
	Macho	27.07	<0.05
Population:	HighLimbo	8.89	<0.05
	HighMacho	40.00	<0.05
	LowLimbo	8.89	<0.05
	LowMacho	16.50	<0.05

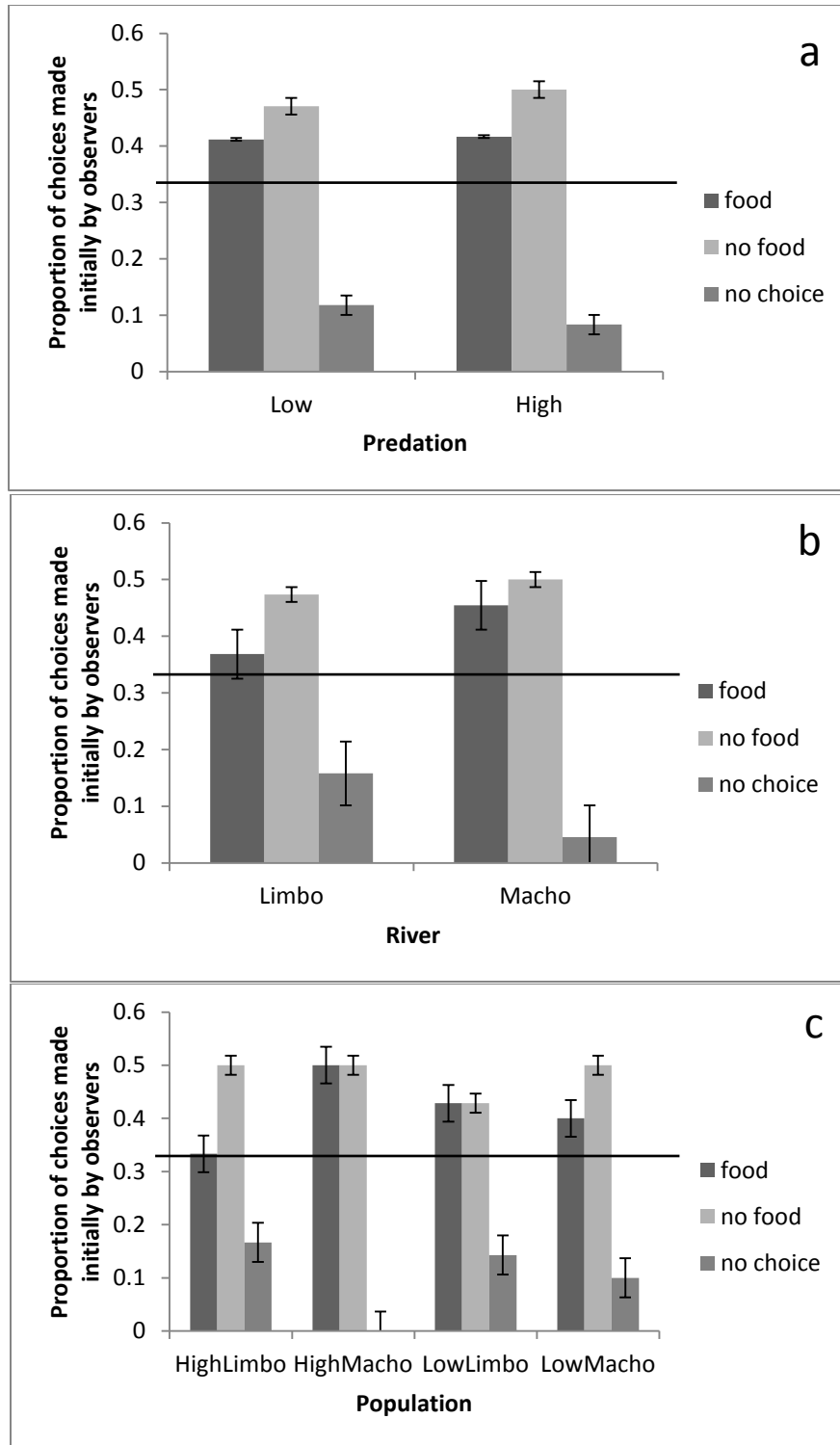


Figure 3.2: The proportion of observer fish that entered the ‘food,’ or ‘no food’ zone initially, or made ‘no choice’ after the demonstrators performed, a) by predation pressure (low, n=16; high, n=24) b) river of origin (Limbo, n=17; Macho, n=22), and c) population (High Limbo, n=11; High Macho, n=12; Low Limbo, n=6; Low Macho, n=10;). Bars show means  $\pm$  standard error. Black line indicates a 0.33 probability of making a random choice.

Table 3.3: The proportion of time fish spent within the ‘food’ zone during the 30 second observer choice period with actual time in seconds in parentheses.

		n	average	median	range
Predation:	Low	16	0.11 (3.38)	0.03 (1.00)	0.00-0.60 (0-18)
	High	24	0.27 (8.04)	0.22 (6.50)	0.00-0.83 (0-25)
River:	Limbo	17	0.11 (3.24)	0.03 (1.00)	0.00-0.50 (0-15)
	Macho	22	0.25 (7.59)	0.23 (7.00)	0.00-0.77 (0-23)
Population:	HighLimbo	11	0.12 (3.55)	0.00 (0.00)	0.00-0.50 (0-15)
	HighMacho	12	0.36 (10.75)	0.37 (11.00)	0.00-0.77 (0-23)
	LowLimbo	6	0.09 (2.67)	0.03 (1.00)	0.00-0.37 (0-11)
	LowMacho	10	0.13 (3.80)	0.03 (1.00)	0.00-0.60 (0-18)

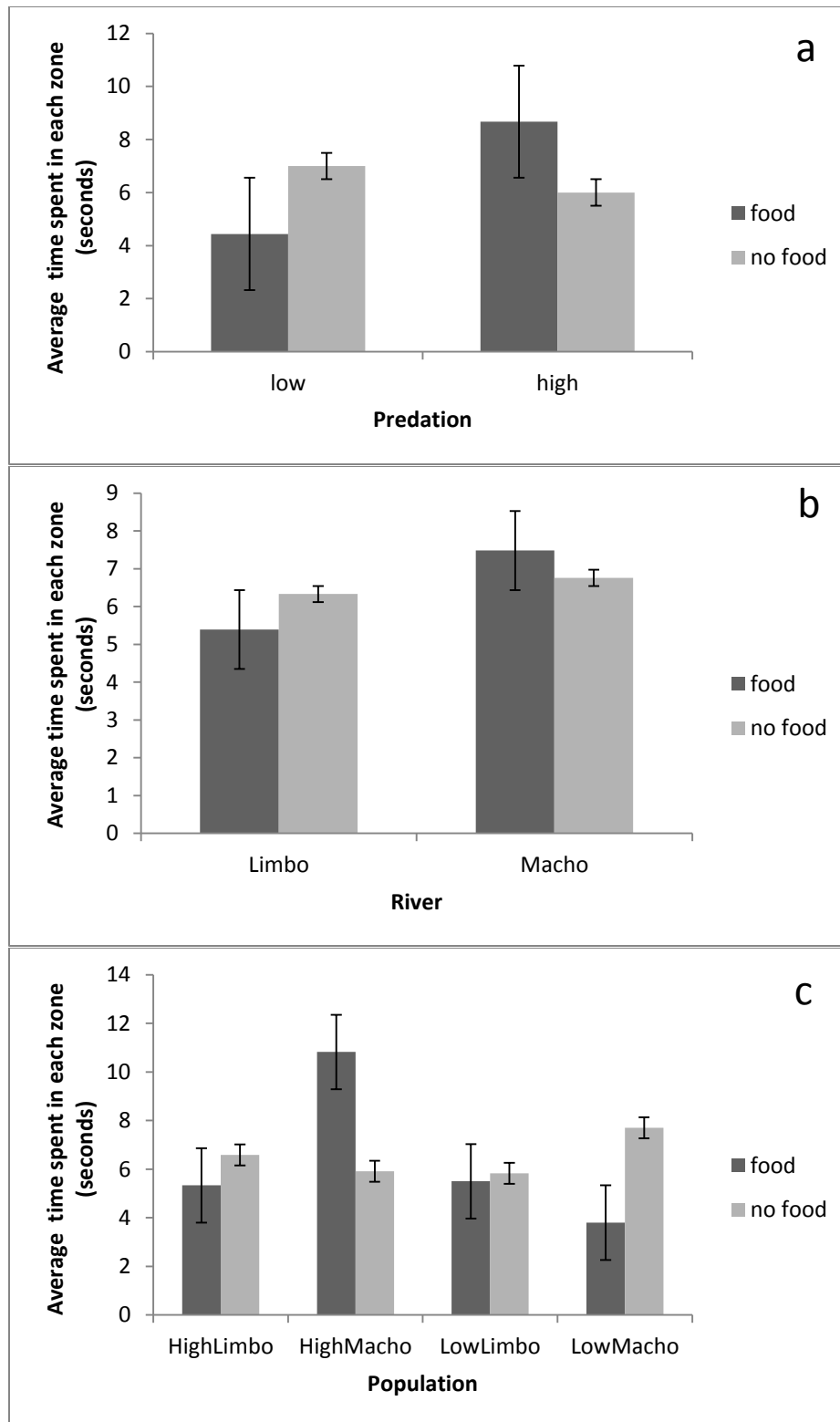


Figure 3.3: The observer's response to the demonstrator's performance as the average time spent in the 'food' or 'no food' zone in seconds for a) predation pressure (low,  $n=16$ ; high,  $n=24$ ), b) river of origin (Limbo,  $n=17$ ; Macho,  $n=22$ ), and c) population (High Limbo,  $n=11$ ; High Macho,  $n=12$ ; Low Limbo,  $n=6$ ; Low Macho,  $n=10$ ). Bars show means  $\pm$  standard error.

## DISCUSSION

During the choice phase of the trials, the observer fish were not randomly positioned, but rather they made an active choice and moved into and spent time in either the ‘food’ or the ‘no food’ zones during the 30 second observer choice period. In terms of where the fish spent their time, the data suggest that the high predation fish spent more time in the ‘food’ zone compared to fish from low predation areas, indicating high predation fish may be more influenced by local enhancement. However this result is inconclusive due to the large river effect that was observed. The observer fish from the high predation population in Rio Macho spent a larger amount of time in the ‘food’ zone than any other population, while the observer fish from the low predation population in Rio Macho spent the least amount of time in the ‘food’ zone than any other population. For the Rio Limbo populations approximately the same amount of time was spent in the ‘food’ zone for observers from both high and low predation sites. Since the observer fish from each of the two rivers acted differently in the experiment overall I cannot determine the ability of *B. episcopi* to use local enhancement in relation to the predation level of their former environment without testing more rivers.

I expected fish from high predation populations to be more influenced by local enhancement because these fish live in a riskier environment and theoretically need to be more aware of their surroundings than fish from low predation populations. It would therefore be more advantageous for these fish to use information gathered from observations, not only to reduce the risk of predation but also to outcompete other foraging conspecifics for the resource (Brown & Braithwaite, 2004). The low predation fish also experience conspecific competition, but very few if any predators, so for them the use of social information may be less important. Other studies have previously found that fish can learn about foraging patches from conspecifics (Brown &

Laland, 2003). Webster & Laland (2008) also report similar results in minnows (*Phoxinus phoxinus*) that were exposed to a ‘high-risk’ environment. When tested in a ‘high-risk’ environment (where there was no cover and artificial predators (models) were present within the testing apparatus), observer minnows had a preference to use socially learned information, gained from watching demonstrator fish, to locate and stay within a goal zone, more than observer minnows exposed to low (cover is present, no predators present), and indirect predation pressure (no cover present, no predators present) (Webster & Laland, 2008).

I may not have seen this result because of the smaller sample size for two of the populations, the difference between the rivers or possibly the variation in observer temperament. I was only able to obtain 7 “good” trials from the high predation Rio Limbo population and 10 “good” trials from the high predation Rio Macho population. In a previous experiment, Brown & Braithwaite (2004) found that high and low predation Rio Limbo fish were not different in the time they took to emerge from a shelter, whereas fish from Rio Macho showed a significant difference in the time to emerge, with the high predation fish emerging quicker than the low predation fish. Variation in the temperament of the observer fish within the different populations screened might also play a role here. Bell (2005) found variability between two populations of threespined stickleback (*Gasterosteus aculeatus*) in the heritabilities and genetic correlations between different behaviors used to measure activity, aggression, and boldness, where these behaviors were correlated in one population and not the other. Lima & Dill (1990) reviewed the effect of predation pressure on individuals and concluded that foraging behavior, predator avoidance, and social interactions all contribute to the decisions fish make. Thus the local enhancement effect I observed may be a result of multiple factors.

One difference in the experiment designed by Coolen et al. (2003) and the one I performed was that Coolen et al. (2003) used groups of three sticklebacks to demonstrate foraging vs. no foraging behavior in their trials. I used only one fish as a demonstrator because during preliminary conditioning trials two *B. episcopi* exhibited aggression and dominance interactions, which distracted the demonstrator fish from feeding. I therefore changed the design so that the demonstrator fish were housed individually so the observer fish could view reliable foraging behavior and I also did not want dominance of the demonstrator fish to be a factor in the observer's choice.

Analysis of the demonstrator fish found no difference in the amount of time the observer fish spent in the 'food' or 'no food' zone according to which fish demonstrated foraging behavior. The demonstrator fish had to start feeding within 30 seconds of food being delivered in the feeding ring and had to continue feeding until the 5 minute observation period was complete for use in the experiment. By using these criteria I was able to expose the observer fish to similar demonstrator fish behavior even though I used multiple demonstrator fish, multiple times during the experiment.

Future studies investigating local enhancement or social learning in *B. episcopi* should include measurements of observer fish temperament traits as this information could be a useful covariate to account for variation in their behavior (see Conrad et al., 2011 and Sih et al., 2004 for a review). The length and weight of the observer fish might also be a helpful covariate to include, because smaller fish tend to be bolder than larger fish (Brown & Braithwaite, 2004; Brown et al., 2005a). Furthermore, measuring the size of the demonstrators and having these fish be of similar size within the same tank, then only having observer fish that are close to the same size in the tank for the trials could reduce any potential intimidation factors. *B. episcopi* can



be very aggressive and large size differences allow the larger individual to dominate smaller fish. Finally, a further useful measure would be to quantify the demonstrator's behavior during the foraging observation period to assess general activity levels within the foraging area and the feeding rate as these factors may also influence the choices made by observer fish (attraction vs. avoidance). Krause (1992) found three-spined sticklebacks were not influenced by group size or feeding rate in their choice of foraging patch but rather were influenced by the excited behavior of the conspecifics while foraging.

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## CHAPTER 4

### CONCLUSIONS

To understand and effectively manage wild populations we need to determine the factors that influence fish physiology and behavior. The research in this thesis has looked at two very different aspects of fish biology within the Panamanian bishop (*Brachyrhaphis episcopi*). This species has already been studied extensively and their natural history and the fact that some populations live with a chronic threat of predation, compared to other populations that experience very little predation has allowed several conclusions to be drawn about the behavior and physiology of this tropical freshwater fish. For example, experiments have found that these fish exhibit clearly different temperament traits and fish from high predation populations tend to be bolder, more active and explore more than fish from low predation areas (Brown et al., 2005a; 2007; Archard & Braithwaite 2011a; Archard et al., 2012). Other studies have found that the decisions these fish make are affected by the predation pressure they experience (Beri, 2012), and their cognitive ability is similarly affected (Brown & Braithwaite, 2005b). While some initial work has started to investigate the physiology of the fish in terms of stress hormone production (Archard et al., 2012) little else is known about changes in physiology that may be affected by different kinds of temperament. I therefore addressed this gap with a study of gill morphology in fish screened for bolder or more timid tendencies described in chapter 2. While some connections have been made between learning and memory in *B. episcopi* (Brown & Braithwaite, 2005b; Beri, 2012), to date, nobody has investigated social learning abilities in this species. So the experiment described in chapter 3 was designed to look for evidence that local enhancement is used by these fish.

In experiment 1 I examined whether or not *B. episcopi* showed plasticity in their gill morphology dependent on their bold or timid temperament state. By measuring the size of their respiratory surface area I found no difference detected between bold and timid fish. I expected bolder fish have a larger respiratory surface area because they are both more active and explore their environments more than timid fish and this may increase their physiological demands, particularly their need for oxygen. A larger lamellae surface was proposed as a more efficient way for bold fish to absorb oxygen from their surrounding water. It is possible that by using a two-dimensional measuring technique on a three-dimensional structure I was not able to accurately capture the differences between the respiratory surface areas.

In experiment 2 I examined whether or not *B. episcopi* would use local enhancement and if so whether the predation background of the fish would affect their use of this kind of information. I was able to show that some *B. episcopi* may rely on local enhancement and high predation fish appear to use this information more than low predation fish, although additional populations from more rivers need to be studied to verify this finding. High predation populations are exposed to more risk than low predation populations, so using social information about the location of foraging patches could be a more efficient strategy and lead to increased chances of survival in a dangerous, predator-rich environment.

There is still a great deal that can be learned by studying *B. episcopi*. In each chapter a number of future directions are suggested. Comparisons between this species and other close relatives such as the guppy (*Poecilia reticulata*) would be useful to further test ideas about the impact that predation pressure has on the behavior and physiology of animals (Burns & Rodd, 2008, Harris et al., 2010). Similarly, other experiments with different species that consider the

effect of temperament on gill morphology would also help to determine how general the effects I report here are for other fish species (Huntingford et al., 2010).

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