THE INFLUENCE OF THE SOCIAL ENVIRONMENT ON PHENOTYPIC
PLASTICITY, FLEXIBILITY, AND EVOLUTION

by

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ABSTRACT

This dissertation describes the ways in which social environment shapes animal phenotype through phenotypic plasticity, flexibility, and evolutionary change. I investigated phenotypic plasticity in response to con specific social environment using the mangrove rivulus fish (*Kryptolebias marmoratus*). I measured reaction norms for growth, behavior, and hormonal physiology for two isogenic lineages of mangrove rivulus raised from hatching to maturity in isolation and with conspecifics. I found that social environment increased growth, particularly in dominant individuals and that animals raised in a social environment exhibited greater flexibility in aggressive behavior towards a mirror or model, but showed no change in boldness or in hormone production. The two isogenic lineages exhibited different reaction norms, with one showing greater response to social environment than the other. I investigated phenotypic flexibility in response to social environment using the nesting behavior of male longear sunfish (*Lepomis megalotis*). I found that male longear sunfish showed a non-significant trend toward reduction of nest construction when potential egg predators (juvenile sunfish) were present. However, sunfish did not alter the size or placement of their nests relative to the location of cover. Finally, I proposed a novel conceptual model to describe how the evolution of sexual signals can be shaped by selection by heterospecific eavesdroppers. This model accounts for selection by traditionally neglected eavesdroppers such as those that avoid signals, prey, and mutualists. I tested the response of bluenose shiners (*Pteronotropis welaka*), a potential mutualist, to a sexual signal (opercular flap length) of their host, longear sunfish. I found that bluenose shiners exhibited a preference for males with longer opercular flaps, the first
experimental evidence of eavesdropping in a nest associate and potential mutualist. I then measured the morphology of the opercular flaps of sunfish from watersheds where bluenose shiners were present, watersheds where shiners were not present, and watersheds distant from shiners. Opercular flap morphology did not vary between these treatments, providing evidence that eavesdropping by bluenose shiners had not exerted strong selection for changes in sunfish sexual signals over a broad spatial distribution.
### LIST OF ABBREVIATIONS AND SYMBOLS

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<tbody>
<tr>
<td>cm</td>
<td>centimeter</td>
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<tr>
<td>df</td>
<td>degrees of freedom</td>
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<td>den df</td>
<td>denominator degrees of freedom</td>
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<td>HD</td>
<td>high definition</td>
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<td>KT</td>
<td>ketotestosterone</td>
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<td>log</td>
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<td>num df</td>
<td>numerator degrees of freedom</td>
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<td>pc</td>
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CHAPTER 1
INTRODUCTION

Interactions between animals influence the expression of phenotypic traits, resulting in variation in behavior, morphology, and physiology. In this dissertation, I examine how the phenotype is modified by interactions that occur across a range of contexts, including intraspecific interactions during early life that result in modified developmental trajectories, interactions between heterospecifics that alter the behavior of adults, and interactions between species that shape the phenotypes present in populations over evolutionary time. Interactions with conspecifics are important for social species (Branchi 2009, Veenema 2009), but even species considered asocial interact during disputes over territories or other resources, and the vast majority of all species interact at least occasionally for the purpose of sexual reproduction (Otto and Lenormand 2002). Interactions with conspecifics during early stages of life can guide the development of appropriate behavioral responses later in life (Branchi 2009, Veenema 2009).

The second chapter of my dissertation examines how the social environment that animals are exposed to during development can alter behavior, physiology, and morphology. Interactions with heterospecifics are also important. Almost all animals are predators, parasites, or prey, with parasites alone accounting for at least 40% of all species (Dobson et al. 2008). For individuals in these categories, interactions with heterospecifics are often a matter of life or death. Important interactions also occur during competition for resources or during mutualisms. The need to interact with or to avoid heterospecifics to gain resources or to prevent being consumed has
resulted in a wide array of adaptations, including developmental plasticity and behavioral and physiological flexibility in response to cues indicating the presence of heterospecifics (Brown et al. 2013, Kendig et al. 2011, Maher et al. 2013). The third chapter of my dissertation examines how heterospecifics could drive flexibility in behavior and in the structures, such as nests, that result from these behaviors. Chapter four provides a comprehensive conceptual framework for understanding selection by heterospecifics on sexual signals specifically, and Chapters five and six test this conceptual framework using behavioral assays to assess interactions between heterospecifics, and museum specimens to investigate the potential for spatially variable heterospecific-driven selection to drive the evolution of sexually selected characters.

**Phenotypic Plasticity in Response to Social Environment During Development**

Phenotypic plasticity is the process by which environmental conditions experienced during early life change the developmental trajectory in ways that result in a permanent change to the adult phenotype (West-Eberhard 2003). These responses can be adaptive in that they allow animals to achieve maximum fitness under different environmental conditions, or they can be maladaptive responses to environments that are unsuitable for development (Ghalambor et al. 2007). Exposure to predator cues can induce changes in behavior and morphology, such as adaptive neophobia, that persist into adulthood (Brown et al. 2013, Kendig et al. 2011) or the development of alternate body forms that deter predatory attack or enhance escape ability (Maher et al. 2013). Interactions with conspecifics during early life can alter future social behavior, morphology, and hormone profiles (Curley et al. 2011, Galef and Laland 2005, Pfennig and Collins 1993, Veenema 2009), and exposure to certain social environments during development contributes to the social competence of adults (Taborsky and Oliveira 2012). When
deprived of appropriate social interaction during development, many species exhibit increased aggression and changes in production of a wide variety of hormones and hormone receptors (Curley et al. 2011, Veenema 2009). Isolation can also increase corticosteroid production in adults and render the animals unable to respond appropriately to social challenges (Earley et al. 2006). Because phenotypic plasticity can significantly alter the phenotype, it has important implications for the ecology and evolution of a species. On an ecological level, phenotypic plasticity mediates interactions between species and determines how individuals respond to environmental variation (Agrawal 2001, Fordyce 2006). On an evolutionary scale, phenotypic plasticity can facilitate the origin of new adaptations upon which selection can act and can buffer populations from rapid environmental change (Moczek 2011, Moczek et al. 2012, Pfennig et al. 2010, Standen et al. 2014).

Phenotypic plasticity is studied using reaction norms, which describe the range of phenotypes that a particular organism can develop under a range of environmental conditions (Dingemanse et al. 2010, Stamps 2003). For example, a reaction norm might describe variation in the growth trajectories of marble trout (*Salmo marmoratus*) experiencing different population densities during the first year of life (Vincenzi et al. 2008). Using reaction norms to study plasticity is complicated by the fact that plasticity is driven by interactions between the genotype and the environment. The shape of reaction norms can vary considerably among genotypes, meaning that testing individuals with unique genotypes under multiple environmental conditions will not provide an accurate depiction of the reaction norm of any particular genotype. Because plasticity describes permanent changes in the phenotype resulting from different early life conditions, it is impossible for the same individual to be raised multiple times under different environmental conditions to see how its developmental trajectory might change.
If, however, multiple genetically identical individuals are available, they can be used to generate an explicit genotype-specific reaction norm. This has allowed reaction norms of growth and physiological function to be tested in plant and invertebrate species where clonal reproduction occurs in the wild and genetically identical individuals can be produced in the laboratory (Shaish et al. 2007, Kroon et al. 2005, Beckerman et al. 2010, Cheplick 2003). However, the study of behavioral plasticity in vertebrates is hampered by the lack of species in which isogenic lineages can be produced. A survey of vertebrates found only 74 asexual species out of more than 42,000 known vertebrate species (Vrijenhoek et al. 1989). Many of these species reproduce only by parthenogenesis, resulting in a population of genetically indistinguishable organisms, which makes it difficult to characterize genotype x environment interactions. Because of this, reaction norms in vertebrates are typically estimated on a population level. Instead of exposing genetically identical individuals to a range of environmental conditions, genetically distinct individuals from the same population are used (Earley et al. 2012). This approach is problematic because it assumes that all individuals within a population have similar reaction norms (e.g., that strong selection has culled within-population variation), and that the function that describes the reaction norm is more variable among populations than within populations. If reaction norms are variable among genotypes within the population, no consistent trends will be seen when using a population-level approach, which would make it more difficult to identify how environmental factors expose or hide phenotypic variation within a population from selection (Killen et al. 2013). As a result, there is a gap in our understanding of how the social environment to which an individual is exposed during development affects behavioral phenotype in vertebrates.
In the second chapter of my dissertation, I examine the effects of the social environment experienced during early life on the development of morphological, behavioral, and physiological traits using mangrove rivulus (\textit{Kryptolebias marmoratus}), a species with an unusual reproductive system allowing for the study of vertebrate reaction norms at the genotype level. Mangrove rivulus have a mixed mating strategy whereby reproduction typically occurs via self-fertilization in hermaphrodites with occasional outcrossing between hermaphrodites and males (Davis et al 1990). Multiple generations of self-fertilization by hermaphrodites produces isogenic lineages that can be used to test how early life experience with different social environments influences various aspects of the adult phenotype while controlling for genetic effects (Earley et al. 2012). I allowed individuals from two isogenic lineages of mangrove rivulus to mature in environments alone or in the presence of conspecifics from the same lineage and observed the effects on behavioral, hormonal, and growth reaction norms. I hypothesized that rivulus housed communally would exhibit increased growth, matching what had previously been observed in the lab under less controlled conditions. I also hypothesized that rivulus would exhibit decreased levels of aggression, reflecting greater experience with social situations, and that isolated rivulus would exhibit increased levels of stress hormones.

**Flexibility of the Extended Phenotype in Response to Predators**

On short timescales, animals exhibit phenotypic flexibility - temporary, reversible changes to behavior, physiology, and morphology in response to changing social or physical environments (Piersma and Drent 2003, Sollid et al. 2003). These changes can be drastic; for example, bluebanded gobies (\textit{Lythrypnus dalli}) reversibly switch between male and female sexes depending on their current social status (Rodgers et al. 2007). However, morphology is typically
less flexible than behavior because changes in behavior do not require growth or remodeling of tissues. The structures an organism builds in its environment, which form a part of its extended phenotype, provide an interesting middle ground between behavior and morphology (Hansell 2000). Although a product of behavior, structures are physical objects that must be constructed and that have measurable physical form. Constructions exhibit flexibility in form as they are built and remodeled; for example, orb weaving spiders (*Argiope keyserlingi*) alter the capture area, mesh size, and level of decoration of their webs in response to changes in prey type and availability (Blamires 2010). Nests are among the most widespread of animal structures (Hansell 2005), and how animals alter nests and other constructions in response to their environment is an area of active research. The locations that animals use for territories may be chosen or modified to enhance defensibility (Earley and Hsu 2013), and nest construction and location can vary depending on environmental influences such as the presence of predators, competitors, and conspecifics (Nilsson 1985, Spencer and Thompson 2003, Peluc et al. 2008). In the third chapter of my dissertation, I examine the flexibility of the extended phenotype, particularly the probability of building nests and the spatial placement of nests, in response to altered predation pressure using longear sunfish (*Lepomis megalotis*). These fish build multiple temporary nests during each breeding season and defend their eggs and offspring from predators (Gill 1906, Witt and Marzolf 1954). I allowed longear sunfish to nest in the presence and absence of juvenile conspecifics, which are egg predators (Keenleyside 1972) and observed nest size, number, and location relative to cover. I hypothesized that sunfish would modify their nest building in response to threat of predation by juvenile conspecifics by decreasing nesting effort and constructing nests in more easily defended locations.
Evolution of Signal Phenotypes in Response to Selection Imposed by Heterospecifics

The ability for interactions between animals to drive phenotypic changes over evolutionary timescales is well known - indeed it is a fundamental aspect of biology. When and how animals interact is determined by the behavioral responses of animals upon detecting each other, and this in turn defines the magnitude and direction of selection that these interactions produce. For example, active ochre sea stars (*Pisaster ochraceus*) select for predator avoidance behaviors in their prey, black turban snails, (*Chlorostoma funebralis*), but inactive sea stars select for snails that do not exhibit predator avoidance (Pruitt et al. 2012). Thus, the magnitude and direction of selection imposed is dependent on the behavioral type of both predator and prey. However, research typically focuses on how behavioral traits affect fitness via interactions with conspecifics. Fewer studies examine how the behavioral traits of one species affect the evolution of traits in another; for example, how responses by prey affect the evolution of predator phenotypes, or how responses by mutualists affect the evolution of characters in their partners. Interactions mediated by heterospecific eavesdropping offer a particularly valuable system for examining how the behavior of one species can shape the evolution of phenotypic traits in another. Because eavesdroppers react to a specific trait (a signal) of another species, they can select for changes in that particular trait. Eavesdropping behavior of predators and parasites is known to select for changes in sexual signals (Peake 2005, Zuk et al. 2006), but eavesdropping occurs in other contexts as well. Competitors eavesdrop on one another (Webster et al. 2010) as do prey on predators (Lohrey 2009, May et al. 2012) and there is more to learn about how these types of interactions and others that have been neglected thus far, like mutualisms, might drive evolutionary changes in signaling phenotypes.
The final chapters of my dissertation examine how eavesdropping heterospecifics can select for changes in sexual signals. Chapter four of my dissertation provides a comprehensive conceptual model describing how interactions between eavesdropper and signaler can drive the evolution of sexual signals. I tested this framework using longear sunfish (*Lepomis megalotis*) whose nests are used as spawning sites by bluenose shiners (*Pteronotropis welaka*) (Johnston and Knight 1999). Male sunfish bear opercular flaps, which are honest signals of male fighting ability and serve as sexual signals to females (Goddard and Mathis 1997, Goddard and Mathis 2000) that might also act as signals to bluenose shiners. In the fifth chapter of my dissertation, I describe a study in which I presented bluenose shiners with sunfish displaying different opercular flap lengths to determine whether shiners eavesdrop on the sexual signals of sunfish and to examine whether shiners demonstrate a similar preference as female sunfish for male sunfish bearing longer opercular flaps. I hypothesized that shiners would exhibit a preference for sunfish bearing longer opercular flaps, as those signals may indicate males that are better fighters and thus may provide better defense of shiner eggs. In the sixth chapter of my dissertation, I looked for selection by shiners on this sexual signal by measuring opercular flap lengths from sunfish derived from watersheds across the Southeastern United States, some containing bluenose shiners, some adjacent to watersheds containing shiners but lacking records themselves, and some outside the coastal plain region where shiners are found. I hypothesized that longear sunfish would exhibit differences in opercular flap morphology between watersheds containing bluenose shiners and nearby watersheds lacking them, with sunfish that co-occur with shiners bearing more elaborate opercular flaps. Distant watersheds were included to allow for comparison with a region where shiners were unlikely to have ever occurred.
References


CHAPTER 2
PHENOTYPIC PLASTICITY IN RESPONSE TO EARLY SOCIAL ENVIRONMENT

Interactions with conspecifics play an important role in shaping adult phenotype in many animals. Social environment can permanently affect personality, social competency, foraging strategies, physiology, growth rates, morphology, and even sex (Curley et al. 2011, Foster 2013, Galef and Laland 2005, Munday et al. 2006, Pfennig and Collins 1993, Stamps and Groothuis 2010a, Veenema 2009). Some of these changes are adaptive, with developmentally plastic responses leading to the expression of phenotypes better suited to helping juveniles survive their current environment or prepare for the environments they will encounter as adults (Stamps and Groothuis 2010b). In other cases, plastic responses can be maladaptive, particularly when they are induced by environmental conditions different from those in which organisms have evolved (Foster 2013, Sih 2013). Plastic responses can help organisms compensate for stressful conditions, but this can come at a cost in other aspects of development (Royle 2005, Veenema 2009).

Plastic responses to early social environment drive both adaptive and maladaptive changes in later social behaviors in many species. In mammals, the quality of early maternal interactions has a large impact, with individuals deprived of sufficient care showing a decreased ability to exhibit aggression levels appropriate to the social context (Veenema 2009). Other conspecifics can also have an important impact; for example, mice raised in communal nests, where more than one mother and litter are present, more quickly settle into a dominant or subordinate role when placed with an unfamiliar individual (Branchi 2009). Fish also learn
appropriate aggressive responses based on their early social environment. Male cichlids
(Astatotilapia burtoni) raised in isolation display species-typical aggressive responses when
presented with a model fish representing another male, but at different rates than control fish
raised in groups (Fernald 1980). Males of this species also exhibited higher levels of aggression
towards heterospecifics (Cichlasoma nigrofasciatium) than conspecifics when reared only with
heterospecifics (Crapon de Caprona 1982). Even interactions well outside the scope of typical
social interactions such as parental care and peer competition can drive important phenotypic
changes; for example, attempted predation by adult guppies on juveniles alters the juveniles’
behavior and morphology in ways that reduce vulnerability to later predation attempts by more
dangerous predators (Chapman et al. 2008).

Plastic changes in behavior and morphology are often driven by changes in hormone
production (Dufty et al. 2002). Exposure to appropriate social stimuli during early development
plays an important role in determining how the brain responds to hormones, producing plasticity
in a variety of adult behaviors (Cushing and Kramer 2005). In mammals, a low quality social
environment during development can alter the development of the hypothalamic–pituitary–
adrenal axis, resulting in changes in adult hormone profiles that are correlated with depression
and anxiety (Veenema 2009). In fish, production of and response to steroid hormones is altered
by social environment (Fox et al. 1997, Oliveira et al. 2001), including the environment
experienced during development (Moretz et al. 2007). Social environment, cortisol, and behavior
all affect each other; social isolation can increase responsiveness to cortisol and reduce the
ability of fish to modulate cortisol response, while increased cortisol levels can induce low social
status (Dibattista et al. 2005, Earley et al. 2006). However, the nature of these relationships
varies from species to species (Dunlap et al. 2002, Øverli et al. 2002). Androgens also affect the
degree to which animals respond to contest experiences (Oliveira et al. 2009) and increase
defensive efforts of parental male fish (Dey et al. 2010).

The examples provided above illustrate that phenotype can vary significantly in response
to the developmental environment. The nature of this variation can be understood using reaction
norms, which incorporate both genetic and environmental influences simultaneously to describe
the factors leading organisms to develop different phenotypes (Dingemanse et al. 2010, Stamps
2003). A reaction norm is the range of phenotypes a single genotype would produce under a
range of different early life environmental conditions (Sarkar 1999). For example, a reaction
norm approach would not simply ask how bold an animal was, but would instead ask how bold
that animal was under a spectrum of different environmental conditions, such as a range of
predator densities, experienced during development. In plants (Tikhonova et al. 2012) and
invertebrates (Beckerman et al. 2010), reaction norms have been investigated using genetically
identical individuals placed under differing environmental conditions. However, it has been
difficult to directly test reaction norms in vertebrates because few vertebrates produce groups of
genetically identical individuals that can be tested at the same time and under different
environmental conditions. Studies of reaction norms in vertebrates have been largely limited to
comparing genetically similar, but not identical individuals such as close relatives or inbred
captive lineages (Stamps 2003). Other researchers have used population level reaction norms that
assume variation in individual reaction norms and their distribution across environmental
gradients can be safely ignored (Earley et al. 2012).

The mangrove rivulus (*Kryptolebias marmoratus*) is a fish species with a highly unusual
mating system that allows for direct testing of genotype-level reaction norms in vertebrates.
Rivulus, along with its sister species *K. hermaphroditus*, are the only two known species of self-
fertilizing vertebrate hermaphrodite (Costa 2011, Tatarenkov et al. 2009). The species exhibits a mixed mating strategy, where most individuals are hermaphrodites but males make up between <1% and 25% of the population (Davis et al. 1990). This results in repeated self-fertilization by hermaphrodites, eventually producing isogenic lineages. Combined with occasional outcrossing between lineages (Mackiewicz et al. 2006), this results in wild populations that contain a large number of genetically distinct isogenic lineages, ideal for studying reaction norms of a variety of genotypes (Earley et al. 2012).

Mangrove rivulus are notable for their ability to survive in the harsh and variable conditions of mangrove habitats, and have a broad range extending from Florida to the Caribbean and Central America (Taylor 2012). The species is typically found in upland mangroves occupying crab burrows, temporary ponds, areas bordering tidal creeks, and mosquito ditches, as well as outside of the water in rotting vegetation (Davis et al. 1990). Mangrove rivulus are tolerant of poor water conditions (Frick and Wright 2002) and can survive out of water (emersed) in a moist habitat for periods of up to 66 days (Taylor 1990). In laboratory and field settings, mangrove rivulus respond aggressively to conspecifics (Earley and Hsu 2008, Hsu et al. 2014, Huehner et al. 1985, Molloy et al. 2011, Taylor 1990) and as a result are not typically considered "social" (Mackiewicz et al. 2006). However, in the wild multiple individuals can be found together in the same small bodies of water, providing an opportunity for interaction with conspecifics (Taylor 1990, Taylor et al. 2008, Taylor 2012). In the lab, mangrove rivulus exhibit reduced aggression when exposed to familiar individuals or individuals of the same genotype, indicating that they can flexibly adjust aggressive behavior in response to differing social environments (Edenbrow and Croft 2012). Rivulus also show significant changes in aggressive and exploratory behavior as well as hormone profiles in response to aggressive encounters, and
these responses differ between winners and losers (Chang et al. 2012, Earley and Hsu 2008, Hsu and Wolf 2001, Hsu et al 2008). The existence of groups of rivulus in the wild indicates that some level of social interaction must occur, and even if most encounters are hostile, laboratory research indicates that the rate or intensity of aggressive interactions can be determined by social relationships and dominance status. Distinct behavioral types also have been documented in this species (Edenbrow and Croft 2011) but more research is needed to determine how social environment experienced during development affects adult behavior and endocrine status.

I investigated the effects of social environment during development (conspecifics present or conspecifics absent from hatching to adulthood) on phenotypic characters in the mangrove rivulus. I measured behavior in the form of aggression (attacks directed towards a mirror and model conspecific) and boldness (latency to emerge from a shelter and begin exploring a novel arena), hormones (cortisol, estradiol, and 11-ketotestosterone), and body size (length). I hypothesized that individuals raised in a social environment would exhibit lower levels of aggression relative to isolated individuals, because they would have developed in a social context with ample opportunity to engage with conspecifics. Mangrove rivulus exhibit plasticity in growth rate in response to a variety of environmental conditions (Lin and Dunson 1999), and because of prior unpublished observations of communally housed individuals, I also predicted that group housed individuals would grow more rapidly than isolated individuals.

I also hypothesized that within group-housed treatments, individual phenotypes would diverge along dominant and subordinate paths. Mangrove rivulus establish dominance within 1 hour when paired in the lab (Hsu et al. 2008) and individuals that recently won an aggressive contest exhibit increased willingness to display to a mirror image when compared to individuals that lost (Chang et al. 2012). Fighting ability in mangrove rivulus contests also is influenced by
cumulative past performance (Hsu and Wolf 1999, Hsu et al. 2013). Individuals with lower cortisol and higher testosterone are better at initiating and winning contests, while losers of escalated fights showed increased levels of cortisol, testosterone, and 11-ketotestosterone (Earley and Hsu 2008). As a result of these reinforcing effects of repeated interactions, I predicted that individuals raised in groups would diverge in their behavior, baseline hormone levels, and growth rates, producing dominant and subordinate individuals with different levels of aggression and different body sizes.

**Methods**

**Experiment 1: Effects of Isolation vs. Communal Housing**

**Acquisition and housing of experimental animals**

The experiment used lab reared mangrove rivulus from two isogenic lineages, DAN2K (n=24) and RHL (n=16). DAN2K was collected in Dangriga (Belize) and RHL was collected in San Salvador (Bahamas) in 2000 and 1997, respectively, by Dr. D. Scott Taylor. I used 10, 38 L aquaria, with five divided into four equal compartments (isolated treatment), and five left unmodified (social treatment). I placed fish into the aquaria within 24 hours of hatching and kept them there for the duration of the experiment (77 days). Each aquarium contained only one lineage. Compartments and aquaria were visually and chemically isolated from each other. Each compartment of the isolated treatment aquaria contained 5 L of 25 ppt saltwater, while undivided social treatment aquaria contained 20L of 25 ppt saltwater to maintain an equal density of fish between treatments. Isolated treatments contained one fish in each compartment, while the social treatment contained four fish. All aquaria were placed under a 12h light: 12h dark photoperiod. The fish were fed once per day with newly hatched brine shrimp (*Artemia* sp.) nauplii. Isolated
fish received 1 ml of shrimp, while each group of four fish received 4 ml of shrimp daily. Experimental measurements of fish were taken between day 51 and day 78 (Chart 1).

**Experimental measurements**

**Body length:** I measured the standard length of each fish four times, at 51, 60, 70, and 77 days post hatch. I measured total length (tip of the snout to posterior margin of caudal fin) and standard length (tip of the snout to posterior margin of caudal peduncle) using calipers while the fish were restrained on a flat surface under plastic wrap. The fish were not tagged because implanting tags or dye was too invasive a procedure for newly hatched fish. As a result, individuals in communal tanks could not be individually identified across time points.

**In-tank aggression estimate:** Fish in the social treatment were filmed four times, on days 56, 59, 67, and 75, for 10 minutes while in their aquaria. Each fish was tracked individually for the duration of the trial by carefully following its movements on the video, and the number of "approaches" and "retreats" was recorded. An approach occurred when a fish swam toward another fish and moved to within one body length, displacing the other fish from its position. A retreat occurred when a fish was displaced from its position by an approaching fish.

**Hormone Collection:** Hormones were collected twice using a water-borne hormone collection method previously validated for rivulus (Earley & Hsu 2008, Earley et al. 2013). Hormones were collected the day prior to conducting behavioral assays, on 70 days post hatch and one week later at 77 days post hatch, and thus approximate the baseline endocrine state. Fish were removed from their housing by hand-net and placed in 500ml beakers. The time taken to remove the fish (net in the water to fish in the beaker) was recorded, and for communal tanks the time between first disturbance and removal of the fish was recorded. The 500ml beakers
contained 400 ml of 25 ppt salt water made by mixing Instant Ocean® synthetic sea salt with
deionized water; beakers were covered with a piece of thick, clear plastic to ensure that the fish
would not jump out. Beakers and plastic covers were rinsed with 95% ethanol and distilled water
prior to use. The fish remained in beakers for one hour and were visually isolated from one
another. After the hormone collection period, the contents of the beaker were poured through a
pre-cleaned net into a second 500 ml beaker pre-cleaned with 95% ethanol and distilled water.
The water from these beakers was passed through Whatman Grade 1 filter paper to remove
debris and feces. The water then was passed over pre-primed (2 x 2 ml methanol then 2 x 2 ml
distilled water) Sep-Pak C18 columns (Waters, Inc., 500 mg bed weight, 3 ml column capacity)
fitted to a 24-port vacuum manifold. Tygon tubing (Saint-Gobain, formulation 2275), previously
rinsed with 95% ethanol and distilled water, was fitted to the column and the other end of the
tubing placed into the water sample. When the vacuum was engaged, the water sample passed
through the tubing and dripped onto the Sep-Pak C18 column, extracting the hormones. Columns
were stored at -20°C for later hormone processing, at which time the columns were thawed and
salts purged with 2 x 2 ml distilled water. The free hormone fraction (not conjugated with
glucuronides or sulphates) was eluted with 2 x 2 ml ethyl acetate into 13 x 100 mm borosilicate
vials. The solvent was evaporated under a gentle stream of ultrapure nitrogen gas in a water bath
at 37°C, resulting in a hormone residue that was resuspended in 5% ethanol: 95% enzyme
immunoassay buffer (25µl ethanol followed by 1 minute vortexing plus 475µl EIA buffer
followed by 30 minutes vortexing). The samples were stored at 4°C and were assayed the next
day. Assay details are provided below.

**Aggression:** After the fish were removed from beakers, they were placed in acrylic fight
tanks for one day to acclimate. Fight tanks measured 20cm tall x 17cm wide x 12cm deep and
were transparent on the front, opaque on the sides and back, contained a gravel substrate approximately 1cm thick, and were filled with 25 ppt water. After one day (day 71-72) the fish were presented with a painted clay model rivulus suspended in the middle of the tank by a transparent fishing line. Fish were filmed with a JVC Everio HD digital camcorder for 10 minutes, and their responses to the model - first orientation toward the model, first approach to the model, and first physical contact with the model - were recorded. After the model test, total and standard lengths were measured as described above and the fish were placed back into their original tanks (isolated or social). The entire procedure – from hormone collection and aggression test to length measurements – was repeated in the exact same fashion one week later (Day 77-78).
TABLE 2.1: Timeline of experimental measurements

The ‘X’ marks indicate that a measurement was taken on a particular day.

<table>
<thead>
<tr>
<th>Experiment 1: Day</th>
<th>51</th>
<th>56</th>
<th>59</th>
<th>60</th>
<th>67</th>
<th>70-71</th>
<th>75</th>
<th>77-78</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-tank aggression</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>Length measurements</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Aggression vs model</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2: Day</th>
<th>42</th>
<th>56</th>
<th>70</th>
<th>77</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length measurements</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Behavioral trials</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Experiment 2: Effects of Isolation vs. Pair Housing

The previous experiment did not allow for tracking of individual animals within a group. However, by placing killifish in pairs I was able to track the larger and smaller individual in a pair between time periods. In this experiment, I also measured boldness (willingness to explore a new environment), in addition to the behavioral, physiological, and growth measures described for Experiment 1. Experimental measurements were taken between day 48 and day 78 (Chart 1).

Acquisition and housing of experimental animals:

This experiment used the same isogenic lineages as described for Experiment 1: DAN2K (n=36) and RHL (n=36). Fish which died and communal fish which lost a partner prior to day 60 of the experiment were removed from the analysis (n=13 DAN2K, n=9 RHL). Fish were placed in 1.5 L units of an Aquatic Ecosystems, Inc. rearing system (#ZF601) within 24 hours of hatching. Adjacent units were paired such that one unit contained one fish (isolated treatment) and one unit contained two fish (social treatment). Each pair of units contained only one lineage, and the location of pairs on the unit was randomized using dice rolls.

Salinity was held between 24 and 25 ppt, pH between 8.6 and 8.7 and temperature between 29 °C and 31.5 °C. All units were visually isolated via opaque baffles, and chemically isolated by virtue of all water being passed over mechanical and biological filtration prior to returning to the separated units. Fish were maintained on a 12h light: 12h dark photoperiod, and were fed daily with newly hatched brine shrimp (Artemia sp.) nauplii. Isolated units were fed 1ml and social units were fed 2 ml of brine shrimp daily.
**Experimental Measurements**

**Body length:** I measured fish length at four ages, 42, 56, 70, and 77 days post hatch. I measured total and standard length using the same protocol as for Experiment 1. Although the fish were not tagged, individuals in the social treatment exhibited obvious size differences, allowing “larger” and “smaller” individuals to be tracked.

**Hormone Collection and Assay Details:** Hormones were collected using the same protocol as described for Experiment 1 at 70 days post hatch. The resuspended hormone residues from Experiment 1 and Experiment 2 were used to assay estradiol, cortisol, and 11-ketotestosterone, each on three Cayman Chemicals, Inc. enzyme-immunoassay plates. Manufacturer’s instructions were followed explicitly. A pooled control, generated by combining 30µl of resuspended hormone from each experimental individual, was run in duplicate at the beginning and end of each plate to assess intra- and inter-assay coefficients of variation. Intra-assay coefficients of variation were: estradiol (plate 1: 2.19%, plate 2: 3.02%, plate 3: 2.38%), 11-ketotestosterone (3.17%, 4.19%, 11.47%), and cortisol (3.23%, 7.40%, 3.18%). Inter-assay coefficients of variation for estradiol, 11-ketotestosterone, and cortisol were 9.39%, 7.42%, and 4.47%, respectively.

**Behavior trials:** After fish were removed from hormone collection beakers they were placed in holding tubs. Fish were removed from the holding tubs for aggression or boldness trials, and then returned to the tubs before being placed in the other trial. All fish from a communal-isolate pair were randomly assigned either aggression or boldness trials first. After testing the fish were returned to their holding units.
For the aggression trials, fish were placed in small chambers with dimensions 9.5 cm long x 7.5 cm wide x 5.5 cm deep containing mirrors at one end covered by opaque removable partitions. Mirrors were used for this experiment because previous research indicated that they induce a stronger response in *Kryptolebias* while still maintaining relevance to outcomes in dyadic contests (Earley et al 2000). Fish were allowed to acclimate for 30 minutes. The partitions were then removed remotely using a fishing line. The fish were filmed for 10 minutes and latencies to approach (move within one body width) and bite the mirror, and the number of bites delivered toward the mirror were recorded. For the boldness trials, fish were confined inside a 3.3 cm diameter translucent white vertical tube in the middle of 30 cm diameter circular opaque white tub. After an acclimation period of 30 minutes, the central tube was removed remotely using a fishing line, and fish movement through the chamber was observed for 10 minutes. The following behaviors were recorded: latency to settle down and cease movement after being released, latency to initiate movement again, and the total time spent moving in the open field, defined as at least one body length away from the walls of the chamber.

**Statistical Analysis**

**Experiment 1**

I conducted principal components analysis on the measurements taken during the test of aggression, including latency to orient towards the model, latency to approach the model, and latency to touch the model. This produced three principal components, one of which (PC1) had an eigenvalue greater than 1.0 and accounted for 66.48% of the behavioral variance (Table 2.2). This principal component was strongly associated with both the time it took fish to swim within one body length of the model and the time it took fish to touch the model. Larger PC1 values
indicate less aggressive fish, with greater latencies to orient towards the model, approach, or physically interact with the model. I used a mixed model with repeated measures to calculate the effects of treatment (social environment – isolated vs. communal), genotype, time, and all interactions on standard length and aggression (PC1 and latency to physically interact with the model). PC1 was log(X+2) transformed to increase achieve normality. I included fish nested by in aquarium as a random effect. The Kenward-Roger method was used to estimate degrees of freedom. I employed a priori linear contrasts to resolve the differences among the levels of significant interaction effects.

**Experiment 2**

I ran a principal component analysis on the measurements taken during behavioral tests, producing six components, two of which had eigenvalues > 1.0 and were used in my analysis: PC1 and PC2 (Table 2.3). PC1 explained 36.05% of the variation and was associated with a high latency to approach and bite the mirror during the aggression test, and was negatively associated with number of bites delivered towards the mirror during the aggression test. Thus, high PC1 scores indicate low aggression levels. PC2 was positively associated with latency to begin movement after being released into a new environment during the boldness test. I interpreted high PC2 scores as indicating low resilience following a stressor.

I used a mixed model analysis with repeated measures to calculate the effects of status (dominant, subordinate, isolate), genotype, time, and all interaction terms on standard length, with fish nested by tank grouping as a random effect, with each grouping each containing one isolated individual and an adjacent pair of dominant and subordinate individuals. Standard length was also included as a continuous predictor for analysis of PC1, PC2, and latency to approach
the mirror. The Kenward-Roger method was used to estimate degrees of freedom. A general linear model was used to determine the effects of treatment, genotype, treatment x genotype, and length on estradiol, cortisol, and testosterone. Fish were again nested by tank grouping, treated as a random effect. Prior to all analysis, I removed all individuals that were paired with a fish that died prior to day 60 (N=22). All principal components analyses were done using JMP version 10, while all other analysis were conducted using SAS version 9.1.
TABLE 2.2: Experiment 1-Principal components analysis

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to orient to model</td>
<td>0.56088</td>
<td>0.70976</td>
<td>0.42620</td>
</tr>
<tr>
<td>Latency to approach model</td>
<td>0.60827</td>
<td>-0.00406</td>
<td>-0.79372</td>
</tr>
<tr>
<td>Latency to touch model</td>
<td>0.56162</td>
<td>-0.70443</td>
<td>0.43400</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>1.9945</td>
<td>0.5895</td>
<td>0.4159</td>
</tr>
<tr>
<td>Percent</td>
<td>66.48</td>
<td>19.65</td>
<td>13.87</td>
</tr>
<tr>
<td>Cumulative Percent</td>
<td>66.48</td>
<td>86.13</td>
<td>100.00</td>
</tr>
</tbody>
</table>

TABLE 2.3: Experiment 2-Principal components analysis

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to approach mirror</td>
<td>0.4709</td>
<td>0.0671</td>
</tr>
<tr>
<td>Latency to bite mirror</td>
<td>0.9984</td>
<td>-0.0558</td>
</tr>
<tr>
<td>Number of bites</td>
<td>-0.8163</td>
<td>0.0194</td>
</tr>
<tr>
<td>Latency to settle</td>
<td>0.1260</td>
<td>0.1026</td>
</tr>
<tr>
<td>Latency to move</td>
<td>-0.0680</td>
<td>0.9977</td>
</tr>
<tr>
<td>Latency to move into center</td>
<td>0.0201</td>
<td>0.1926</td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>2.1629</td>
<td>1.2376</td>
</tr>
<tr>
<td>Percentage</td>
<td>36.048</td>
<td>20.626</td>
</tr>
<tr>
<td>Cumulative Percentage</td>
<td>36.048</td>
<td>56.674</td>
</tr>
</tbody>
</table>
Results

Experiment 1

There was a significant main effect of both treatment and time on body length (Table 2.4). Communal fish were significantly larger than isolates, and fish grew over time. Communal fish showed a significant increase only between the first and last measurement (linear contrasts, day 51 vs. day 77; $F_{1,69.9}=13.06$, $P=0.0006$) while isolated fish showed significant growth between first and second as well as second and fourth measurements (linear contrasts, day 51 vs. day 60: $F_{1,68.3}=10.5$, $P=0.0018$; day 60 vs. day 77: $F_{1,68.3}=7.6$, $P=0.0075$). Isolated fish were significantly smaller than communal fish at every time-point (linear contrasts days 51, 60, 70, 77; $F_{13,3} > 8.12$, $P<0.05$).

For aggression (PC1), there were no significant main effects of treatment, genotype, or time but there was a significant effect of genotype x time (Table 2.5). Genotype DAN2K showed significantly longer latencies to approach and physically interact with the model (increased PC1 scores) on the second trial relative to the first (linear contrast: $F_{1,64.6}=5.9$, $P=0.018$), while genotype RHL showed no temporal change in the reaction to a model opponent (linear contrast: $F_{1,64.6}=1.8$, $P=0.18$) (Figure 2.2); these same trends were shown for latency to physically interact with the model. There was a non-significant tendency for the latency to physically interact with the model to vary with treatment x time (Table 2.5), an effect driven by increased latencies shown by communal animals at the second time point (linear contrast day 70 vs 77; communal: $F_{1,33.3}=6.8$, $P=0.01$; isolate: $F_{1,33.1}=0.1$, $P=0.72$; Figure 2.3).

A mixed model similar to the one described above, but also including length and handling time as fixed effects, was used to estimate the effects of the experimental variables on estradiol,
cortisol, and testosterone. All hormones were natural-log transformed to achieve normality. Estradiol and cortisol both increased between the first and second measurements (time effect – Table 2.6, Figure 2.4) but no other effects were significant. Testosterone did not vary as a function of any of the main effects or their interactions (Table 2.6). There were no correlations between behavior, hormones, and body size.
FIGURE 2.1: Experiment 1 - Effect of treatment on standard length
FIGURE 2.2: Experiment 1-effect of genotype on response to model

a) Latency for genotypes to touch model

b) PC1 scores of genotypes
FIGURE 2.3: Experiment 1-Effects of treatment on response to model

FIGURE 2.4: Experiment 1-Hormone levels over time
Table 2.4: Experiment 1-Standard length

<table>
<thead>
<tr>
<th>Effect</th>
<th>F(\text{Num DF, Den DF})</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>15.67(1, 6.29)</td>
<td>0.0068</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.10(1, 6.29)</td>
<td>0.7589</td>
</tr>
<tr>
<td>Time</td>
<td>16.05(3,135)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Treatment*Genotype</td>
<td>0.23(1, 6.29)</td>
<td>0.6447</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td>0.41(3,135)</td>
<td>0.7442</td>
</tr>
<tr>
<td>Genotype*Time</td>
<td>0.35(3,135)</td>
<td>0.7875</td>
</tr>
<tr>
<td>Treatment<em>Genotype</em>Time</td>
<td>0.08(3,135)</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

Table 2.5: Experiment 1-Behavior

<table>
<thead>
<tr>
<th>Effect</th>
<th>Latency to touch model</th>
<th>PC1 (\text{log}(PC1+2))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(\text{Num DF, Den DF})</td>
<td>P-value</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.11(1,6.04)</td>
<td>0.7535</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.09(1,6.04)</td>
<td>0.7779</td>
</tr>
<tr>
<td>Time</td>
<td>1.91(1,63.7)</td>
<td>0.1720</td>
</tr>
<tr>
<td>Treatment*Genotype</td>
<td>0.04(1,6.04)</td>
<td>0.8431</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td>3.74(1,63.7)</td>
<td>0.0567</td>
</tr>
<tr>
<td>Genotype*Time</td>
<td>4.95(1,63.7)</td>
<td>0.0296</td>
</tr>
<tr>
<td>Treatment<em>Genotype</em>Time</td>
<td>2.91(1,63.7)</td>
<td>0.0928</td>
</tr>
</tbody>
</table>
Table 2.6: Experiment 1-Hormones

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estradiol (ln estradiol)</th>
<th>Cortisol (ln cortisol)</th>
<th>11Ketotestosterone (ln KT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(Num DF, Den DF)</td>
<td>P-value</td>
<td>F(Num DF, Den DF)</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.84(1,8.45)    0.0838</td>
<td>0.01(1,9.58)    0.9237</td>
<td>1.75(1,10.2)   0.2146</td>
</tr>
<tr>
<td>Genotype</td>
<td>2.78(1,5.5)     0.1512</td>
<td>0.01(1,6.25)    0.9195</td>
<td>0.11(1,7.16)   0.7532</td>
</tr>
<tr>
<td>Time</td>
<td>29.68(1,63.9)   &lt;0.0001</td>
<td>0.26(1,63.5)    0.0021</td>
<td>0.02(1,60)     0.8791</td>
</tr>
<tr>
<td>Treatment*Genotype</td>
<td>0.22(1,5.87)    0.6561</td>
<td>0.01(1,6.66)    0.9364</td>
<td>0.11(1,7.55)   0.7500</td>
</tr>
<tr>
<td>Treatment*Time</td>
<td>1.62(1,65.3)    0.2082</td>
<td>0.17(1,65)      0.6846</td>
<td>0.00(1,62.3)   0.9537</td>
</tr>
<tr>
<td>Genotype*Time</td>
<td>0.55(1,63.7)    0.4598</td>
<td>0.05(1,63.3)    0.8251</td>
<td>0.50(1,59.5)   0.4809</td>
</tr>
<tr>
<td>Treatment<em>Genotype</em>Time</td>
<td>3.83(1,65.2)</td>
<td>0.0547</td>
<td>0.19(1,64.9)</td>
</tr>
<tr>
<td>Standard Length</td>
<td>5.29(1,68.2)    <strong>0.0245</strong></td>
<td>0.09(1,66.9)    0.7634</td>
<td>0.01(1,60.9)   0.9166</td>
</tr>
<tr>
<td>Total Handling time</td>
<td>0.19(1,38.2)    0.6666</td>
<td>0.57(1,40.1)    0.4530</td>
<td>2.66(1,34.9)   0.1119</td>
</tr>
</tbody>
</table>
Experiment 2

There were significant effects of status, time, and genotype x status on standard length (Table 2.7). Standard length increased over time and dominants were larger than subordinates or isolates (time effect, status effect – Table 2.7, Figure 2.5). For both genotypes, dominants were significantly larger than isolates (linear contrasts; DAN2K: $F_{1,186}=30.1$, $P<0.0001$; RHL: $F_{1,180}=99.5$, $P<0.0001$) and subordinates (linear contrasts; DAN2K: $F_{1,167}=48.8$, $P<0.0001$; RHL: $F_{1,168}=70.9$, $P<0.0001$)(Figure 2.6). Subordinates and isolates did not differ significantly in size for either genotype (linear contrasts; DAN2K: $F_{1,186}=3.2$, $P=0.07$; RHL: $F_{1,181}=3.4$, $P=0.07$)(Figure 2.6). Fish increased in size over time, but DAN2K were significantly smaller than RHL in both dominant (linear contrasts: $F_{1,47.3}=4.92$, $P=0.03$) and subordinate (linear contrasts: $F_{1,41.2}=4.91$, $P=0.03$) but not isolate (linear contrasts: $F_{1,32.1}=0.1$, $P=0.76$) status classes.

There were significant effects of genotype, time, genotype x time, and status x time on latency to approach the mirror (Table 2.8). DAN2k had a higher latency to approach the mirror than RHL (genotype effect – Table 2.8) and latency decreased from time 1 to time 2 (time effect - Table 2.8). Dan2k showed a significant decrease in latency to approach the mirror between time 1 and time 2 (linear contrasts: $F_{1,73.7}=9.15$, $P=0.003$), while RHL remained at a steady low latency at both timepoints (linear contrasts: $F_{1,74}=0.05$, $P=0.8319$; Figure 2.7). There were no significant differences between statuses at time 1 (linear contrasts, day70; dominant vs subordinate: $F_{1,82.5}=1.28$, $P=0.262$; dominant vs isolate: $F_{1,84.5}=0.02$, $P=0.887$; subordinate vs isolate: $F_{1,75.2}=2.54$, $P=0.115$; Figure 2.7), but at time 2 subordinates showed significantly lower latency to approach the model than isolates and dominants showed a similar nonsignificant trend relative to isolates (linear contrasts, day77; dominant vs subordinate: $F_{1,81}=0.18$, $P=0.671$; dominant vs isolate: $F_{1,85.9}=2.79$, $P=0.099$; subordinate vs isolate: $F_{1,75.1}=6.74$, $P=0.011$; Figure
2.7); this was driven by decreases in latency among subordinates and dominants and lack of change for isolates (Figure 2.7). PC1 showed a significant effect only for status*time (status*time effect-Table 2.8). This was driven by a larger PC1 score for isolates than subordinates at 77 days (linear contrasts, dominant vs isolate time 2; F_{1,75.1}=10.32, P=0.0010; Figure 2.8). PC2 showed no significant effects (Table 2.8). No significant effects were observed for estradiol or cortisol, but genotype did have a significant effect on ketotestosterone levels (Table 2.9), with Dan2k having significantly higher levels than RHL (genotype effect - Table 2.9). There were no correlations between behavior, hormones, and size.
FIGURE 2.5: Experiment 2-Effect of status on standard length
FIGURE 2.6: Experiment 2-Effect of genotype and status on standard length

Asterisk * denotes statistically significant difference at P>0.05
FIGURE 2.7: Experiment 2-Latency to approach mirror

a) Effect of genotype on latency to approach mirror
b) Effect of social status on latency to approach mirror

FIGURE 2.8: Experiment 2-Effect of status on PC1
TABLE 2.7: Experiment 2-Standard length

<table>
<thead>
<tr>
<th>Effect</th>
<th>$F_{(\text{Num DF, Den DF})}$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status</td>
<td>75.02(2, 178)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Genotype</td>
<td>3.16(1, 18.2)</td>
<td>0.0922</td>
</tr>
<tr>
<td>Time</td>
<td>112.30(3, 167)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Status*Genotype</td>
<td>4.48(2, 178)</td>
<td>0.0126</td>
</tr>
<tr>
<td>Status*Time</td>
<td>0.60(6, 167)</td>
<td>0.7278</td>
</tr>
<tr>
<td>Genotype*Time</td>
<td>0.41(3, 167)</td>
<td>0.7463</td>
</tr>
<tr>
<td>Status<em>Genotype</em>Time</td>
<td>0.32(6, 167)</td>
<td>0.9265</td>
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</tbody>
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TABLE 2.8: Experiment 2-Behavior

<table>
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<tr>
<th>Effect</th>
<th>$F_{(\text{Num DF, Den DF})}$</th>
<th>P-value</th>
<th>$F_{(\text{Num DF, Den DF})}$</th>
<th>P-value</th>
<th>$F_{(\text{Num DF, Den DF})}$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td></td>
<td></td>
<td>PC2</td>
<td></td>
<td>Latency to approach mirror</td>
<td></td>
</tr>
<tr>
<td>Status</td>
<td>2.08(2, 80.8)</td>
<td>0.1314</td>
<td>1.01(2, 82)</td>
<td>0.3672</td>
<td>0.58(2, 85.3)</td>
<td>0.5631</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.45(1, 21.8)</td>
<td>0.5117</td>
<td>0.35(1, 82)</td>
<td>0.5552</td>
<td>5.14(1, 16.8)</td>
<td>0.0369</td>
</tr>
<tr>
<td>Time</td>
<td>2.70(1, 77)</td>
<td>0.1043</td>
<td>0.25(1, 82)</td>
<td>0.6166</td>
<td>5.14(1, 77.6)</td>
<td>0.0262</td>
</tr>
<tr>
<td>Status*Genotype</td>
<td>0.4(2, 75.9)</td>
<td>0.6691</td>
<td>1.19(2, 82)</td>
<td>0.3097</td>
<td>0.63(2, 79.6)</td>
<td>0.5331</td>
</tr>
<tr>
<td>Status*Time</td>
<td>4.34(2, 68.6)</td>
<td>0.0167</td>
<td>1.67(1, 82)</td>
<td>0.2416</td>
<td>4.50(2, 69.1)</td>
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<tr>
<td>Genotype*Time</td>
<td>2.0(1, 69.5)</td>
<td>0.1592</td>
<td>1.45(2, 82)</td>
<td>0.1995</td>
<td>4.92(1, 68.9)</td>
<td>0.0298</td>
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<tr>
<td>Status<em>Genotype</em>Time</td>
<td>0.24(2, 68.8)</td>
<td>0.7877</td>
<td>0.20(2, 82)</td>
<td>0.8175</td>
<td>1.04(2, 68.6)</td>
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<tr>
<td>Standard Length</td>
<td>3.07(1, 65.7)</td>
<td>0.0842</td>
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<td>0.6631</td>
<td>0.27(1, 85)</td>
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Table 2.9: Experiment 2-Hormones

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<th>Effect</th>
<th>Estradiol (log(_{10})estradiol)</th>
<th>P-value</th>
<th>Cortisol log(_{10})(cortisol)</th>
<th>P-value</th>
<th>Ketotestosterone log(_{10})(KT)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(F_{(\text{Num DF}, \text{Den DF})})</td>
<td></td>
<td>(F_{(\text{Num DF}, \text{Den DF})})</td>
<td></td>
<td>(F_{(\text{Num DF}, \text{Den DF})})</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>0.10(2, 45)</td>
<td>0.9089</td>
<td>1.85(2, 45)</td>
<td>0.1683</td>
<td>2.30(2, 45)</td>
<td>0.1123</td>
</tr>
<tr>
<td>Genotype</td>
<td>0.24(1, 45)</td>
<td>0.6270</td>
<td>0.30(1, 45)</td>
<td>0.5891</td>
<td>7.78(1, 45)</td>
<td>0.0077</td>
</tr>
<tr>
<td>Treatment*Genotype</td>
<td>0.70(2, 45)</td>
<td>0.5017</td>
<td>0.44(2, 45)</td>
<td>0.6476</td>
<td>2.77(2, 45)</td>
<td>0.0731</td>
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<tr>
<td>Standard Length</td>
<td>1.51(1, 45)</td>
<td>0.2250</td>
<td>3.28(1, 45)</td>
<td>0.0768</td>
<td>0.00(1, 45)</td>
<td>0.9658</td>
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</tbody>
</table>
Discussion

I hypothesized that social environment would have a significant effect on behavior, morphology, and physiology in mangrove rivulus. I found that social environment did increase body size and flexibility in aggressive response. Interestingly, there were no significant effects of social environment on baseline hormone levels. The two genotypes I studied responded differently to social environment, emphasizing the importance of interactions between genes and environment in producing final phenotype.

In both experiments, I found that fish kept communally grew larger than fish kept in isolation. In the second experiment, dominants in communal tanks were much larger, while subordinates were the same size as isolates. This is similar to the pattern of growth observed in juveniles of a social cichlid *Pelvicachromis taeniatus*, where group-housed individuals grew more than isolates, but isolates grew more than the smallest individuals in groups (Hesse and Thünken 2014). The larger size observed in communal individuals are interesting, as group-housed individuals compete for food and interact aggressively with each other. Avoidance of these potential costs did not appear to benefit isolated fish, perhaps because food was supplied in sufficient quantities that social fish were not limited by food acquisition or energy expenditure. Size differences between communal and isolated fish were apparent by the first observation (Experiment 1, day 51; Experiment 2, day 42), indicating that social environment started to affect growth early in development. Due to concerns about excessive disturbance affecting fish growth, this study did not examine growth trajectories with earlier time points. An experiment that sacrificed fish and measured length and mass beginning early in development would make it possible to determine, with greater resolution, how the social environment affects growth trajectories between hatching and 40-50 d of age.
In experiment 2 I also found that dominant fish were significantly larger than isolated fish at all time points. Low social status decreases growth rate in territorial male cichlids (Hofmann et al. 1999), and stress can cause reductions in growth (Barton 2000, Jentoft et al. 2005). Take together, this may indicating that isolated and subordinate fish exhibited decreased growth due to greater stress. Although baseline cortisol levels were not significantly higher in isolated and subordinate fish than in dominants, our experimental procedure did not measure the sensitivity of animals to cortisol or post-encounter cortisol levels, which could drive differences in behavioral responses even in the absence of differences in baseline cortisol levels (Earley et al. 2006).

Rivulus also exhibited changes in behavior in response to social environment. Communal animals showed a reduction in aggression between the first and second behavioral tests of trial 1, but an increase in aggression between the first and second behavioral tests in trial 2. Although the direction of change was different between experiments, in both cases communal fish showed increased flexibility in aggressive behavior when compared to isolated individuals. Social isolation can decrease the ability of animals to modulate their behavior in response to aggressive interactions, often increasing aggression (Earley et al. 2006, Hesse and Thünken 2014, Veenema 2009). In experiment 2, subordinate individuals showed a greater decrease in aggression between timepoints than dominant individuals, agreeing with a prior study which found that losers, but not winners, had the ability to alter their aggressive behavior during contests (Earley et al. 2006). Measurements of exploratory behavior did not change based on social environment, indicating that those behaviors form a separate module from social behaviors.

The response of social rivulus to aggression tests differed between experiments. When presented with a model (experiment 1), rivulus showed a trend toward decreasing aggression between the first and second experiment. When presented with a mirror (experiment 2), they
increased aggression. The reason for this variable response may be the differing stimuli offered by models and mirrors. Models are inert and do not exhibit any behavior, while mirror images reflect the aggressive behavior of the target fish. As a result, interactions between rivulus and mirrors escalate more than interactions between rivulus and models (Earley et al. 2000). As a result, social rivulus may have interpreted model fish as less threatening or aggressive than mirrors images, and modified their behavior in the next encounter to match the perceived threat from the first encounter. This is consistent with the idea that social experience during development leads to increased capability to respond with appropriate level of aggression to different social situations (Fernald 1980, Veenema 2009, Hesse and Thünken 2014, Toth et al. 2011).

Both experimental trials included two genetic lines, Dan2K and RHL, so that I could compare reaction norms between different genotypes. This experiment confirms that these genotypes respond differently to social environment. Dan2K, but not RHL, showed changes in behavior between timepoints in both experiment 1 and 2. This indicates that Dan2K are more flexible than RHL in response to aggressive interactions. Dan2K also exhibited different growth patterns than RHL in experiment 2. Communally housed Dan2K (both dominant and subordinate) were smaller than communally housed RHL, but isolated fish of both genotypes were the same size. In this case, plasticity in response to social environment exposes cryptic variation in body size that would not be visible if all fish were isolated. Exposure of cryptic variation in response to environmental change is one mechanism by which plasticity may produce rapid evolutionary change in response to selection (Foster 2013).

The results of my experiments show conclusively that early social environment can have significant impacts on behavior and morphology, and that the reaction norms of the genotypes I
examined differed. This research shows that social environment can be important even in
mangrove rivulus, a species that is not usually considered social (Mackiewicz et al. 2006). This
should be remembered when using these species as model organisms. Other questions still
remain to be asked about this system. The two genotypes used in this experiment were chosen
based on availability, not for their traits or the location from which they were collected.
Comparing the reaction norms of genotypes from different locations would provide insight into
how reaction norms have evolved in different populations, while comparing reaction norms from
genotypes known to follow different life history strategies could show if plasticity responses
differ between genotypes that put more effort into growth vs. genotypes that put more effort into
reproduction. The fish exhibited different behavioral responses to mirrors and models in this
experiment, perhaps due to the differing threat levels provided by these experiences. Those
matched against a mirror experienced an opponent that matched their aggression, and thus may
have been perceived as more threatening than the nonresponsive model. Mangrove rivulus could
be presented with larger or smaller conspecifics to induce winning or losing experiences and
determine if communally housed individuals are also able to adjust their aggressive behavior to
more ecologically relevant scenarios involving live opponents.
Literature


Hsu, Y., Huang, Y. Y., & Wu, Y. T. (2014). Multiple contest experiences interact to influence each other’s effect on subsequent contest decisions in a mangrove killifish. *Animal Cognition, 17*(2), 165-175.


Taylor, D. S. (2012). Twenty-four years in the mud: what have we learned about the natural history and ecology of the mangrove rivulus, Kryptolebias marmoratus?. *Integrative and Comparative Biology, 52*(6), 724-736.


CHAPTER 3

PHENOTYPIC FLEXIBILITY IN NEST CONSTRUCTION IN RESPONSE TO THE PRESENCE OF EGG PREDATORS

Behavioral and phenotypic flexibility allows animals to react appropriately to changing environmental conditions (Piersma and Drent 2003). Flexibility can provide fitness dividends in variable environments when animals have good information about their environment and can rapidly and adaptively alter their behavior or physical phenotype (Gabriel et al. 2005). Flexibility is not only important on the level of individual fitness, but also can influence large-scale ecological processes, determining which species tolerate environmental change or invade new environments (Wright et al 2010). Behavioral flexibility produces flexibility in the extended phenotype of animals, including the morphology and location of the structures that they build (Lima 2009, Lynch 1974, Schaedelin and Taborsky 2009). Nests are among the most widespread of animal structures (Hansell 2005), and are very important because they provide a habitat for young during the vital first days of life when mortality is often very high (Hansell 2005). As a result of this, variability in nest construction and placement can have large impacts on the survival of offspring (Martin 1993, Mori and Saito 2004), and many species exhibit flexibility in nest construction and placement in response to risk of predation (Caldwell 1992, Lima 2009, Jones and Reynolds 1999, Spencer and Thompson 2003).

The presence of other organisms, particularly potential predators, is among the most important environmental components determining nest construction and placement. Many animals have nests with characteristics that clearly serve to deter predation, either on adults or
offspring. For example, sticklebacks (Candolin and Voigt 1998) and turtles (Spencer and Thompson 2003) establish their nests in locations that strategically reduce predation risk on adult nest-makers, while warblers (Forstmeier and Weiss 2004, Peluc et al. 2008) and neotropical frogs (Murphy 2003) establish nests in locations that reduce predation on offspring. These countermeasures sometimes have trade-offs that cause nests to be placed in otherwise suboptimal positions or constructed in otherwise suboptimal ways (Forstmeier and Weiss 2004, Jones and Reynolds 1999, Tieleman et al. 2008). For example, dusky warblers (Phylloscopus fuscatus) place nests in colder microclimates higher off the ground and farther from food sources, but only when predators are present. As a result, many organisms exhibit flexibility in nest placement or construction, allowing them to maximize fitness by using costly countermeasures only in response to the presence of predators (Forstmeier and Weiss 2004).

I examined nest building in the longear sunfish *Lepomis megalotis* to determine how the presence of egg predators affects nest location and size. Male longear sunfish dig shallow, disc shaped depressions in which females lay eggs that are then guarded by males (Gill 1906, Witt and Marzolf 1954). Sunfish nests are subject to a variety of egg predators, including conspecifics (Keenleyside 1972). I tested nest-building behaviors in response to juvenile longear sunfish, which are known predators of eggs (pers. obs.), but are not dangerous to adult sunfish. I provided adults with the opportunity to build nests closer to cover (aquatic plants) or further from cover and tracked the size and number of nest excavation areas produced. Nests placed closer to cover provide greater concealment for the nest, but also reduce visibility, making it easier for potential predators to approach undetected (Götmark et al. 1995, Magana et al. 2010). Because juveniles can be driven off if observed, I hypothesized that sunfish would position their nests further from cover when egg predators were present to maximize visibility of potential intruders. I also
hypothesized that individuals would dig fewer, smaller nests in high-predation treatments, because the costs of defending nests and eggs would be higher when predators are present, reducing the expected benefit of reproduction and increasing the difficulty of defending nests.

Methods

Experimental Arenas

Nest building arenas (n=16) were constructed from blue plastic pools 80 cm in diameter and 17 cm deep. The substrate of the pools was a 1cm deep layer of gravel. Pools were delineated into two sections by a nylon string running in a chord 30cm from one side, marking off two sections of the pool - a larger gravel-filled area and a smaller area filled with aquatic plants (Anachris sp.). A total of 16 pools were used, filtered in blocks of 8 by two separate filtration systems. Each filtration system was equipped with a sump filter containing fibrous filter pads and activated carbon, as well as a chiller and heaters for temperature control. Arenas were housed in a heated greenhouse containing metal-halide lights, which provided supplemental lighting on a 14 h light: 10 h dark photoperiod, to mimic summertime light conditions and stimulate breeding behavior, which is dictated by light cycle (Smith 1970). Water temperature fluctuated on a diurnal cycle, varying between 20 and 26ºC. Ammonia and nitrite levels were kept at 0, and nitrates below 20 ppm by large water changes between experimental trials.

Experimental Trials

Male longear sunfish were photographed and placed in the pools. Half of the pools also contained 5 juvenile longear sunfish less than 7cm in length. Sunfish were allowed to acclimate
for 5 days with (n=17) or without (n=18) juvenile sunfish. At the end of this time period, a female sunfish was introduced. Previous observations had shown that the addition of a female stimulates nest building in males (unpublished pers. obs.). Females were left in the pools for two days. Each pool was photographed twice, once on each day the female spent in the pool. Photographs were taken in the evening (1700-1900 h), the nests were photographed with a US quarter placed adjacent to the nest for scale. Sunfish nests were easily visible as the thin layer of gravel was displaced by the sunfish’s digging efforts, allowing the blue bottom of the tubs to become visible. After two days, fish were removed from experimental chambers and chambers were cleaned. Between experimental trials fish were kept communally in 750L holding tanks filtered by trickle - filters, with males, females, and juveniles in separate tanks. These tanks were in the same room and under the same lighting and temperature conditions as the experimental pools, and water quality was maintained by changing water as necessary. These tanks included broken pottery and aquatic vegetation as refuges. Fish were fed daily in the afternoon ad libitum with a mixture of frozen Artemis and chironomid larvae. Each male and female was used twice, once in each treatment (predator or no predator), while new juveniles were drawn the pool of juvenile individuals for each trial replication.

**Image analysis**

Images were analyzed with ImageJ (http://imagej.nih.gov/ij/). I identified nests in each pool (multiple nests were sometimes dug in a single pool). The area of each nest was measured using the freehand and wand tools in ImageJ to obtain the area of blue showing through the gravel. The area of all nests in a pool was summed to provide a measure of total digging effort. I also measured the shortest distance from the center of the cleared area of each nest to the vegetated side of the pool as well as the shortest distance to the nearest wall (all nests were
constructed on the side of the pool containing no vegetation). The measure 'minimum distance from vegetation' was the minimum distance between any nest in the pool and the vegetated area. The measure 'minimum distance from structure' was the minimum distance between any nest in the pool and either the pool wall or vegetated area.

**Statistical analysis**

I used a non-parametric Wilcoxon rank sums test performed in JMP version 10 to determine if there were significant differences between treatments in nest number, total area of all nests, minimum distance from vegetation, and minimum distance from nearest structure. Because this statistical test does not support repeated measures, nest construction on days 1 and 2 were analyzed separately.

To determine whether the likelihood of nest construction changed as a function of predator presence/absence, I used a repeated measures analysis on categorical data (nest presence or absence) using the Glimmix procedure in SAS (version 9.1). Because the experiment had two mutually exclusive outcomes (nest presence or absence) I used a binary response distribution and a logit link function. The model included treatment (predators or no predators) and day (1 or 2), along with the interaction between these factors. Tank ID was included as a random factor.

**Results**

Predation had a non-significant but biologically interesting effect on nest production, while there was no effect of day or the interaction between treatment and day (Table 3.1). In the presence of nest predators 17.65% of fish constructed a nest, while 36.11% constructed a nest in the absence of nest predators (Figure 3.1).
I found no significant effect of treatment on nest number, total area of all nests, minimum distance from vegetation, or minimum distance from nearest structure (Table 3.2).
### TABLE 3.1: Effect of nest predators on the probability of nest construction

<table>
<thead>
<tr>
<th>Effect</th>
<th>$F_{(\text{Num DF, Den DF})}$</th>
<th>$P$-value</th>
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<tr>
<td>Treatment</td>
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</tr>
<tr>
<td>Day</td>
<td>0.04$_{(1,66)}$</td>
<td>0.8375</td>
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### TABLE 3.2: Effect of nest predators on nest construction and location

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</tr>
</thead>
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<td>Number of nests Day 1</td>
<td>-0.9923</td>
<td>0.3211</td>
</tr>
<tr>
<td>Number of nests Day 2</td>
<td>-1.4336</td>
<td>0.1517</td>
</tr>
<tr>
<td>Minimum distance Day 1</td>
<td>0.0000</td>
<td>1.0000</td>
</tr>
<tr>
<td>Minimum distance Day 2</td>
<td>-0.6864</td>
<td>0.4941</td>
</tr>
<tr>
<td>Distance to Vegetation Day 1</td>
<td>-0.3873</td>
<td>0.6985</td>
</tr>
<tr>
<td>Distance to Vegetation Day 2</td>
<td>-0.6836</td>
<td>0.4941</td>
</tr>
<tr>
<td>Area Day 1</td>
<td>-0.1291</td>
<td>0.8973</td>
</tr>
<tr>
<td>Area Day 2</td>
<td>0.6838</td>
<td>0.4941</td>
</tr>
</tbody>
</table>
FIGURE 3.1: Probability a nest would be constructed in each treatment on day 1 and 2

Error bars show standard error of the mean
Conclusions

I hypothesized that the presence of juvenile conspecific nest predators would cause adult male longear sunfish to alter nest construction and location. However, my results largely failed to support this hypothesis, and instead showed that sunfish did not modify nest location, number, or area based on the presence of predators. Sunfish were less likely to construct nests in the presence of predators, but this reduction was statistically non-significant. The sample size in this experiment was small due to difficulties in acquiring suitable experimental animals, and this hindered our ability to detect potential effects. A power test showed that increasing sample size to 100 would be required to detect an effect of the size we observed, and the lack of statistical significance may not be reflective of a lack of biological significance but instead may reflect a lack of sample size.

It is reasonable that male sunfish would be less likely to construct nests in the presence of egg predators if those predators are able to reduce the economic defendability of the nest. For an organism to benefit from defending a territory, the benefit gained must outweigh the costs of defense (Brown 1964). The presence of egg predators may increase the probability that offspring will be lost or that the nest will fail entirely, reducing the benefits to be gained by constructing and defending a nest. Even if sunfish are able to successfully defend their nests, the need for increased defensive activity may result in increased energy expenditure by the parental male, raising the costs of defense. Because of these increased costs, individuals that reduce nesting efforts when predators are present – that is, individuals with a flexible behavioral phenotype - may experience higher long-term fitness despite sacrificing immediate fitness returns. Similar reductions in nesting have been seen in other species when predators were prevalent; red-backed shrikes (*Lanius collurio*) show decreased nesting in areas near nest predator species, and prey
birds nest at lower rates in European kestrel (*Falco tinnunculus*) territories (Roos and Pärt 2004, Suhonen et al. 1994).

Although sunfish decreased the probability that a nest would be constructed, they did not reduce the effort they spent on nest construction. This failure to vary nesting effort could occur because once a fish has committed to nesting, particular nest sizes or distances from cover are optimal whether or not predators are present. For example, a certain nest area might be optimum for attracting females or fanning eggs, or a certain distance from cover might be preferred to allow adult males to avoid predatory attacks. However, the variability observed in these traits seems to indicate that nests are not constrained to a narrow range of characteristics. It is also possible that the small sample size of nests constructed obscured any pattern in construction; few individuals (18-39%) constructed nests, so only a subset of the data could be used in this analysis.

I hypothesized that sunfish would establish nests further from cover in high predation treatments, and that this would serve to reduce the opportunity for nest predators to approach the nest undetected. An alternative possibility was that sunfish might establish nests closer to cover to reduce the probability that the nest was detected. However, sunfish showed no tendency to build nests either closer to cover or further from cover. This could mean that cover does not affect the success of nest predators attempting to access the nest. Alternatively, nesting arenas may have been small enough that all locations were considered “close to cover” by sunfish.

A reduction in the probability of nest construction by longear sunfish when juvenile sunfish are common could have implications for sunfish population dynamics in the wild. Observations in other systems have made it clear that reactions by prey to potential predators can
play a larger ecological role than actual mortality due to predation (Fill et al. 2012, Ripple and Beschta 2004, Schimits et al. 2004). This “ecology of fear' can buffer predator-prey interactions and structure ecosystems by allowing relatively small numbers of predators to have a large effect (Brown et al. 1999). If the presence of juvenile sunfish reduces reproductive output of adults by deterring adults from constructing nests, then population growth might be limited at lower populations than would otherwise be expected as adults reduce reproductive rates when the population of juveniles rises. Alternatively, sunfish could compensate for the presence of nest predators by altering nesting behavior in ways not measured in this experiment, such as by clustering nests together in colonies or nesting in different areas. Colonial nesting appears to have an antipredator function in some Lepomis species (Dominey 1981, Gross and MacMillan 1981). However, a study of Lepomis megalotis found that both colonial and noncolonial nests suffered similar amounts of nest losses due to predation (Jennings 1991), so more remains to be learned about the nature of antipredator tactics in this species. It would be useful to assess the effects of juveniles in a more natural environment by stocking ponds with different densities of juvenile sunfish and adults, and observing changes to nest location, structure, and population density in a more natural situation.
References


CHAPTER 4

THE ROLE OF HETEROSPECIFIC EAVESDROPPERS IN THE EVOLUTION OF SEXUAL SIGNALING

Sexually selected structures are often very conspicuous, consisting of bright colors (Olson and Owens 1998), elaborate structures (Emlen et al. 2005), loud calls (Searcy and Andersson 1986), distinctive movements (Byers et al. 2010), or other traits that attract mates of the opposite sex or deter competitors of the same sex (Andersson and Simmons 2006, Jones and Ratterman 2009). The form of these sexual signals is thought to reflect a balance between intrasexual or intersexual selection favoring more conspicuous signals and selection by heterospecific eavesdroppers (hereafter, ‘eavesdroppers’; see glossary below) favoring less conspicuous signals. Empirical and theoretical studies have focused on how eavesdropping predators and parasites can favor the evolution less of elaborate sexual signals (Endler 1980, Haynes and Yeargan 1999, Komarova and Levin 2010, Peake 2005, Zuk and Kolluru 1998, Zuk et al. 2006).

Heterospecific eavesdropping is observed across a wide variety of taxa; even fungi eavesdrop on sexual signals, producing sticky nets to trap soil nematodes when their pheromones are detected (Hsueh et. al. 2013). These eavesdroppers all fit a similar mold: they are predators or parasites that are attracted to conspicuous traits and that exert negative fitness effects on the signaler (see Table 4.1). We contend that this focus is too narrow. Eavesdroppers may be repelled by sexual signals as well as attracted by them, and eavesdroppers can have positive as well as negative effects on signaler fitness. It is not safe to assume that eavesdroppers always penalize
conspicuous signalers. Our ideas about how eavesdroppers mediate the evolution of sexual signals may need to be revised.

There is reason to expect that these ‘other’ eavesdroppers are more prevalent than is currently appreciated and thus, their potential effects have not often been considered in theoretical models focused on the evolution of sexual signals. Predators and parasites respond to some sexual signals by avoiding them, rather than approaching them. Conspicuous signalers exposed to this type of eavesdropping would have enhanced, rather than diminished, fitness. The dewlap displays of anoles deter predatory snakes and serve as sexual signals (Driessens et al. 2014, Leal and Rodriguez-Robles 1997), and the flashes of fireflies deter bat predators and attract mates (Moosman et al. 2008). In some systems, prey will eavesdrop on sexual signals emitted by predators and parasites. Hosts of parasitic cowbirds avoid building nests in areas where cowbird songs are played (Forsman and Martin 2009), spiders exhibit antipredator behaviors when the songs of predatory birds are played (Lohrey 2009), and various species exhibit antipredator behavior when exposed to scent markings of predators (Amo et al. 2008, May et al. 2012). Eavesdropping also occurs between species that utilize the same environment, and can influence habitat selection (Fletcher 2007, Mönkkönen and Forsman 2002). For example, competitively dominant least flycatchers are attracted to the songs of redstarts, but many other small migratory birds avoid the songs of flycatchers (Fletcher 2007, 2008). These kinds of behaviors result from eavesdropping on heterospecific signals - behavior is modified in response to information obtained from the sexual signals of heterospecifics – but their potential effects on the evolution of sexual signals has been sorely understudied. The fitness effects imposed by eavesdroppers that avoid sexual signals also may be underestimated; because they avoid signalers, it is inherently more difficult to observe them eavesdropping in the wild.
If we are to fully understand the magnitude and direction of evolutionary change of sexual signals in response to selection imposed by heterospecific eavesdroppers, a unified approach that accounts for all types of eavesdroppers is needed. This approach must identify how eavesdroppers respond to sexual signals, the ecological relationships between signalers and eavesdroppers, and how these factors interact to exert selection on sexual signaling. We describe the full array of possible categories of eavesdroppers, most of which have been understudied, and the selection pressures they could impose, to explain patterns of evolution in sexual signals that do not make sense when considering only those eavesdroppers that are attracted to sexual signals and that exert negative fitness effects on the signaler.
TABLE 4.1: A diversity of eavesdroppers

We examined 85 eavesdropping systems documented in the literature. The table illustrates how many eavesdroppers of each type were documented, while the number in parentheses indicates how many studies actually examined the effect of eavesdropping on the evolution of sexual signals. It is common for studies to document that eavesdropping is occurring and to document behavioral changes in signaling by individuals in the presence of eavesdroppers, but experimental or field studies that document changes in signaler fitness are rare and mostly limited to predatory eavesdroppers.

<table>
<thead>
<tr>
<th>Eavesdropper category</th>
<th>Response to sexual signal</th>
<th>Class of Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predator/Parasite</td>
<td>Approach</td>
<td>Class 1</td>
</tr>
<tr>
<td></td>
<td>64 (2)</td>
<td>67 (2)</td>
</tr>
<tr>
<td>Prey</td>
<td>Retreat</td>
<td>Class 2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Competitor</td>
<td>Assess</td>
<td>Class 3</td>
</tr>
<tr>
<td></td>
<td>3 (1)</td>
<td>3 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Class 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent systems where selective effects on the signaler were explicitly studied

Note: some systems could not be assigned definitively to a category and are excluded from some columns.
Glossary

**Magnitude:**

Variation in sexual signal phenotype that can be numerically arranged along an axis from “low” values below the population mean to “high” values above the population mean. Changes in size, volume, frequency, chemical concentration, length, and amplitude all can be considered examples of change in magnitude. Physiological costs, reproductive benefits, and signal detectability often, but not always, increase with magnitude.

**Eavesdropping:**

Following Peake (2005), we define eavesdropping as “the use of information in signals by individuals other than the primary target.” This is a broad definition that makes no assumptions about the sensory modality through which the signal is perceived and allows that eavesdroppers may not always impose a cost on signalers.

**Baseline benefits and costs:**

The benefits and costs of sexual signaling that are not imposed by interactions with eavesdroppers. Baseline benefits of a signal include attracting more mates or deterring competitors of the same species. Baseline costs include the physiological costs of producing and maintaining a signal.

**Signaler and Eavesdropper**

Our model considers the interaction between two types of organism: signalers and eavesdroppers. Signalers produce sexual signals and eavesdroppers respond to those signals and affect signaler fitness. However, not all individuals of the signaler species are actively engaged
in producing sexual signals at all times, and individuals of the eavesdropper species may encounter signalers even when sexual signals are not being produced. It is important to consider interactions between signalers and eavesdroppers in the absence of signaling so that these can be compared to interactions when signaling and eavesdropping are occurring. In some cases, most interactions may happen when no signaling is occurring, because eavesdroppers avoid actively signaling signalers.

*Interaction*

Any form of encounter between an eavesdropper and signaler that alters signaler fitness. Interactions are not necessarily the result of eavesdropping on a signal – eavesdropper and signaler also may interact with each other when no signaling is occurring if they stumble upon signalers randomly or track them down using other cues. Eavesdropping may increase the likelihood that an interaction will take place by attracting eavesdroppers to signalers, or decrease the likelihood that an interaction will take place by causing eavesdroppers to avoid signalers that they would have otherwise stumbled upon or attacked. Interactions may be positive or negative from the perspective of the signaler. For example, signalers have negative interactions with predatory eavesdroppers, but positive interactions with prey.
A conceptual framework for eavesdropper-driven selection on sexual signals

We forward a conceptual framework that illustrates how eavesdroppers alter the economics of sexual signaling, a unique selection pressure that might drive the evolutionary response of sexually selected traits towards different optima than would be expected under baseline (eavesdroppers absent) conditions (Figure 4.1).

Baseline costs and benefits change as signal magnitude increases (Johnstone 1995). Baseline benefits increase because these signals are easier for conspecifics to detect and are either more attractive to mates or intimidating to rivals (Kujipur et. al. 2012). These benefits are subject to diminishing returns; receivers are less able to perceive differences between high magnitude signals (Weber's law; Akre and Johnsen 2014) and signalers eventually approach their maximum reproductive potential. Production and maintenance costs also increase as signal magnitude increases, but the exact nature of this increase depends on the type of signal. If the signal is a physical structure or is produced by a physical structure, the cost of producing the structure will increase in a manner proportional to the dimensionality of the structure. For linear structures like tails or ribbon feathers, costs will increase on a roughly linear scale. For 2 dimensional structures like fans or color patches on the skin, costs will square for each increase in magnitude, while costs will increase on a cubic scale for 3 dimensional structures. If the signal is audible, the cost will increase by a factor of 10 for each 10-decibel increase. This occurs because the decibel scale is logarithmic, and the energy contained in a sound increases by a factor of ten as volume increases by ten decibels. We choose an intermediate representation with costs increasing proportionally to the square of signal magnitude. The combination of diminishing returns and increasing costs prevent the magnitude of sexual signals from increasing
indefinitely (Akre et. al. 2011). Peak net fitness occurs at a signal magnitude where benefits outweigh costs by the greatest margin (Figure 4.1).

Eavesdroppers add costs or benefits to the baseline costs and benefits described above, changing the fitness curve. The direction of the change depends on two factors: 1) whether high or low magnitude signalers are most likely to interact with eavesdroppers; 2) whether signalers receive costs or benefits when interacting with eavesdroppers. The first factor results from the way that eavesdroppers respond to sexual signals; their attraction to or repulsion by signals of different magnitudes causes them to interact differently with different signalers at different frequencies. The second factor results from the ecological relationship between signaler and eavesdropper; predators, competitors, and parasites impose costs on signalers, prey and mutualists provide benefits.

The two factors can be combined to produce four classes (Figure 4.2) that describe how eavesdroppers drive directional selection on sexual signals: Class 1) costs are imposed on high magnitude signalers, raising the relative fitness of low magnitude signalers; Class 2) costs are imposed on low magnitude signalers, raising the relative fitness of high magnitude signalers; Class 3) Benefits are provided to high magnitude signalers, raising their relative fitness; Class 4) Benefits are provided to low magnitude signalers, raising their relative fitness. These represent simplified cases, where eavesdropper interaction is clearly skewed toward one end or the other of the spectrum of signal magnitudes, and where eavesdroppers are always harmful or beneficial. Other, more complicated patterns are possible, but can be considered variations on the theme described here.
FIGURE 4.1: A visual model of sexual selection in the absence of eavesdroppers

This graph illustrates baseline selection in the absence of eavesdroppers. The x axis shows the magnitude of the signal, a quantity which reflects aspects of the signal such as decibel volume, length, mass, area, chemical concentration, or duration. The blue line shows the baseline benefit of signaling, such as increased mating opportunities. Benefits increase with magnitude, but face diminishing returns at higher magnitudes. The red line illustrates baseline costs, such as the physiological costs of producing signals or maintaining signaling structures. These costs also rise with increasing magnitude, but the rate of increase does not diminish. The black line illustrates the baseline fitness of signalers, produced by subtracting costs from benefits. Fitness is highest at the magnitude where benefits exceed costs by the greatest margin, and signaling at this magnitude is favored by selection.
FIGURE 4.2: A visual model of sexual selection with 4 classes of eavesdroppers

These graphs illustrate the four classes of eavesdropper driven selection on sexual signals. Dashed blue lines indicate the benefits of signaling after interactions with eavesdroppers have been added. Eavesdroppers can provide benefits in the form of mutualisms, or by serving as a food source. Dashed red lines indicate the costs of signaling imposed by eavesdroppers. These costs include increased mortality or damage from predation or parasitism, or increased competition from competitive dominants. Eavesdropper-derived costs and benefits can increase or decrease with signal magnitude depending on how eavesdroppers respond signals. Dashed black lines indicate the fitness of signalers after eavesdroppers have been included. The magnitude of signal providing the greatest net fitness changes when eavesdropper effects are included, as indicated by arrows.
Class 1:

Class 1 selection occurs when eavesdroppers interact most frequently with high magnitude signalers and have a negative effect on signaler fitness, imposing costs on the most conspicuous signalers and favoring the evolution of lower magnitude signals. Class 1 eavesdroppers are often predators or parasites that are attracted to prey sexual signals. Eavesdroppers of this type have been documented across diverse taxa and eavesdrop on signals using a wide variety of sensory modalities. Because high magnitude signalers often are more conspicuous, eavesdroppers are more likely to detect and locate them. Some eavesdroppers also show a preference for individuals that signal at high magnitude (Bernal et al. 2006, Page and Ryan 2008), further increasing the probability that high magnitude signalers will encounter eavesdroppers. Studies into the evolutionary effects of Class 1 eavesdroppers generally match our predictions, showing reductions in or even elimination of sexual signaling in populations where eavesdroppers are present (Endler 1980; Zuk et al. 2006; see Beckers and Wagner 2012 for counterexample). For example, the wing morphology required for calling to females was almost completely lost in male field crickets *Teleogryllus oceanicus* on the island of Kauai under high risk of parasitism by the eavesdropping parasitoid fly *Ormia ochracea*. Non-signaling males had higher relative fitness due to the high costs faced by signaling males, and as a result the mean signal magnitude of the population decreased precipitously (Zuk et al. 2006). Instead of completely losing a signal, other organisms may develop behavioral adaptations that allow them to reduce signaling when eavesdroppers are present (Cade et al. 1996, Remage-Healey et al. 2006).

While the consequences of eavesdropping by predators and parasites are well known, our model also predicts that Class 1 selection should occur if high magnitude sexual signals attract
competitively dominant heterospecifics. Like predators and parasites, eavesdropping competitors would lower the fitness of signalers. Animals use a variety cues, including sexual signals, provided by heterospecifics to select habitats (Fletcher 2007, Goodale et. al. 2010, Mönkkönen and Forsman 2002, Parejo et al. 2005), but more research is needed to determine the extent to which sexual signal evolution is affected by these species. Species that compete over breeding territories provide a particularly good opportunity to study eavesdropping by competitors. Sexual signals are often broadcast from breeding territories, and if they attract competitive dominants the signalers may experience reduced reproductive success. For example, least flycatchers eavesdrop on competitively inferior American redstarts and preferentially settle in territories where redstart songs are played (Fletcher 2007). If least flycatchers reduce the fitness of breeding redstarts, then selection should favor reductions in redstart singing that reduce competition, just as other organisms evolve reductions in signaling that reduces predation or parasitism.

Class 2:

Class 2 selection occurs when eavesdroppers interact most frequently with low magnitude signalers and impose the greatest fitness costs on them, thereby favoring the evolution of higher magnitude signals. Eavesdropping predators and parasites also drive Class 2 selection, but unlike Class 1 selection, their response to sexual signals causes them to interact with low magnitude signalers most. Class 2 selection requires that eavesdropping predators or parasites interact less with prey or hosts displaying high magnitude sexual signals, even though these signalers are more conspicuous and thus usually easier to locate. This seemingly counterintuitive response can occur if eavesdroppers are repelled by sexual signals, in which case organisms bearing the most conspicuous signals are most likely to be spotted and avoided. It can
also occur if eavesdroppers exhibit a preference for low magnitude signalers and actively target them.

Why would eavesdroppers be repelled by sexual signals? Some sexual signals identify chemically defended organisms that are otherwise similar to suitable prey species. For example, bats can distinguish palatable and unpalatable frogs on the basis of their vocalizations (Tuttle and Ryan 1981), and another species of bat is deterred by the flashes of fireflies (Moosman et. al. 2009). Essentially, sexual signals double as aposematic signals under this form of selection. Eavesdroppers may also be deterred by the signals themselves. Some intrasexual signals are used to intimidate conspecifics, and may even act as weapons to injure them. These signals are sometimes used to deter predators as well. For example, male frilled lizards fan out their neck display, dramatically increasing their apparent size, both when confronting other males and when facing predators (Shine 1990), and ungulates sometimes use their horns as defensive weapons as well as in male-male combat (Stankowich 2012). In these cases, more elaborate sexual signals may also be better able to deter predators. In fact, it is not always easy to determine whether the predator deterrent or sexual signaling component of a signal came first.

Eavesdroppers might also use sexual signals as an index of signaler quality, allowing them to target weak or sickly individuals unable to display high quality signals. As with the examples above, this would result fewer attacks on strong signalers. Some predators are known to preferentially prey on old or sick individuals that are easier to catch (Genovart et. al. 2010, Krumm et. al. 2010, Mesa et. al. 1994), and some prey signal their ability to evade or resist predation (FitzGibbon and Fanshawe 1988). Sexual signals can be repurposed to signal predators in this way. For example, male Jamaican anole lizards present dewlap displays that are honest signals of bite strength to both conspecific males and predatory snakes, and these displays may
be under selection by both conspecifics and heterospecifics (Driessens et al. 2014). Sexual signals that act as honest indicators of other traits associated with vulnerability to attack, like health, size, or parasite resistance (Hall 2013, Loyau et al 2005), may be particularly likely to gain a secondary function as signals of quality to heterospecific eavesdroppers.

Class 3:

Class 3 selection occurs when eavesdroppers have a positive effect on signaler fitness and interact most with high magnitude signalers, bringing the greatest benefits to the most conspicuous signalers and favoring the evolution of higher magnitude signals. Class 3 selection can occur when mutualists respond to sexual signals and when prey are lured by sexual signals.

Mutualists are just as capable of detecting and responding to those signals as any other organism. However, their responses to heterospecific sexual signals have not been investigated and the prevalence of this type of eavesdropping is unknown. If mutualists are attracted to sexual signals, they will provide extra benefits to conspicuous signalers, raising their relative fitness and favoring more elaborate signaling. Reproductive mutualisms such as nest association in fishes are particularly promising systems where eavesdroppers may be both attracted to sexual signals and provide benefits for high magnitude signalers. Nest associates are often beneficial to hosts, and they must locate hosts during spawning, precisely when sexual signals are being displayed most prominently. Eavesdropping need not be limited to mutualisms resulting from direct interactions between organisms, but could also help facilitate indirect mutualisms between organisms in the same food web, such as indirect mutualisms, food chain mutualisms, and competitive mutualisms (see Vandermeer 1980 for description of these mutualisms).
Another form of Class 3 selection occurs when prey are attracted to the sexual signals of predators. This kind of maladaptive response has been documented in fireflies (Box 3), where predator and prey both signal using flashing patterns of light. In this system, predatory females utilize flashing signals to attract both conspecific males and heterospecific prey (Lloyd 1986, Woods et al. 2007). While the pattern of signaling differs between contexts, the same structure is used to make both forms of signals. Females with brighter, more conspicuous light should benefit by attracting more mates and more prey. Similar dual-use signaling structures could exist in other bioluminescent organisms.

Class 4:

Class 4 selection occurs when eavesdroppers have a positive effect on signaler fitness and interact most with low magnitude signalers, bringing the greatest benefits to the least conspicuous signalers and favoring the evolution of lower magnitude signals. Class 4 eavesdroppers are prey or hosts, and thus have a positive impact on the fitness of their predators and parasites by providing them with food. Because these same interactions harm eavesdropping prey, they avoid sexual signals indicating the presence of predators or parasites. As a result, they interact most with low magnitude signalers who are harder to detect and avoid. Prey are known to avoid cues indicating predator presence, and predators are known to reduce or modify these cues to reduce cue production when eavesdropping prey are present (Fenton 2003, Riesch and Deecke 2011). The sexual signals of predators also serve as cues for prey (Emmering and Schmidt 2011, Ferrerro et al. 2011, Forsman and Martin 2009, May et al. 2012), however, in the literature prey responses are rarely referred to as “eavesdropping” despite qualifying for this label.
Prey eavesdropping should constrain sexual signals produced by predators, but the level of constraint depends on the method predators use to capture prey. Ambush predators rely on cryptic coloration and shape to avoid being detected by prey. This precludes the use of permanent conspicuous sexual signals like prominently displayed color patterns or persistent odors that would negate camouflage. Instead, ambush predators should rely on sexual signals that can be produced intermittently, like visual signals that can be hidden, specific movements, and mating calls.

Markings and calls of predators also are subject to eavesdropping by prey. Many species of birds and mammals respond to the vocalizations of predators (Blumstein et al. 2008). Although predators are often quiet while hunting, vocalizations and scent markings can still be used as territorial signals. In this case, sexual signals deter prey from a predator's hunting territory rather than alerting them to the immediate presence of a predator. Scent cues used by carnivores as territorial markings also induce aversive or defensive behaviors in rodents (Ferrerro et al. 2011, May et al. 2012). Songs of brood parasites (Forsman & Martin 2009) and vocalizations of nest predators (Emmering and Schmidt 2011) deter songbirds from nesting near signalers. While territorial signals are not necessarily sexual signals, they often are used for both intersexual and intrasexual signaling (Baum and Kelliher 2009, Gosling and Roberts 2001). If territorial signals are displayed in the same areas where predators hunt, they could reduce predation success by alerting prey, causing them avoid those areas or to become more vigilant. The effects of selection might be visible in the spatial and temporal distribution of signals across predator territories, with markings and calls displayed at places and times least likely to alert prey in favored hunting territories.
Implications of the model

The classes of selection described above highlight previously unappreciated ways that eavesdropping could drive the evolution of sexual signals. Class 2 selection has the potential to drive signals to become increasingly elaborated even in the presence of eavesdropping predators, and could help account for the existence of sexual signals that appear to be more elaborate than female preference alone would dictate. Even more intriguingly, this form of eavesdropping could provide one explanation for the origin of aposematic signals. The origins of aposematic signaling have been difficult to explain because conspicuous animals would initially face higher predation. Sexual selection often favors the evolution of conspicuous signals even in undefended species, and thus could help “jump-start” aposematic signaling by favoring conspicuous traits in chemically defended species even before any predators are deterred by those traits (Bonduriansky 2011, Mallet and Singer 1987). Eavesdropping predators would then begin, through learning or selection, to treat the sexual signal as an aposematic warning. This would further select for conspicuous coloration and might eventually even result in a loss of sexual dimorphism as both sexes come to display coloration in order to benefit from predator deterrence. This might render the signal uninformative from a mate choice perspective, resulting in a complete transition of a display from sexual signal to interspecific signal. Or the signal might continue to fill both roles. Signals are known to serve both aposematic and sexual signaling functions in several species such as fireflies (Moosman et. al. 2009), poison dart frogs (Siddiqi et. al. 2004), and butterflies (Mallet and Singer 1987), but whether these signals originated as sexual signals, aposematic signals, or developed both uses simultaneously is currently unknown. More information is needed about the origin of conspicuous coloration in these groups.
Eavesdropping in reproductive associations has not been extensively studied, but has the potential to provide interesting information about how selection on signals changes as the impact of eavesdroppers on fitness changes. Reproductive associations range from the clearly parasitic, as observed with cowbirds (Payne and Payne 1998), to the apparently mutualistic, as observed in nest associations of some species of shiner (Johnston 1994). Indeed, interactions may range from parasitic to mutualistic within the same species pair because hosts often benefit from reductions in predation (Johnston 1994, Peoples and Frimpong 2013, Canestrari et al. 2014), and these benefits are only available when predators are in the environment. By collecting information about selection under different contexts, it may be possible to compare both class 1 and class 3 eavesdropping in the same system and assess if outcomes match our predictions.

Class 4 eavesdropping may play an important role in limiting the evolution of certain types of sexual signals among predatory organisms. If only class 1 selection existed, we would predict that top predators would tend to display elaborate sexual signals, because they are not at risk of predation as adults. But signaling in these species may be constrained by the need to avoid detection by prey, and a full understanding of how sexual signals in predators have evolved must account for the response of prey to those signals.
Potentially interesting model systems

Eavesdropping and reproductive mutualisms: Nest associations by cyprinid minnows provide a number of promising systems for the study of eavesdropping by mutualists. Nest associations occur when two or more species share the same nest while spawning (Wisenden 1999). Typically, a male of the host species constructs and guards the nest, while simultaneously displaying sexual signals to attract conspecific females. Associate species also gather around the nest and spawn. Often, host males continue to guard the eggs and fry of both species. In several species, this association seems to be mutually beneficial due to dilution of predation on eggs (Johnston 1994, Peoples and Frimpong 2013), although in other species the relationship may be parasitic (Fletcher 1993).

Because host males produce a variety of sexual signals to attract females at the time (spawning) when associates must locate hosts, sexual signals are available for eavesdroppers to detect. Because sexual signals can provide information on the quality of parental care (Knapp and Kovach 1991), eavesdroppers also have incentive to utilize this information to choose suitable hosts. Selection on host sexual signals by associates depends on the fitness effects of associates on hosts. Mutualistic associates should select for more elaborate sexual signals, while parasitic associates should select for less elaborate signals. Because the same species of associate could potentially be parasitic or mutualistic depending on the threat of egg predation (and therefore, the value of the dilution effect), it might be possible to directly test how selection on sexual signals changes as the same eavesdropper species transitions from type 1 to type 3 as a result of changing predator density.
**Fireflies and selection on sexual signaling:** *Photinus* and *Photuris* fireflies offer an opportunity to investigate many kinds of eavesdropping in a single system. Both groups of firefly are common in North America, and both males and females use flashing lights for sexual signaling. However, *Photinus* fireflies do not feed as adults (Lloyd 1997) while adult *Photuris* females are predatory. *Photinus* are subject to eavesdropping by many predators, including bats and some *Photuris* fireflies (Moosman et. al. 2009, Woods et. al. 2007). Bats find the chemically defended *Photinus* fireflies unpalatable, and are deterred by the same flashes of light that serve as sexual signals, making this system a candidate for Class 2 selection (Moosman et. al. 2009). *Photuris* fireflies specialize in consuming other fireflies and respond to *Photinus* sexual signals, promoting Class 1 selection (Lloyd 1986). Male *Photinus* fireflies are attracted to flashes produced by the same light-emitting organ used for by *Photuris* females for sexual signaling (Woods et. al. 2007). Because the “eavesdropping” *Photinus* males deliver a nutritional benefit to female *Photuris* with a conspicuous bioluminescent signal, this system represents a potential example of Class 3 selection on the light-producing organs of *Photuris* females. Because so many different classes of selection occur in this system, it provides an excellent opportunity to discover how counteracting sources of selection by multiple eavesdroppers affect signal evolution. Fireflies also offer an opportunity to investigate the potential for sexual signals to transition into aposematic signals via class 2 selection.

**Rodent eavesdropping on carnivore scent markings:** Rodent responses to carnivore scent cues offer a well documented example of prey eavesdropping on predatory sexual signals that could be extended to study how prey select for changes in predatory sexual signaling. Rats respond to a variety of feline kariomones in a lab context by initiating a variety of defensive or avoidance behaviors (Ferrero et al 2011, May et al 2012). These chemicals are left by cats when
scent marking using urine and rubbings from scent glands, and some are used for many types of
intraspecific communication, including sexual signaling (Verberne and Boer 1976).

If rodents’ responses to predatory sexual signals allow them to reduce the odds of being
captured, then selection should favor predators that reduce the availability of those signals to
prey. Rodents could potentially select for changes in the placement, frequency, timing, or
composition of the scent markings of predatory carnivores, but these possibilities have not been
investigated. If rodent prey effectively eavesdrop on the sexual signals of felines and other
carnivores, then predators that have been prevented by experimental manipulation from marking
their territory should have higher rates of prey capture, provided that competition from invading
conspecifics is prevented. Comparison of sexual signaling between closely related members of
carnivora that subsist on different prey types also could be informative. Species or populations
that consume a greater proportion of aquatic prey should require fewer countermeasures against
prey eavesdropping on terrestrial scent marks, and comparing signaling in a phylogentic context
between predators with different diets should help highlight what aspects of signals are under
selection by terrestrial eavesdroppers.

Conclusions

We have shown how heterospecific eavesdroppers have the potential to affect the
evolution of sexual signals in a variety of ways. However, there is a need for more
documentation of eavesdropping behavior, especially outside of the type of eavesdropping that
leads to Class 1 selection. Predators that are deterred by sexual signals may be overlooked
because they exhibit behavior opposite of what is normally considered eavesdropping, but they
also are potentially important drivers of the evolution of elaborate sexual signals. Prey are known
to avoid the sexual signals of predators, but these systems are rarely referenced as eavesdropping in the literature, and little work has been done to document how selection by prey affects predators. The potential impact of eavesdropping in mutualistic systems and between competitors is still largely unknown.

It is important to understand how signalers alter their behavior in the presence of eavesdroppers. Apparent adaptations to eavesdropping provide an indirect form of evidence for selection by eavesdroppers on signalers. For example, individuals that detect Class 1 eavesdroppers may cease signaling or switch to a private channel (Römer et al. 2010). The inverse of this phenomenon occurs when prey actively display intrasexual threats toward predators, which might be indicative of Class 2 selection favoring detectability of signals by predators (Shine 1990, Vanhooydonck et al 2005). Relatively few studies have documented cessation of signaling during hunting (but see Riesch and Deecke 2011), but such behaviors should be common because of the risk of prey eavesdropping (Blumstein et al. 2008). Reductions or shifts in predatory signaling may be adaptations resulting from eavesdropper-driven selection. The existence of such responses is not conclusive evidence of such selection because these behaviors could be exaptations, maladaptive, or merely coincidental. However, their presence still is an important indicator of systems where eavesdroppers may be having a large effect on signaler fitness.

The fitness effect of eavesdroppers on signalers can be directly measured by comparing signaling in populations where eavesdroppers are present and absent. Endler's (1980) research on guppy sexual signals provides a good example; guppies were allowed to reproduce for several generations in mesocosms containing predators and mesocosms lacking them. A comparison of male color patterns between the two groups showed clear evidence of class 1 selection, with
predators consuming the most brightly colored males and selecting for populations of less colorful males. Field crickets in the Pacific islands have similarly evolved reduced signaling in the presence of eavesdropping parasitic phoronid flies (Zuk et al. 2006), although mainland species did not show an equivalent response, perhaps because other countermeasures limited the effectiveness of the parasitic flies (Beckers and Wagner 2012). Selection by mutualists, competitors, or prey could be estimated using similar methods, comparing sexual signaling in artificial mesocosms or naturally isolated populations that exist under different eavesdropping regimes.

Such populations offer an opportunity to study not only changes to sexual signals, but also changes spatiotemporal distributions of, and behavioral interactions between, individuals of the signaler species. Selection by phoronid flies for a non-signaling morph in male field crickets has changed the distribution of males in the population. When signaling males were abundant, they were distributed widely but, following strong eavesdropper-driven selection, the resulting nonsignaling males tend to cluster near the few remaining signaling males and attempt to intercept females (Zuk et al. 2006). Females of many species change their preference in the presence of predators, preferring less conspicuous males (Gong and Gibson 1996, Johnson and Basolo 2003), or simply becoming less choosy (Forsgren 1992). However, selection by eavesdroppers on sexual signals could also indirectly alter the ways that females choose between males. If eavesdropper-driven selection reduces the variance of sexual signals or favors their complete elimination, males of different quality would not differ as greatly in signal quality, and females might have a harder time distinguishing high and low quality mates. Such populations might be expected to shift to different forms of sexual signaling.
Phylogenetic analysis is needed to understand how eavesdroppers have affected signal evolution over evolutionary timescales. For example, we predict that prey eavesdropping can limit the sexual signals of predators. Ambush predators should be especially vulnerable to eavesdropping by prey, because their hunting strategy relies heavily on being inconspicuous. If eavesdropping prey constrain sexual signaling, then elaborate sexual signals that are difficult to hide should be lost in lineages that adopt this hunting strategy. Alternatively, such hunting tactics might be unable to evolve in species with conspicuous signals. Phylogenetic analysis can also be helpful in determining whether sexual signals are modified to convey information to heterospecifics, or vice versa. For example, poison dart frog coloration serves a clear aposematic function (Vences et al. 2003) and also a mate choice function. Although it is generally thought that aposematism drove the evolution of conspicuous coloration in this species, it is possible that female choice played a role in the development of conspicuous coloration by favoring brighter males. Female bias for brightly colored males has been observed in some toxic aposematic species (Maan and Cummings 2009). If a combination of sexual selection and Class 2 selection by eavesdropping predators helped to initiate the evolution of this aposematic signal, female bias for conspicuous coloration should predate the evolution of toxicity in this group, and should be present in some of the related nontoxic and cryptic species as well.

Eavesdroppers are important, not only in their own right as organisms with interesting methods of information gathering, but also as sources of selection on the sexual signals of their targets. Many kinds of organisms eavesdrop, and they exhibit a wide variety of responses to the sexual signals they encounter. The fitness of signalers faced with many potential heterospecific eavesdroppers will depend on the net costs and benefits of eliciting responses from conspecifics and heterospecifics. Eavesdropper-driven selection can favor strategies allowing prey to hide
their signals from predators, predators to hide their signals from prey, or competitors to hide signals from other competitors. It can result in investment in highly conspicuous signals to ward off predators or competing males and/or to attract beneficial heterospecifics and mates.

Understanding the ecology and evolution of a sexual signal may require paying close attention to the fitness consequences of eliciting a response from conspecifics as well as eavesdropping heterospecifics. Eavesdropper driven selection may help explain why some sexual signals are highly conspicuous and others are not, how aposematic signals could become widespread, or why some organisms shift to different forms of sexual signal over evolutionary time. Selection by eavesdroppers can also shape interactions between conspecifics by altering the context in which signals are displayed and changing the correlation between signals and the quality of signalers. We encourage researchers to capitalize on opportunities to study various forms of eavesdropper-driven selection on sexual signals.
References


CHAPTER 5

INTERSPECIFIC ASSESSMENT OF SEXUAL SIGNALS:
CAN HETEROSPECIFICS CHOOSE THE BEST HOSTS?

Many species use sexual signals to communicate with potential mates or competitors for mates (Andersson and Simmons 2006). However, these signals also are vulnerable to interception by heterospecifics that use signals to gain information about signalers, a process known as eavesdropping (Peake 2005). For example, predators and parasites eavesdrop on heterospecific sexual signals to locate food or hosts (Bernal et al. 2006, Haynes and Yeargan 1999, Stoddard and Markham 2008, Virant-doberlet et al. 2011, Zuk and Kolluru 1998), and competitors and prey also use heterospecific signals as cues indicating the presence of competitors or predators (Emmering & Schmidt 2011, Ferrero et al. 2011, Fletcher 2007, Forsman and Martin 2009). Heterospecific eavesdropping is important to study because eavesdropping behaviors can affect the ecology and evolution of both species involved; eavesdropping can allow signalers to be more easily located and increase the rate of interaction between signalers and eavesdroppers, and these interactions with eavesdroppers can drive selection on both signaling and eavesdropping.

Eavesdropping in predator-prey systems has been well studied, but less is known about eavesdropping in other types of systems. One such system is nest association, which occurs when a fish of one species (donor) leaves its eggs or young in the care of another species of fish (host) (Wisenden 1999). Eavesdropping in nest association systems is particularly interesting because
these associations can be mutualistic. Donors benefit because of the care and protection their
eggs receive from hosts, while hosts benefit because the addition of donor eggs dilutes predation
on their own eggs (Johnston 1994b, Shao 1997, Wallin 1992), although in some cases nest
association is costly for hosts because of disease spread or egg predation by donors (Baba &
Karino 1998, Fletcher 1993, Sato 1986). Eavesdropping by donors could determine which hosts
encounter donors and influence how frequent those encounters are by attracting or deterring
donors from particular hosts. Because the fitness of a donor depends directly on the quality of
parental care provided by the host, donors have an incentive to assess sexual signals for
information about the quality of care provided by hosts. This contrasts with many other
documented instances of eavesdropping, where predators or parasites utilize sexual signals to
locate hosts (Bernal et al. 2006, Camp 2006, Page and Ryan 2008). The mutualistic nature of
some nest associations also provides an interesting contrast with documented instances of
eavesdropping in predatory or parasitic systems driving reductions in sexual signaling. Because
mutualists are beneficial to the organisms they interact with, eavesdropping mutualists might
select for, rather than against, conspicuous sexual signals.

Our study aimed to establish the presence and nature of eavesdropping in a potentially
mutualistic nest association between bluenose shiners (*Pteronotropis welaka*) and longear
sunfish (*Lepomis megalotis*). Nest associations are relatively common between shiners and
sunfish (Fletcher 1993, Johnston 1994a, Hunter and Hassler 1965, Shao 1997, Wallin 1992,
Wisenden 1999). In these systems, a male sunfish is the host, digging and guarding a nest of
eggs, and shiners are donors (Gill 1906, Johnston 1994a, Shao 1997). Longear sunfish are
sexually dimorphic, with males displaying brighter colors, darker fins, and large opercular flaps
– long, black extensions of the gill cover (Goddard & Mathis 1997). Longer flaps are sexual signals; when presented with a choice between males that have had artificially lengthened flaps and individuals with flaps that have not been lengthened, female sunfish prefer to spend time near males with longer flaps and also avoid males with artificially shortened flaps (Goddard and Mathis 1997). Furthermore, flap length conveys information to other males during aggressive interactions; males with artificially lengthened flaps win more fights than those with smaller flaps (Goddard & Mathis 1997, Goddard & Mathis 2000). Nest defense relies on behaviors similar to those seen in male aggressive interactions (Keenlyside 1972, Magee & Neff 2006), so opercular flap length likely also indicates that the male is a competent nest defender. Information about nest defense is valuable to shiners because their own reproductive success depends on the capacity of sunfish to defend the nest, and the shiner eggs within it, from intruders and egg predators. Shiners in the wild would have the opportunity to assess this information and compare opercular flap lengths of potential male hosts because sunfish often cluster their nests and spawn simultaneously (Jennings 1991).

Other cues could potentially draw bluenose shiners to longear sunfish. The movements of mating sunfish and the release of reproductive fluids have been found to attract nest redfin shiners (Notropis umbratilis) to green sunfish (Lepomis cyanellus) nests (Hunter & Hassler 1965). Other aspects of sunfish morphology such as size or color patterns could also contribute to shiner choice. We attempted to exclude these potential influences in order to focus solely on the signal of interest. Shiners were given the opportunity to choose between isolated male sunfish, eliminating chemical and behavioral cues produced during mating. Opercular flap
length was artificially modified to eliminate possible confounding correlation between flap length and other aspects of morphology.

We hypothesize that bluenose shiners eavesdrop on the sexual signals of longear sunfish, preferring sunfish with longer opercular flaps that indicate greater success in aggressive interactions and competence in nest defense. We provided shiners with pairs of sunfish and allowed them to choose between individuals of three opercular flap length classes (long, medium, and short) or alternatively provided them with isolated sunfish and allowed them to choose between animals with long or short opercular flap lengths and an empty compartment. We predicted that shiners would assess sunfish flaps rather than exhibiting simple attraction to all individuals possessing long flaps, meaning that an attraction to longer flapped fish would only occur when shiners were given the opportunity to compare paired sunfish.

**Methods**

**Collection of specimens**

Bluenose shiners were collected with seine net from the middle Tombigbee-Lubbub watershed in Pickens County, Alabama (33° 17.523' N, -87° 55.538' W; 33° 19.150' N, -87° 55.930' W; 33° 20.079' N, -87° 55.549' W; 33° 20.608' N, -87° 55.054' W; 33° 22.998' N, -87° 59.631' W). Longear sunfish were collected with hook and line or by electrofishing from several locations in the Black Warrior watershed in Tuscaloosa County, Alabama (33° 12.854' N, -87° 34.300' W; 33° 13.167' N, -87° 33.000' W; 33° 13.300' N, -87° 33.901' W; 33° 18.508' N, -87° 28.646' W; 33° 17.460' N, -87° 31.206' W). Both species were collected under a scientific collecting permit (#2014035648668680) issued by the state of Alabama Department of
Conservation and Natural Resources. Fish were transported in 18 L buckets from the field to the laboratory at the University of Alabama.

**Animal Husbandry**

All fish were housed in a climate-controlled room at 25°C with a 14h:10h photoperiod, mimicking summertime breeding conditions. Bluenose shiners were held communally in 375 L tubs with 2 cm gravel substrate. Longear sunfish were kept individually in visually isolated 37 L aquariums with 2 cm gravel substrate, PVC tube hides, and hang on back power filters. Both species were fed a mixture of frozen bloodworms, brine shrimp, and flake food daily. Target water quality parameters (ammonia and nitrite=0, nitrate <20 ppm, pH between 6.5 and 7.5) were maintained with weekly water checks and changes. All fish were kept in the lab for at least 1 week prior to experimentation. All procedures were approved by the University of Alabama Institutional Animal Care and Use Committee (Protocol #11-356).

**Experimental design**

We presented shiners with pairs of stimuli to assess their response to sunfish with differing opercular flap lengths. To determine the relative preference of shiners for sunfish opercular flap length, shiners were presented with one of three treatments allowing them to choose between pairs of sunfish that had been modified to have different opercular flap lengths: long vs. short-flapped sunfish (n=16); long vs. medium-flapped sunfish (n=14); and short vs. medium-flapped sunfish (n=13). To determine the absolute response of shiners to sunfish with different opercular flap lengths, shiners were presented with one of two treatments allowing them to choose between a single sunfish or an empty chamber: long-flapped sunfish vs empty chamber
(n=15), and short-flapped sunfish vs. empty chamber (n=16). Trials conducted within these five treatments were partitioned across three blocks run in sequence with at least 1 week separating them.

Shiners were housed communally in one of two identical 375 L tubs before use in each experimental block and transferred to the other identical tub after experimentation, to prevent mixing between used and unused shiners. Shiners were never reused within an experimental block, but were reused after random mixing between blocks. This was done to limit the total number of shiners used in the experiment, because the species is rare. A total of 26 sunfish were housed individually in 37 L tanks, opaque barriers prevented visual contact with sunfish in other tanks, and each individual was assigned a unique identifier. Sunfish pairs were randomized as much as possible using dice rolls within the constraints imposed by the need to avoid repeating identical pairings with identical flap lengths and to ensure pairs were size matched to within 10% standard length. Sunfish assigned to different opercular flap length categories did not differ significantly in body length, body depth, or original opercular flap length (Table 5.1).
Table 5.1: Sunfish size across treatment classes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Size Class</th>
<th>Body length Mean ± Std. Err.</th>
<th>Body depth Mean ± Std. Err.</th>
<th>Original relative opercular flap length Mean ± Std. Err.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Pooled</td>
<td>Long</td>
<td>11.04 ± 0.16</td>
<td>4.882 ± 0.090</td>
<td>0.1035 ± 0.0019</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>10.74 ± 0.20</td>
<td>4.853 ± 0.110</td>
<td>0.1016 ± 0.0024</td>
</tr>
<tr>
<td></td>
<td>Short</td>
<td>10.95 ± 0.16</td>
<td>4.841 ± 0.090</td>
<td>0.1049 ± 0.0015</td>
</tr>
<tr>
<td>Long vs. Short</td>
<td>Short</td>
<td>11.05 ± 0.28</td>
<td>4.899 ± 0.147</td>
<td>0.1024 ± 0.0030</td>
</tr>
<tr>
<td>Long vs. Medium</td>
<td>Long</td>
<td>11.06 ± 0.27</td>
<td>4.828 ± 0.165</td>
<td>0.2013 ± 0.0036</td>
</tr>
<tr>
<td>Medium vs. Short</td>
<td>Medium</td>
<td>10.89 ± 0.30</td>
<td>4.926 ± 0.163</td>
<td>0.1009 ± 0.0035</td>
</tr>
<tr>
<td>Long or Short vs. No Fish</td>
<td>Short</td>
<td>10.99 ± 0.33</td>
<td>4.918 ± 0.165</td>
<td>0.1072 ± 0.0034</td>
</tr>
</tbody>
</table>

Body length
- Wilcoxon / Kruskal-Wallis* Chi-Square(df)  P-value
  - All Pooled 1.0168(2)  0.6015
  - Long vs. Short 0.0646(1)  0.7994
  - Long vs. Medium 0.0276(1)  0.8680
  - Medium vs. Short 0.0132(1)  0.9084

Body Depth
- Wilcoxon / Kruskal-Wallis* Chi-Square(df)  P-value
  - All Pooled 0.0655(2)  0.9678
  - Long vs. Short 0.2583(1)  0.6113
  - Long vs. Medium 0.3383(1)  0.5608
  - Medium vs. Short 0.3307(1)  0.5653

Original relative opercular flap length
- Wilcoxon / Kruskal-Wallis* Chi-Square(df)  P-value
  - All Pooled 1.2079(2)  0.5466
  - Long vs. Short 0.1977(1)  0.6565
  - Long vs. Medium 0.0155(1)  0.9008
  - Medium vs. Short 0.1190(1)  0.7301

*Kruskal-Wallis test for “All Pooled”, Wilcoxon for others
Generating opercular flap size categories

We generated three opercular flap length categories (long, medium, and short) by measuring the opercular flaps and bodies of 81 sunfish collected from the upper Tombigbee watershed, where the bluenose shiners used in this experiment were collected. These fish were obtained from the University of Alabama’s museum collections. We photographed fish and measured fish dimensions (Figure 5.1) including opercular flap length (the distance from the anterior base of the black portion of the flap to the posterior tip of the flap), opercular flap depth (the distance from the middle of the upper edge to the middle of the lower edge of the flap) and fish standard length (the distance from the tip of the upper lip to the posterior part of the caudal peduncle) using ImageJ software (http://rsbweb.nih.gov/ij/). Size-adjusted opercular flap lengths and depths were calculated by dividing opercular flap length by standard length (distance from upper lip to base of the tail fin); the size-adjusted measurements are dimensionless numbers. Size-adjusted opercular flap length had a bimodal distribution, (Figure 5.2), with the greater peak representing nest-guarding male sunfish and the lesser representing females or sneaker males, which can be found in some longear sunfish populations (Jennings and Philipp 1992). After all females and sneaker males were excluded, we derived the three experimental flap size categories from the distribution of size-adjusted male flap lengths (n=40). We defined “long” opercular flaps as equal to two standard deviations above the mean size-adjusted flap length (0.140), “medium” flaps as equal to the mean size-adjusted flap length (0.118), and “short” opercular flaps as equal to two standard deviations below the mean size-adjusted flap length (0.092).
FIGURE 5.1: Sunfish measurements

(A) Red line indicates opercular flap length as measured from the anterior base of the black region to the posterior tip of that region (B) Yellow line indicates opercular flap depth, measured from the upper edge of the black region to the lower edge (C) blue line indicates sunfish body length measured from the tip of the top lip to the posterior portion of the caudal peduncle.
FIGURE 5.2: Distribution of relative opercular flap lengths

Histogram of opercular flap lengths of longear sunfish in the Upper Tombigbee illustrating distribution of flap length/body length.
Opercular flap manipulations

Field-caught male sunfish were photographed and their standard length and opercular flap dimensions were measured in the manner described above (Figure 5.1). After measuring the fish we used sterilized surgical scissors to reduce the opercular flap length of all sunfish by trimming the end of the opercular flaps to be slightly shorter than the “short” length category. To modify opercular flap length, black plastic flaps made of transparency film printed solid black were attached to the operculum. These flaps were rectangular in shape, with the length equivalent to the appropriate size-adjusted flap length and the depth equivalent to the average size-adjusted flap depth (body length multiplied by 0.08). We stitched these flaps to the base of the opercular flap using a sewing needle (0.6 mm diameter) and black cotton sewing thread. Following this procedure sunfish and shiners were placed into the experimental tank for 18 h of acclimation prior to the experiment. After the trials were concluded, plastic flaps were removed from sunfish and sunfish were reassigned new flap lengths. We never reassigned the same sunfish with the same opercular flap lengths to the same pair.

Experimental Trials

Experimental tanks (108 L, 120 cm long x 30 cm wide x 30 cm high) were divided into five sections - a central acclimation chamber for shiners (20 cm wide) and two treatment chambers at the far ends of the tank (30 cm wide), separated from the acclimation chamber by two intermediate chambers (20 cm wide) (Figure 5.3). The treatment chambers were separated from the rest of the tank by an open mesh with 2.5 cm holes, large enough to allow shiners to pass but small enough to prevent sunfish from leaving the chambers to which they were assigned. The
central chamber was divided from the intermediate chambers by opaque half-walls and removable transparent dividers, staggered to block direct line of sight from one end of the tank to the other. Thus, acclimating shiners could see both male sunfish, while neither sunfish could see the other. The transparent dividers could be lifted to allow shiners to move throughout the entire tank. One male and two female shiners (males are easily distinguishable by their enlarged dorsal fins) were placed in the central chamber and male treatment sunfish were placed in the treatment chambers at the same time. Fish were not fed while in the experimental tanks. All sunfish pairs were matched to be within 10% standard length. After 18 h of acclimation, we removed the dividers and allowed the shiners 30 minutes to swim between chambers, recording which chamber they were in every 20 seconds. We videotaped the trials using a JVC digital videocamera placed approximately 4 feet from the aquarium at the beginning of filming. Experimenters left the room during filming.
FIGURE 5.3: Experimental tank design

Tanks used in shiner choice experiment. Chambers are indicated by the letters below. (A) Treatment chambers holding either sunfish with modified opercular flaps or left empty (B) Intermediate chambers separated from treatment chambers by open mesh passable by shiners (C) Central chamber holding shiners for acclimation before the experiment, separated from intermediate chambers by removable transparent dividers and opaque permanent dividers.
Data Analysis

Model overview

For each trial, the data consisted of the number of shiners observed in each of the five sections at twenty-second intervals. To model the choice process we treated these data as a summary of the true locations of individual fish along the length of the tank. After removal of the barriers we used the first observation to assign a distribution of initial locations to the fish (e.g. two fish in section 3, one fish in section 2). After the initial observation, we modeled the location \( x_{i,t} \) of each individual as a random walk with a treatment-specific drift term \( \Delta_{j(i)} \) among other parameters.

\[
x_{i,t} \sim \text{RW}(x_{i,t-1} + \Delta_{j(i)}, \ldots)
\]

To find a model that effectively represented the distribution of shiner movement distances within the tank, we used three distributions to model this random walk: 1) normal distribution; 2) student's t distribution; and 3) a mixture of student's t distribution with an effectively flat distribution. In all cases we used truncated distributions to account for the fact that fish movements were constrained by tank dimensions. To connect the models to the data, we used an observation process that summed the inferred number of fish in each section. We assumed the observation process was perfect (if a fish was counted in section 3, it was definitely in section 3), so we constrained the model to only produce estimated locations that summed to the correct count in each section. Due to this observation model, the locations inferred were always compatible with the observed data, but because observations were relatively coarse, the inferred location within a section was strongly dependent on the process model. We coded this
model in the BUGS language and used the program JAGS (Plummer 2014) to infer model parameters. The program JAGS produced posterior samples from the distribution of model parameters, which we used for 1) model checking; 2) to estimate mean treatment effects; and 3) to generate credible intervals.

Model checking

Our main concern with goodness of fit was that the model might not correctly account for the step size distribution of shiner movements and therefore bias the estimates of the drift parameters. Therefore we chose to focus on whether the model, with its inferred parameters, was able to reproduce the step size (counted in number of sections moved) distribution from our data. To check whether the models sufficiently represented the data, we applied a Bayesian diagnostic procedure for goodness-of-fit known as a posterior predictive check (Gelman 1996). First we used an R function to map the counts of individuals per section to the location of each individual. Based on individual locations we calculated the observed step sizes, and summarized the data as the number of steps of each size. For comparison, we also simulated repeated draws of individual locations from each model, and summarized those in the same way. If the model reproduced the step size distribution generated from the data, we expect a plot of counts of data-derived and model-derived step size counts to cluster along the 1:1 line. Deviation from that pattern would indicate some degree of lack of fit.

Results

We found that bluenose shiners respond to the opercular flap length of male longear sunfish hosts. This response is illustrated in figures 5.4 and 5.5. Figure 5.4 shows a heat map of
shiner location over time for each of the five treatments. Each column is divided into five columns, representing each of the five chambers in the experimental tanks. All 270 time records are placed in sequence, starting with t=0 at the top of each column. The percentage of black in each cell indicates the percentage of shiners that were found in a particular chamber at a particular time point; a 100% black area (such as the central chamber at t=0) indicates that all shiners were present at that chamber at that time, while a 50% black areas indicates that 50% of shiners were observed in that chamber across all replicates.

When presented with pairs of sunfish, shiners tended to move towards long or medium flapped individuals, but did not distinguish between long and medium flapped individuals when those were paired (Fig 4 columns a, b, c; Fig 5 row a, b, c). Shiners showed significant preference for medium over short and long over short, but the strength of that preference was not significantly different between treatments. Shiners were equally likely to associate with long and short-flapped sunfish when the animals were presented alone. (Fig 4 column c, d; Fig 5 row c, d).
FIGURE 5.4: Shiner movement

A heatmap illustrating shiner location across experimental tanks over time. This graph was generated by counting the total number of shiners in each of the five chambers at each observation time-point. The first observation is located at the top, the final at the bottom. Darkness of color indicates what proportion of shiners across all replicates were present in a chamber, with black indicating 100% and white indicating 0%.

Darker color indicates more shiners were present. Columns A-E each illustrate different treatments: A) Short (S) vs Long (L) B) Medium (M) vs Long (L) C) Small (S) vs Medium (M) D) None vs Long (L) E) None vs Short (S)
FIGURE 5.5: Diffusion models

Three diffusion models estimating displacement of shiners towards sunfish during the experiment. Shiners movement was treated as a random walk with an additional directional bias, shown on the graph. Rows illustrate different treatments. An expected displacement of 0 represents no movement bias toward either side, increasingly negative numbers indicate a tendency to move toward longer flapped sunfish.

A) Long vs Short B) Long vs Medium C) Medium vs Small D) Long vs None E) Small vs None
Discussion

Our results demonstrate that bluenose shiners respond to the sexual signals of their hosts, longear sunfish. Shiners showed a preference for individuals bearing the longest opercular flaps, but this attraction toward elaborate signalers was dependent on the other options available to shiners. When shiners were presented with a short flapped sunfish paired with a long flapped sunfish, or a short flapped sunfish paired with a medium flapped sunfish, they favored the sunfish with the longer flap (long or medium option). However, they did not show a preference for long flapped sunfish over medium flapped sunfish.

This could be interpreted to mean that shiners were repelled by short flapped fish instead of attracted to long flapped fish, since they always avoided short flapped fish in a pair, but did not distinguish between long and medium flapped sunfish. This might occur if shiners wished to avoid other, more predatory, *Lepomis* species with shorter opercular flaps, such as *Lepomis cyanellus*. Similarly, shiners may have wished to avoid female sunfish, which have shorter flaps than males. However, a closer look at the data shows that this is unlikely to be the case. When shiners were presented with sunfish in isolation, they did not avoid short flapped fish. Instead, they actually approached these individuals marginally more than they approached isolated long flapped fish. If shiners were simply avoiding short flapped fish, they would avoid them when paired with an empty chamber as well. Furthermore, when shiner movement was graphed over time, shiners in “long vs short” and “medium vs short” treatments clustered in the chamber containing the longer flapped male of a pair (right side of columns A and C, Figure 5.4), but did not especially avoid the chamber containing the shorter-flapped male (left side of columns A and C, Figure 5.4), as would have been the case if shiners were avoiding that fish. Indeed, at some
time points the chamber containing the shorter flapped fish was most populated by shiners. Thus it does not appear that fish were specifically avoiding short-flapped individuals, but were instead choosing longer flapped males.

Why do shiners then fail to distinguish between long and medium flapped sunfish? One possibility is that shiners are unable to perceive the difference between long and medium flapped sunfish. It is easier for organisms to distinguish between small magnitude stimuli that differ by a set amount than to distinguish between higher magnitude stimuli that differ by the same amount (Bisazza et al. 2010, Gómez-Laplaza and Gerlai 2010) and this can limit preference for elaborate signals (Akre 2011, Akre and Johnsen 2014). Thus this response pattern may be due to perceptual limitations of shiners. Alternatively, the lack of preference for long over medium flaps may be adaptive. If long and medium flapped fish do not differ significantly in their ability to care for shiner eggs, then there would be no advantage for shiners to exhibit a preference for one over another. In this case, a lack of preference between long and medium flapped fish would prevent fish from needlessly rejecting suitable hosts. Unfortunately, no research has yet been performed on the perceptual abilities of shiners or the care quality provided by males with different opercular flap lengths.

The response of shiners to sunfish changed drastically when shiners were presented with a single sunfish in isolation, rather than a pair. Instead of approaching the longer flapped males more quickly than the short-flapped males, they responded in a similar way to both types of sunfish, and failed to show a strong affinity for either long or short flapped individuals over the empty side of the tank. This contrasts with a tendency to approach longer flapped sunfish when those sunfish are presented as one of a pair. Sunfish in the wild are colonial nesters (Keenlyside
and shiners may have been unwilling to commit to a single host without comparing its quality to that of other potential hosts. If this is the case, the dispersal of shiners throughout the tank might have been an attempt to sample more hosts before making a choice. Females have also been shown to exhibit sampling behavior rather than simply approaching a single male (Gibson and Langen 1996, Murphy and Gerhardt 2002). Alternatively, shiners might have been responding to the fact that the male was isolated. Colonially nesting *Lepomis macrochirus*, a related species, suffer lower rates of egg predation and fungal infection, possibly due to the benefits provided by cooperative defense (Côté and Gross 1993, Gross and MacMillian 1981). Shiners may similarly have found lone sunfish unattractive because they lacked the benefits colonial nesting provides. Interestingly, several studies have found that colonial nesting and isolated males receive similar numbers of eggs from female sunfish in the wild, implying that females do not prefer colonial nesters to isolated sunfish (Dupuis and Keenleyside 1988, Neff et al 2004). However, females do prefer males in larger colonies in some other colonially nesting species (Tyler 1995). Further research is needed to see if shiners in the wild also avoid isolated males. If they do, perhaps it indicates that female sunfish experience some cost when laying eggs in colonies that counteracts the potential benefit of better care.

Shiners appear to be utilizing heterospecific signals differently than many other eavesdroppers. Many heterospecifics use what Peake describes as interceptive eavesdropping to gather locational information about the signaler (Peake 2005, Zuk and Kolluru 1998). In contrast, longear sunfish signals are not visible unless the fish itself is visible, and are therefore not likely to be useful in locating signalers. Instead, shiners may be using opercular flap length to assess the characteristics of males, in particular their skill at nest defense. The preference of shiners is
similar to that of female sunfish, which also prefer to associate with males bearing longer opercular flaps (Goddard and Mathis 1997). Because female sunfish and bluenose shiners of both sexes rely on longear sunfish to protect their eggs, it is not surprising that both groups prefer males bearing a signal (longer opercular flaps) that reflects greater success at aggressive interactions (Goddard and Mathis 2000) similar to those used to defend nests (Keenlyside 1972, Magee & Neff 2006). Assessment of sexual signals has been observed in avian brood parasites (Parejo and Avilés 2007), another group of species where fitness may vary with the quality of parental care provided by the sexually signaling hosts on which they eavesdrop.

A comparison can be made between the response of eavesdroppers with the response of females. Broadly speaking, the response of shiners matched the response that Goddard and Mathis (1997) found in females—both shiners and female sunfish were attracted to males with longer opercular flaps. This is consistent with the idea that opercular flaps indicate some trait of male sunfish, like quality of parental care, which affects the fitness of both female sunfish and shiners. However, the response we observed in shiners did not perfectly match the response Goddard and Mathis observed in female sunfish. Female sunfish exhibited a preference for males with artificially elongated (long) opercular flaps over individuals with standard (medium) length flaps, while bluenose shiners did not prefer fish with long flaps over medium flapped fish. These differences could simply result from differences in experimental design or procedure, but they could also point to differences in the way that females and eavesdroppers perceive signals or utilize the information found in them. Perhaps female sunfish, with their larger eyes, are less limited in their ability to perceive the smaller relative difference in size between medium and large flapped male sunfish. Alternatively, large and medium flapped sunfish might differ in some
way that effects the fitness of female sunfish, but not shiners. For example, while both sunfish and shiners benefit from parental care provided by male sunfish, only female sunfish benefit from the genetic contribution that male sunfish provide when spawning. If medium and long flapped sunfish differ in the quality of their genetic contribution, but not in the parental care they provide, we would expect female sunfish but not shiners to exhibit a preference for the longest flapped males. Further research on the visual systems of both species and the correlation between opercular flap length and genetic quality as well as parental care is needed to determine if either of these explanation might account for the difference in response between female sunfish and bluenose shiners. Comparing the response of eavesdroppers with the response of females highlights avenues for the study of how perception varies across species and how signals can transmit multiple types of information.

Although the effects of bluenose shiners on longear sunfish fitness have not yet been determined, a number of other similar nest associations have been shown to be mutually beneficial to both hosts and donors. Eavesdropping by mutualists is particularly interesting because of the potential for mutualists to select for increases rather than decreases in sexual signals. Predators and parasites typically select for reductions in sexual signals because they harm the signalers that they eavesdrop on (Peake 2005). Since mutualists benefit rather than harm signalers, they should impose selection favoring increases in signaling rather than decreases. This is an intriguing potentially new explanation for some elaborate sexual signals, but more research is needed to determine if shiners or other nest associates can actually deliver sufficient benefits to signalers to drive the evolution of enhanced sexual signaling in sunfish.
References


CHAPTER 6

DO THE SEXUAL SIGNALS OF LONGEAR SUNFISH SHOW VARIATION CONSISTENT WITH SELECTION BY BLUENOSE SHINERS?

Female preference and male-male competition play an important role in shaping phenotypic evolution (Andersson and Simmons 2006, Emlen and Oring 1977, Jones and Ratterman 2009, Kokko et al. 2003). Studies focusing on the evolution of sexual signals typically examine conspecific interactions; preferences by females or competitive advantages in conflicts between competing males that drive the evolution of elaborate sexual signals. Conspecifics, however, are not the only individuals capable of detecting sexual signals. Heterospecifics can also eavesdrop on sexual signals and respond to them (Brandley et al. 2013, Peake 2005), impacting the fitness of signalers and selecting for changes in the form and function of sexual signals (Endler 1980, Zuk et al. 2006). Of particular interest are cases of eavesdropping in systems involving interspecific alloparental. Alloparental care occurs when individuals care for non-descendent young (Wisenden 1999). Interspecific alloparental care is known in birds (Payne 1977, Stevens 2013), insects (Akino et al. 1999, Strohm 2008), and fish (Johnston 1994b, Wisenden 1999), and can be harmful (Fletcher 1993, Hoover 2003) or beneficial (Johnston 1994a, Röder et al. 2014) to host fitness. Heterospecifics that utilize alloparental care (donors) need to choose quality hosts. Like females, donors can assess the quality of potential caregivers by the signals they produce (Parejo and Avilés 2007, Soler et al 2014). Eavesdropping donors that have a positive impact on signaler fitness would impose a heterospecific analog to sexual selection, favoring signals indicative of high quality parental care. In these cases, eavesdropping
donors might also play an important but underappreciated role in shaping the evolution of sexual signals in the species that host their offspring.

Females exhibit preferences for male signals for many different reasons (Andersson and Simmons 2006, Emlen et al. 2012, Jones and Ratterman 2009, Kokko et al. 2003), but one important factor behind female preference for certain traits is the ability for those traits to honestly indicate qualities of the signaler, including size, metabolism, immune function, lipid reserves, functioning of important endocrine signaling pathways (e.g., insulin/insulin-like growth factor), and probability of survival (Amorim et al. 2013, Bertram et al. 2011, Emlen et al. 2012, Gavassa et al. 2011, Gingras et al. 2013, Griggio et al. 2010, Hill 1994, Rantala et al. 2000, Simons et al. 2012). Many of these traits can serve as indices of overall genetic quality. By choosing males with high quality signals, females can gain indirect benefits by mating with males who carry the highest quality genes (Møller and Alatalo 1999, Moore 1994). However, females may also gain direct material benefits by choosing males with high quality signals (Møller and Jennions 2001). When males provide parental care, females can benefit by choosing males capable of providing quality care that will result in increased offspring survival. When the quality of parental care that a male is able to provide correlates with age, size, or health, signals indicative of maturity and good genetic quality can also be useful for indicating proficiency in parental care. Although the relationship between paternal care and male sexual signaling is somewhat complicated by the trade-off between allocating resources to care and to signals that indicate quality (Kokko 1998), evidence for it has been observed in wild populations (Amorim et al. 2013, Møller and Jennions 2001).
Eavesdropping donors could also respond to sexual signals that correlate with parental care, via behaviors akin to heterospecific ‘mate’ choice and resulting in selection on sexual signals (Chapter 4). Eavesdropping heterospecifics of many species utilize sexual signals to locate signalers (Camp 2006, Page and Ryan 2008, Sakaluk and Belwood 1984), and some species also utilize signals to assess qualities of the signaler (Parejo and Avilés 2007, Tuttle and Ryan 1981). We examined the potential for selection driven by heterospecifics on ornamental traits using a nest association between two North American fishes, longear sunfish (Lepomis megalotis) and bluenose shiners (Pteronotropis welaka) (Johnston and Knight 1999). Nest associations are a form of interspecific parental care that occurs when donor fish lay their eggs in the nests of hosts that then provide care for eggs and offspring (Johnston 1994b, Wisenden 1999). In this system, shiners are donors and male sunfish are hosts. Eavesdropping is particularly likely in these nest associations because sexual signals possessed by the host are potentially good indicators of the quality of parental care it may provide; a meta-analysis by Möller and Jennions (2001) found that sexual signals explained 23.6% of the variance in paternal care in ectotherms, much higher than any other direct benefit investigated in the study. Nest associations also provide benefits to hosts when the addition of donor eggs dilutes predation on host eggs (Johnston 1994a, Shao 1997, Wallin 1992), though some donors may impose costs by preying on host eggs or spreading disease (Baba & Karino 1998, Fletcher 1993). This raises the possibility that some nest associations are mutualistic and that donors may be selecting for enhanced signals of quality in male sunfish by providing benefits, like dilution of predation risk, that increase the reproductive success of hosts possessing more elaborate traits.
We examined the morphology of longear sunfish (*Lepomis megalotis*) opercular flaps for evidence of selection by bluenose shiners (*Pteronotropis welaka*). Longear sunfish exhibit significant sexual dimorphism in coloration as well as in the length of the opercular flap—black extensions of the gill cover that are longer in males (Gill 1906). These flaps serve as both intrasexual and intersexual signals; males with the longest flaps are better able to win aggressive contests with other sunfish (Goddard & Mathis 2000) and are more attractive to female sunfish (Goddard & Mathis 1997). Because nest defense involves aggressive interactions with conspecifics and congenerics (Magee & Neff 2006), opercular flaps may be an honest signal of nest defense capability. Because shiners rely on sunfish to defend their eggs, they might also utilize opercular flap length as a signal of the quality of potential hosts. Indeed, we found that when presented with a choice between sunfish with opercular flaps of differing lengths, shiners preferentially approached males with long or average opercular flaps over males with short opercular flaps (Chapter 5).

The distribution of shiners and sunfish offers an excellent opportunity to test whether characteristics of the opercular flap, a known intraspecific sexual signal, have responded to selection imposed by shiners. Bluenose shiners exist in isolated populations across the southern coastal plain, while longear sunfish are widespread across much of the eastern United States (Boschung & Mayden 2004). This distributional pattern means that longear sunfish are found in watersheds containing bluenose shiners and in nearby watersheds with similar physical and ecological characteristics that lack bluenose shiners. There are also sunfish located well outside the historical range of bluenose shiners. This provides an opportunity to examine sunfish opercular flaps in areas where shiners are present and are potentially imposing
significant positive selection on opercular flap length as well as in areas where shiners are not present and selection by shiners is not possible.

Because shiners exhibit a preference for male sunfish possessing longer opercular flaps, individuals with long and medium-sized flaps should be more likely to experience fitness effects associated with shiner nest association. If bluenose shiners have a mutualistic relationship with host sunfish, as has been shown in some other nest associations (Johnston 1994a, Peoples and Frimpong 2013, Shao 1997a, Yamane et al. 2013), then male sunfish with long and medium-sized flaps should experience increased fitness relative to those possessing shorter flaps. We hypothesized that the opercular flap of longear sunfish would act as a morphological module capable of varying in shape relative to overall body shape, and that bluenose shiners would select for changes in opercular flap morphology in longear sunfish. We predicted that sunfish that coexist with bluenose shiners would exhibit longer opercular flaps compared to sunfish that do not coexist with bluenose shiners.

Methods

To obtain data on sunfish morphology we photographed 904 preserved sunfish from collections at the University of Alabama, University of Florida, Mississippi Museum of Natural History, University of Georgia, and University of Tennessee. These sunfish were collected from 65 watersheds, which we classified into three watershed classes: Present (n=19), Nearby (n=25), and Distant (n=21). Watersheds classified as ‘Present’ had records of bluenose shiners. Those classified as ‘Nearby’ had no records of bluenose shiners but either fed directly into watersheds known to have shiners or were parts of river systems on the South Atlantic Gulf region that were adjacent to watersheds with shiners. Watersheds classified as ‘Distant’ were not adjacent to
watersheds containing shiners and were typically found outside the South Atlantic Gulf region (Figure 6.1). The genetic structure of longear sunfish populations is not well understood, but I attempted to reduce the potential impact of confounding population differences. All of the sunfish were collected east of the Mississippi river, as sunfish on the western shore may be a separate population or subspecies _L. megalotis breviceps_, and well south of the range of the morphologically, but not genetically, distinct _L. megalotis peltastes_ (Jennings 1991). However, it is possible that there are genetic differences between sunfish of the “Distant” watersheds from Tennessee and the more southern “Present” and “Nearby” watersheds (Jennings 1991). The interspersed arrangement of “Present” and “Nearby” watersheds makes it unlikely that individuals in these two categories are part of two distinct subspecies or long-separated lineages that might exhibit consistent regional variation in morphology for reasons unrelated to selection by shiners.

Sunfish were photographed alongside a scale. Images were landmarked at 16 points using TPS Dig (Figure 6.2). We also marked two points on the scale allowing us to determine the size of the fish. The output from TPS Dig was analyzed in R (version 3.1.2) using the Geomorph package to obtain principal components and Escoufier's RV coefficients.

I used Escoufier's RV coefficients to determine if opercular flap morphology formed a separate module of morphological variation compared to body shape (Klingenberg 2009). Landmarks on the opercular flap (#10-14; Figure 6.2) were compared to all other points on the sunfish. The RV analysis compared shape variation captured in the 5 opercular landmarks to variation captured in 1000 iterations of 5 randomly chosen landmarks. This test is intended to determine if the variation within opercular flap landmarks was different than the typical variation.
in any 5 random landmarks, thus showing if the opercular flap is acting as a separate module. I obtained procrustes distance, which is a measure of the total variation in shape of a set of points after the points have been scaled and rotated to equivalent size and orientation. We also performed a principal component analysis on opercular flap morphology to identify relevant shape variation, using points 10-14. This generated 8 principal components (Table 6.1), of which we analyzed only the first two. PC1 described shape variation in the opercular flap ranging from elongate to short, while PC2 described shape variation ranging from square to round (Figure 6.3).
This map shows the watersheds from which our fish were obtained. Green watersheds contained bluenose shiners, yellow watersheds had no shiner records but were near watersheds containing shiners, and orange watersheds were distant from any known shiner locations. Map of distribution obtained from explorer.natureserve.org (retrieved Sept 2014)
FIGURE 6.2: Landmark positions

1. Tip of the lip
2. Anterior attachment of dorsal fin
3. Posterior attachment of dorsal fin
4. Dorsal attachment of caudal fin
5. Central attachment of caudal fin
6. Ventral attachment of caudal fin
7. Posterior attachment of anal fin
8. Anterior attachment of anal fin
9. Anterior attachment of pelvic fin
10. Dorsal anterior of opercular flap
11. Dorsal inflection of opercular flap
12. Distal tip of opercular flap
13. Ventral inflection of opercular flap
14. Ventral anterior of opercular flap
15. Posterior limb of eyeball
16. Anterior limb of eyeball
### FIGURE 6.3: Deformation grids for PC1 and PC2

<table>
<thead>
<tr>
<th>a) PC 1 Low</th>
<th>b) PC 1 High</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Deformation Grid PC1 Low" /></td>
<td><img src="image" alt="Deformation Grid PC1 High" /></td>
</tr>
<tr>
<td>c) PC 2 Low</td>
<td>d) PC 2 High</td>
</tr>
<tr>
<td><img src="image" alt="Deformation Grid PC2 Low" /></td>
<td><img src="image" alt="Deformation Grid PC2 High" /></td>
</tr>
</tbody>
</table>

This figure shows the deformation grids for our two major principal components, PC1 and PC2. A low score for PC 1 indicates a more elongate opercular flap, while a high score indicates a flap with a less elongate morphology. A low score for PC2 indicates a flap with a squared-off tip, while a high score indicates a flap that was more circular in shape.
**TABLE 6.1: Principal Components of opercular flap shape**

This table shows the principal components produced from the opercular flap data. Only the first two principal components were used.

<table>
<thead>
<tr>
<th></th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
<th>PC5</th>
<th>PC6</th>
<th>PC7</th>
<th>PC8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proportion of Variance</strong></td>
<td>0.4557</td>
<td>0.1997</td>
<td>0.1321</td>
<td>0.1176</td>
<td>0.0576</td>
<td>0.0373</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td><strong>Cumulative Proportion</strong></td>
<td>0.4557</td>
<td>0.6554</td>
<td>0.7875</td>
<td>0.9051</td>
<td>0.9627</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
</tr>
</tbody>
</table>
Results

Sunfish opercular flap variation had an RV coefficient of 0.7395, with a corresponding P value of 0.3087. The minimum RV coefficient was 0.451. This provides no evidence that opercular flap morphology is acting as a morphological unit. Using PC1 and PC2 as well as Procrustes distance we analyzed opercular flap shape using a linear mixed model to determine if opercular flap shape was different between watershed classes. Our fixed effects were watershed class (shiners present, nearby, or distant). We included size as a covariate because sunfish shape changes allometrically, and we wished to avoid the possibility that any observed differences were due to differences in sunfish size among the watershed classes. We excluded watersheds where less than 4 specimens could be photographed. We found no significant differences in procrustes distance or either principal component (Table 6.2) among sunfish in different watershed classes, although as expected, size was highly negatively correlated with PC1 (larger fish had more elongate flaps). A graph showing the PC1 and PC2 scores of all individuals showed no obvious clustering of individuals in different watershed categories in different areas of opercular flap space shape (Figure 6.4).
### TABLE 6.2: Statistical analysis of opercular flap shape using ANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>PC1</th>
<th></th>
<th>PC2</th>
<th></th>
<th>Procrustes Distance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(Num DF, Den DF)</td>
<td>P-value</td>
<td>F(Num DF, Den DF)</td>
<td>P-value</td>
<td>F(Num DF, Den DF)</td>
<td>P-value</td>
</tr>
<tr>
<td>Watershed</td>
<td>1.48(2,702.8)</td>
<td>0.862</td>
<td>1.45(2,620.3)</td>
<td>0.235</td>
<td>0.01(2,638.8)</td>
<td>0.991</td>
</tr>
<tr>
<td>Size</td>
<td>108.79(1,856.3) &gt;0.0001</td>
<td>45.05(1,867.8) &gt;0.0001</td>
<td>0.19(1,834.4)</td>
<td>0.666</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watershed x Size</td>
<td>0.11(2,853.3)</td>
<td>0.893</td>
<td>1.89(2,866.9)</td>
<td>0.151</td>
<td>0.15 (2,824.5)</td>
<td>0.860</td>
</tr>
</tbody>
</table>
FIGURE 6.4: Graph of principal components 1 and 2

This graph shows the principal component scores of individuals in the three watershed classes, with each dot representing one individual. Sunfish from watersheds with shiners present are represented by red dots, nearby by black, and distant by green. The X axis shows PC1 scores, the Y axis PC2 scores.
Conclusions

We found no evidence to support our hypothesis that the presence of shiners on the watershed level affects sunfish opercular flap morphology. Given that we previously observed a preference of shiners for sunfish with longer opercular flaps, our results may indicate that shiners do not have a significant effect on host sunfish fitness. If shiners had a strong positive effect on host fitness, we would expect them to select for even longer opercular flaps. If they had a strong negative impact on host fitness, we would expect them to select for shorter opercular flaps. But if shiners have little effect on the fitness of their hosts, they will not impose any selection pressure at all, and would not have an effect on opercular flap morphology. It is also possible that shiners are too scarce or scattered to have a detectable impact on sunfish morphology. This could occur on two scales. On local scales, if shiners are not sufficiently abundant then only a fraction of sunfish nests will contain shiner eggs, and as a result only a fraction of sunfish will benefit from longer (or shorter) opercular flaps and the strength of selection on the trait may be much weaker. On larger scales, shiners may be present only within certain areas of a watershed, and sunfish collected from other parts of that watershed may experience little or no selection. This could obscure any selective effects by shiners on sunfish at the watershed level. Unfortunately, bluenose shiners are difficult to capture (Albanese et al. 2007) and thus we lack a full understanding of their exact distributions. However, they are known to have a scattered and uneven distribution (Albanese et al. 2007). Because the sunfish I analyzed were captured as far back as the 1950’s, it is particularly difficult to know if shiners were present in the streams they inhabited at the time they were collected. Smaller-scale selective effects could be examined in the future by more in-depth surveys of waterways known to contain bluenose shiners. Because of
the spotty distribution of the species, it is likely that in many watersheds they are present in some water bodies and absent in others. By extensively sampling these waterways it would be possible to determine exactly where bluenose shiners are located, and then to compare the opercular flap length of sunfish in areas with and without shiners.

It is also possible that, despite showing a preference for longer opercular flaps in the lab, shiners do not show such a preference in the field, thereby eliminating any strong selection by shiners on sunfish opercular flap morphology. In locations with suitably clear water, it would be possible to observe shiner response to sunfish with different opercular flap lengths under natural conditions. It might also be possible to survey sunfish nests for presence and quantity of shiner eggs, and determine if a correlation exists between this data and sunfish phenotype. If sunfish nests are highly scattered under natural conditions, it could be argued that shiners would simply not have the opportunity to choose hosts based on opercular flap morphology, but because longear sunfish typically nest in colonies (Keenlyside 1972) it is likely that shiners in the field would at least have the opportunity to make a choice of hosts.

The presence of other nest associates might also complicate the selective forces acting on sunfish. Five other minnows that occur in my study area have been documented laying their eggs in the nests of Lepomis species, including golden shiners (*Notemigonus crysoleucas*), redfin shiners (*Notropis umbratilis*), dusky shiners (*Notropis cummingsae*), pretty shiners (*Lythrurus bellus*), and red shiners (*Cyprinella lutrensis*) (Fletcher 1993, Hunter and Hassler 1965, Johnston and Page 1992, Shao 1997b, natureserve.org). Golden shiners are widely distributed across all watersheds included in my study and thus they are likely to have the same selective effect on sunfish from all watersheds. Redfin shiners and red shiners are found mostly to the west of my
watersheds, but both occurred in the same 4 “distant” watersheds. Dusky shiners occur mostly to the east of my study region, but are found in 3 watersheds classed as “present”. Pretty shiners were found in 6 “present” and 9 “near” watersheds but no “distant” watersheds. They did not overlap in range with dusky shiners. Although it is possible that these shiners could be selecting for changes in opercular flap length, the fact that they were not highly concentrated in any specific watershed category makes it unlikely that they would skew my results by selecting disproportionately on any one watershed category.

Finally, it is possible that opercular flap length has low heritability in this system, making it insensitive to selection by bluenose shiners. Unfortunately, no research has been done on the heritability of this trait in any *Lepomis* species. Opercular flap length varies substantially between different *Lepomis* species and between *L. megalotis* populations (Jennings 1991), indicating that *Lepomis* has evolved differences in flap length in the past, but the heritability of this trait at present is not known. Although we did not reveal evidence to support our hypothesis that shiners select for changes in sunfish opercular flap morphology across watersheds in the southeastern United States, further research into this system is warranted to distinguish between the possible explanations for why no selective effects were observed. Documentation of the heritability of opercular flap length in longear sunfish would clarify how strongly selection could affect this trait. Experimental testing of bluenose shiner effects on host fitness similar to what has been done in other nest associates (Fletcher 1993, Johnston 1994a) could directly determine if shiners a strong or minimal effect on sunfish fitness. If shiners have a minimal effect on sunfish fitness that is likely to explain the lack of observed changes in sunfish opercular flap shape. If shiners have a strong effect on sunfish fitness, a survey of sunfish nests in areas known to
contain shiners would allow for an estimation of rates of interaction between shiners and sunfish. 

If shiners prove to be rare visitors to sunfish nests, this could explain the lack of selective effects. 

If shiners are locally abundant and visit a large proportion of sunfish nests, comparisons of 
opercular flap morphology of sunfish in specific water bodies known to have a large population 
of shiners with those in water bodies known to lack shiners might pick up localized effects of 
selection that are obscured at the watershed level.
References:


CHAPTER 7
CONCLUSION

The goal of this dissertation was to determine how conspecifics and heterospecifics shape the morphology, physiology, and behavior of individuals with whom they interact over the course of development, during adult interactions, and over evolutionary time. I utilized the unique reproductive biology of the mangrove rivulus (Kryptolebias marmoratus) to compare the development of genetically identical individuals in social and nonsocial environments, and found treatment differences in growth and behavior. I examined the effect of the social environment on the extended phenotype of fish by measuring parameters of nest construction by longear sunfish (Lepomis megalotis), finding some non-significant but potentially biologically relevant decreases in nesting frequency in response to egg predators. Finally, I generated a novel conceptual model for explaining how heterospecific eavesdropping selects for changes in sexual signals and tested its predictions in a potential mutualism, a type of system that had not previously been examined for eavesdropping. I found evidence of eavesdropping by bluenose shiners (Pteronotropis welaka) on their hosts, longear sunfish, but failed to find evidence that selection had driven the type and magnitude of morphological change predicted by my model.

Effects of social environment on development

The first chapter of my dissertation examined how the social environment affects morphological, behavioral, and physiological development in the mangrove rivulus. In two
parallel experiments, I raised isogenic lines of mangrove rivulus from hatching to near maturity in social environments and in isolation. Because self-fertilization among mangrove rivulus produces lineages of genetically identical individuals (Earley et al. 2012), I was able to generate reaction norms for both genotypes used in the experiment in response variation in social conditions experienced during development. Vertebrate-specific behavioral or physiological processes cannot be fully understood using clonal lines of plants, invertebrates, or cells, but genotype-level reaction norms are difficult to obtain from vertebrate organisms because most species do not naturally produce isogenic lineages (Vrijenhoek et al. 1989), which forces researchers to rely largely on population-level reaction norms, twin studies, or inbred laboratory lineages (Earley et al. 2012, Stamps 2003). My research adds to our knowledge of how differences in social environment can produce differences in phenotype in vertebrates when genetic variance is completely controlled.

This research is also valuable because it sheds light on the social structure of mangrove rivulus. These fish are widely considered to be asocial (Mackiewicz et al. 2006) and are often housed in complete isolation in laboratory settings. However, we found that isolated individuals grew more slowly and resembled subordinate individuals kept in groups. This may indicate that isolation is stressful to these fish, or reduces growth in some other way. Together with field research (Taylor 2012) the data that I collected suggest that mangrove rivulus are not as asocial as previously understood, which has implications for how relevant their behavior in laboratory tests of aggression (following maintenance in isolation) is to the behavior they exhibit in the field. It also highlights the need to consider the impact of social interactions even in organisms that do not live in cohesive groups. Further research is needed to determine the mechanism
driving decreased growth in subordinate individuals, as there was no obvious increase in baseline stress hormones and food was available to all fish in equivalent amounts. Other measurements of stress such as metabolic rates would be useful, as would observations of growth in rivulus kept at different densities under more natural conditions.

Phenotypic plasticity can drive the evolution of novel phenotypic variants and can promote speciation. An interesting implication of this research is that plastic responses to the social environment might produce variation in a population that would act as a starting point for the evolution of new traits or for population divergence (Pfennig et al. 2010). Both of my experiments began with fish that were same in nearly every respect: genetically identical, the same age, and often from eggs laid in the same clutch. Despite this, not only did fish exposed to social environments differ from fish held under isolation, but social fish showed greater variation between individuals in a group. The variation produced by the social environment could potentially lead to the evolution of different phenotypes in rivulus if previously plastic traits become fixed regardless of social environment (Foster 2013).

There is much more that could be done using this promising model organism to study topics related to the long-term impact of social interactions. Since my experiments were performed, other research has shown that different lineages of mangrove rivulus exhibit different life history strategies and vary in growth rate and reproductive output (Garcia 2014). By testing these strains we could gain insight into how life history traits correlate with individual responses to the social environments that they experience during early life. Rivulus in the wild also occur at highly variable population densities (Taylor 2012), and now that we have genotypes from a greater variety of locations we could see if the response of rivulus to conspecifics is related to the
population density at which they are typically found, perhaps indicating some level of density-dependent selection on phenotypically plastic responses. Our research also followed mangrove rivulus to the cusp of maturity, but social environment is also likely to have interesting impacts on the phenotype of sexually mature animals. Mangrove rivulus occur as either males or hermaphrodites. Males are produced either through development (primary males) or via sex change from hermaphrodites (secondary males)(Harrington 1967), and it would be interesting to understand how the social environment might affect sexual differentiation and transitions between the sexes, as it does in various other fish species (Black et al. 2005, Mackie 2003, Hobbs et al. 2004).

**Effects of social environment on extended phenotypes**

The second chapter of my dissertation examined how the presence of egg predators affected nest construction by longear sunfish. Male longear sunfish dig nests, which house eggs and young fry, and defend these from predators (Gill 1906, Witt and Marzolf 1954). I hypothesized that when egg predators were present sunfish would alter the frequency at which they dug nests and the locations where those nests were placed. I allowed fish to construct nests in the presence of egg predators (juvenile sunfish) and observed the frequency, size, and placement of nests constructed. Many other studies of how nest construction by fish is affected by predators have focused on predators that threaten adult fish (Candolin and Voigt 1998, Jones and Paszkowaki 1997, Winkelman 1996). My study instead provides information about responses to predators of fry and eggs. Because adult and hatchling fish vary dramatically in size, fry and eggs are threatened by a different set of predators than adults. Host fish are likely to have a different response to these predators, and this study helps fill a gap in our knowledge.
about that response. Although this experiment found no significant results, it was still one of the few experiments documenting changes in nest production by fish in response to nest predators, as opposed to predators on adults.

Although I failed to find significant results in this particular experiment, I did see a non-significant but potentially ecologically relevant decrease in nest construction when predators were present. Repeating the experiment at a different time of year might increase nest-building rates. I performed my experiments in late fall, outside the normal breeding season, although I did alter light cycle and temperature to match what fish would have experienced during the breeding season. More replication might also have helped detect effects that were obscured by the low rate of nest building in our sample. A power analysis indicated that a sample size of 100 would be sufficient to detect statistically significant differences between treatments with the effect size that we observed. The experimental procedure I used could also prove useful for understanding changes in nest building in response to other types of organisms. For example, egg predators such as snails or crayfish, which move along the substrate, might induce a different type of response, as might cues from predators on adult fish, such as basses, water snakes, and herons. The response of sunfish to potential nest associates could also be tested.

**Effects of heterospecific eavesdroppers on sexual signals**

The third chapter of my dissertation described a new conceptual framework for understanding how heterospecific eavesdropping drives selection on sexual signals. Previous descriptions of heterospecific eavesdropping focused on specific types of eavesdropping, often eavesdropping used by predators and parasites to locate prey (Komarova and Levin 2010, Peake
However, prey and competitors also eavesdrop, and some eavesdroppers avoid signals (Fletcher 2007, May et al. 2012, Moosman et al. 2009). My work is the first to look at all these phenomena under a single framework, allowing predictions to be made regarding how a full range of eavesdropping interactions might exert selection on sexual signals. This is valuable because it highlights significant gaps in our knowledge of the selective forces imposed by eavesdroppers. While a few studies have demonstrated selection by predators attracted to prey signals (Endler 1980, Zuk et al. 2006), no studies have yet examined selection by predators that avoid prey signals or selection by prey on predator sexual signals. The selective effects of eavesdropping by competitors or by mutualists have also not been investigated.

Chapters 4 and 5 of my dissertation tested the conceptual framework that I developed using a pair of species, bluenose shiners (nest associate and potential mutualist) and longear sunfish (host), in which the shiner spawns in the nests of longear sunfish. The sunfish then care for their own eggs and the shiner eggs (Johnston and Knight 1999, Shao 1997). In Chapter 4, I tested for eavesdropping by bluenose shiners on the sexual signals of their hosts, longear sunfish. I uncovered evidence that bluenose shiners exhibit a preference for male sunfish with longer opercular flaps, which mirrored the preference of female sunfish (Goddard & Mathis 1997). This is both the first observation of eavesdropping by a potential mutualist and the first observation of eavesdropping in a nest association system. The nature of eavesdropping we found was also noteworthy: instead of merely exhibiting greater taxis toward larger opercular flaps, shiners exhibited a context-dependent response that indicated they were using signals to assess signaler quality rather than merely locate signalers. This is one of the few eavesdropping systems where assessment of this kind has been observed.
Based on the eavesdropping observed in Chapter 4, I predicted in Chapter 5 that bluenose shiners should exert strong selection on longear sunfish opercular flap lengths. Using preserved specimens, I compared opercular flap morphology between sunfish from watersheds that contained bluenose shiners, watersheds lacking shiners, and watersheds outside of the described shiner range. Although I failed to find morphological variation indicative of strong selection by shiners on the sexual signals possessed by sunfish, this research was an important attempt to examine the effects of heterospecific eavesdropping-driven selection in the wild. As I noted in Chapter 3, many studies document the behavior of eavesdropping, but relatively few look for evidence of selection resulting from eavesdropping interactions. This is especially true for systems where eavesdroppers are not clearly predators or parasites. My research helps to fill this gap, although our lack of significant results implies that not all eavesdropping interactions exert strong enough selection to produce observable divergence in sexually selected characters among populations. There are a few possible reasons for this. Shiners may not alter the reproductive success of sunfish enough to produce strong directional selection, or may be too rare to have an impact on the population as a whole. Alternatively, gene flow between areas with shiners and without might obscure any selective effect.

There is much more to be learned about the fitness effects of heterospecific eavesdroppers on signalers. Direct measurements of eavesdropper impacts on signaler fitness are vitally important to understand the ecological and evolutionary implications of heterospecific eavesdropping. Comparisons of eavesdropper phenotypes in populations under different eavesdropping pressures provide insight into how strongly heterospecifics affect signals under natural conditions. These research techniques have already provided valuable information about
the effects of eavesdropping predators and parasites on sexual signals, but they still need to be applied to competitors, prey, and mutualists. Only then will a full picture of heterospecific selection on sexual signals emerge.
References


