

Article

# The pervasive effects of lighting environments on sensory drive in bluefin killifish: an investigation into male/male competition, female choice, and predation

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Handling editor: Zhi-Yun Jia

Received on 19 February 2018; accepted on 17 May 2018

## Abstract

Sensory drive predicts that the conditions under which signaling takes place have large effects on signals, sensory systems, and behavior. The coupling of an ecological genetics approach with sensory drive has been fruitful. An ecological genetics approach compares populations that experience different environments and asks whether population differences are adaptive and are the result of genetic and/or environmental variation. The multi-faceted effects of signaling environments are well-exemplified by the bluefin killifish. In this system, males with blue anal fins are abundant in tannin-stained swamps that lack UV/blue light but are absent in clear springs where UV/blue light is abundant. Past work indicates that lighting environments shape genetic and environmental variation in color patterns, visual systems, and behavior. Less is known about the selective forces creating the across population correlations between UV/blue light and the abundance of blue males. Here, we present three new experiments that investigate the roles of lighting environments on male competition, female mate choice, and predation. We found strong effects of lighting environments on male competition where blue males were more likely to emerge as dominant in tea-stained water than in clear water. Our preliminary study on predation indicated that blue males may be less susceptible to predation in tea-stained water than in clear water. However, there was little evidence for female preferences favoring blue males. The resulting pattern is one where the effects of lighting environments on genetic variation and phenotypic plasticity match the direction of selection and favor the expression of blue males in swamps.

**Key words:** *Lucania goodei*, adaptive plasticity, intrasexual selection, male competition, private communication, predation

Twenty-five years ago, John Endler published his seminal paper on sensory drive in the American Naturalist (Endler 1992). In that paper, Endler introduced concepts developed in the field of sensory ecology (Duntley 1951; Mertens 1970; Lythgoe 1979; Lythgoe

1988) to an ecological and evolutionary audience. Endler particularly emphasized the role of the environmental conditions on signaling dynamics. Applied to a sexual selection context, sensory drive states the following: Males have traits that they use as signals to obtain

mates. These signals are displayed at various times and places and then must travel through the environment and be detected by the sensory system of the receiver. These sensory properties have strong influences on a number of behaviors including (a) non-mating traits such as the ability to find food, find proper habitat, avoid predators, etc. and (b) mating behaviors such as female mate choice and male/male competition. Mating behaviors create sexual selection favoring certain male traits over others. The evolution of particularly conspicuous male traits also has the potential to increase male susceptibility to predation. Since its publication, sensory drive has been cited over 1,300 times and in varying ways, ranging from mechanistic studies of signal generation and sensory system properties (Stieb et al. 2016; Escobar-Camacho et al. 2017; Phillips and Derryberry 2017) to comparative studies of signal and sensory system properties across broad phylogenetic distances (Ryan and Keady-Hector 1992; Gomez and Thery 2007; Stuart-Fox et al. 2007; Ord et al. 2015; De Lanuza and Font 2016; Gawryszewski et al. 2017; Buchinger et al. 2017; Strauss et al. 2017; Stanger-Hall et al. 2018).

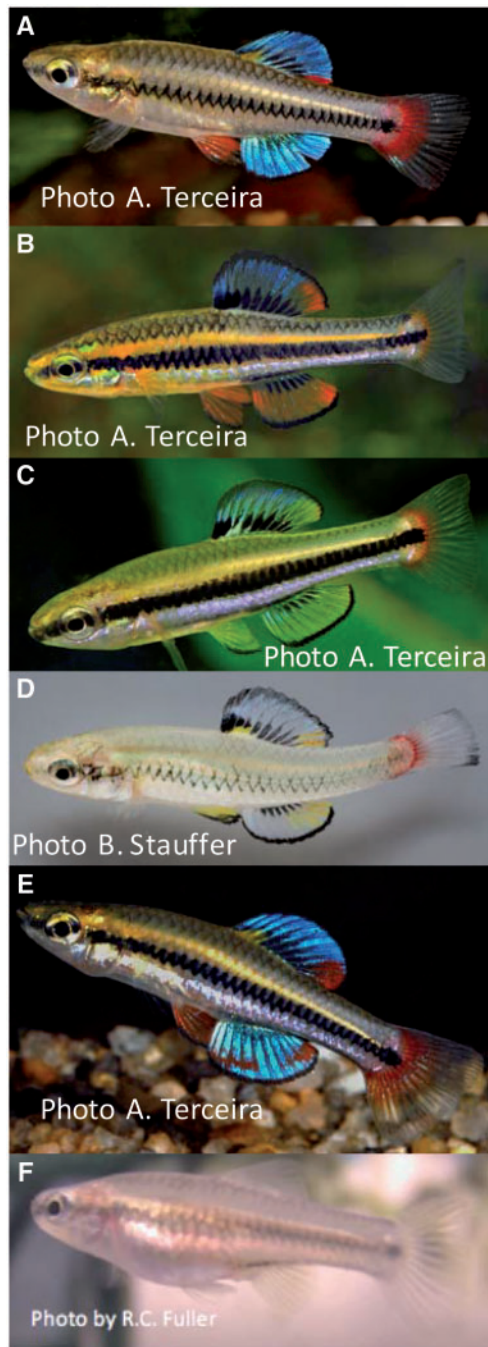
The application of an ecological genetics approach to questions surrounding sensory drive has been fruitful (Marchetti 1993; Endler and Houde 1995; Endler 1995; Houde 1997; Boughman 2001; Leal and Fleishman 2002; Seehausen et al. 2008; Servedio and Boughman 2017). An ecological genetics approach relies on identifying traits that co-vary with environmental variables across populations or closely related species and asking (a) whether the correlation between trait values and environment are driven by natural and/or sexual selection and (b) whether trait variation is due to genetic and/or environmental effects (Travis and Reznick 1998; Reznick and Travis 2001). The merger of sensory drive and ecological genetics highlights the multi-faceted effects of environmental variation. Variation in environmental signaling conditions can result in genetic differentiation among populations and closely related species (Lythgoe et al. 1994; Cronin et al. 1996; Endler et al. 2001; Seehausen et al. 2008; Knott et al. 2017; Nandamuri et al. 2017); it can alter the development of signals, sensory systems, and associated behaviors (Kroger and Fernald 1994; Cronin and Caldwell 2002; Fuller and Travis 2004; Hofmann and Carleton, 2009; Knott et al. 2010; Ziegler et al. 2011; Ehlman et al. 2015; Sandkam et al. 2016; Wright et al. 2017; Wright et al. 2018); and it can alter the immediate perception of signals by altering their transmission and the backgrounds on which they are perceived (Long and Houde 1989; Seehausen and Van Alphen 1998; Maan et al. 2006; Reichert and Ronacher 2015). Differences in lighting environments can even alter survival in the absence of predation (Maan et al. 2017). Hence, signaling environments can affect among population genetic variation, phenotypic plasticity, and the direction of selection.

These multi-faceted effects are well-exemplified by the variation in color patterns, color vision, and visually based behaviors present in the bluefin killifish *Lucania goodei*. In this article, we first review the previously published literature on (a) the among population correlations between color patterns, visual systems, and signaling environments and (b) the multi-faceted effects of lighting environment on the phenotypic expression of male coloration, visual system properties, and visually based behaviors. We then present the results of three new experiments that examine the effects of lighting environment on male/male competition, female mate choice, and predation risk. We note that many studies examine the effects of lighting environments on female mating preferences (Long and Houde 1989; Gamble et al. 2003; Maan et al. 2006; Maan and Cummings 2009), but few examine the effects of lighting environment on male/male competition. Of course, in many systems, male coloration is

associated with the outcome of male competition (Andersson 1994; Santos et al. 2011; Crothers and Cummings 2015; Johnson and Fuller 2015; Zhou and Fuller 2016). However, the role of natural variation in lighting environments on the outcome of male/male competition has received little attention. Tinghitella et al. (2015) recently tested whether three-spined stickleback males with red throat color had a competitive advantage over males lacking red throat color in full spectrum versus red-shifted light (where red throats are absent), but found no effect of lighting environment. Several studies have manipulated lighting environments to disrupt male/male signaling by eliminating the ability of animals to use specific wavelengths and color contrasts (Evans and Norris 1996; Baube 1997; Braun et al. 2014; Zhou and Fuller 2015), but few have mimicked natural variation in lighting environments to assess its likely effects on male competition in the wild. The pattern that emerges is one where the direction of selection due to predation, male competition, and to a far lesser extent, female mate choice coincides with the patterns of phenotypic plasticity and genetic variation that favor the presence of killifish with blue anal fins in swamps.

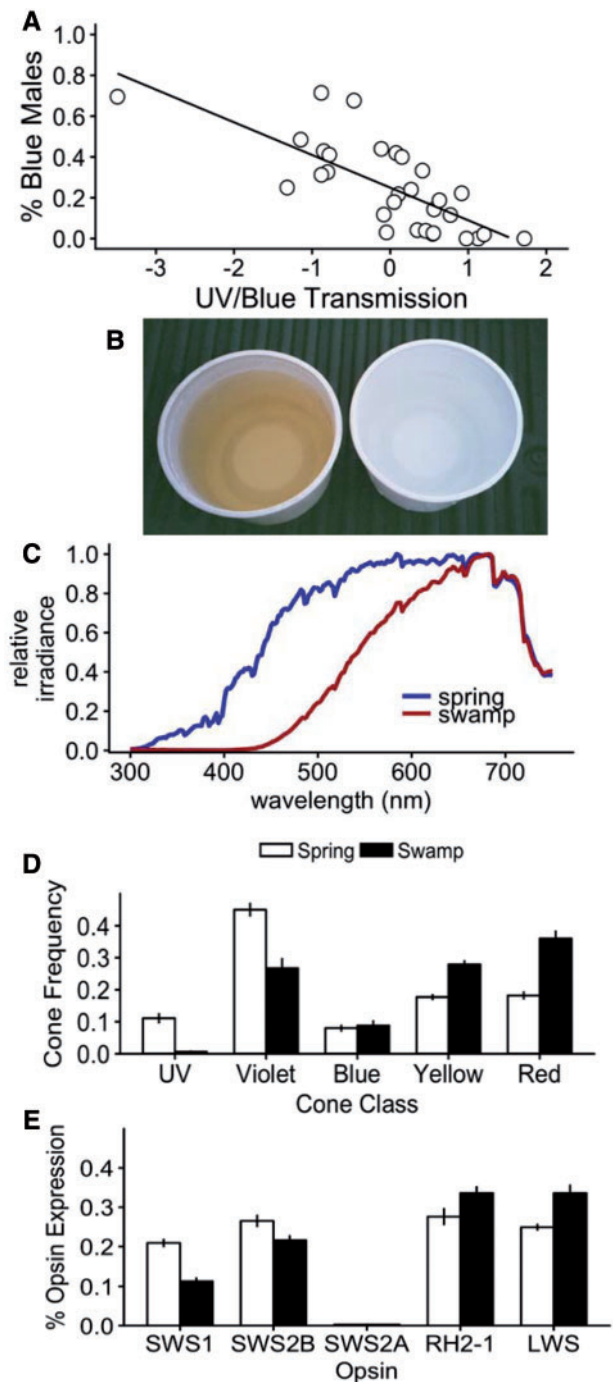
Male color patterns are extremely variable within and among populations of bluefin killifish, *Lucania goodei* (Foster 1967; Arndt 1971; Fuller 2002). Figure 1 illustrates the variation in male coloration. The other intriguing aspect of this system is that bluefin killifish populations are found in a variety of lighting habitats including crystal clear springs and tannin-stained swamps (Figure 2). Here, we focus on the patterns in male anal fin coloration, which, at first glance, present a paradox: blue males are abundant in tannin-stained swamps that have low abundance of UV/blue wavelengths and where males and females are less sensitive to those wavelengths of light. Figure 2A shows the relationship between the abundance of males with blue anal fins and the relative transmission of UV/blue wavelengths across 30 populations in Florida. Blue males are common in swamp populations that have low transmission of UV/blue wavelengths, and are rare or absent in springs with high transmission of UV/blue (Fuller 2002). Figure 2B shows samples of swamp and spring water in white buckets. Figure 2C shows the relative down-welling irradiance spectrum in a spring and a swamp population (see Supplementary materials and Supplementary Figure 1 for details and absolute irradiance). The visual systems also vary between springs and swamps (Fuller et al. 2003, 2004). Bluefin killifish possess five broad classes of cones: UV, violet, blue, yellow, and red. Spring animals have a higher relative frequency of UV and violet cone cells, whereas swamp animals have a higher relative frequency of yellow and red cone cells (Figure 2B). The pattern of opsin expression reflects this variation in cone cell types with higher proportional expression of the SWS1 and SWS2B (the opsins involved in UV and violet cone cells) in springs and high proportional expression of RH2-1 and LWS (the opsins involved in yellow and red cone cells) in swamps (Figure 2C). Electroretinogram studies also indicate that swamp animals are less sensitive to UV/blue light than spring animals (Fuller et al. 2003). These population patterns present somewhat of a paradox, because blue males are common in habitats that lack UV/blue light and where animals are less sensitive to those wavelengths.

This paradox is potentially resolved when we consider that color patterns can create high chromatic contrast without being particularly bright. In fact, similar phenomena have been noted in birds where brighter warbler species live in darker habitats (Marchetti 1993), in sticklebacks where males with red throats are found in clear water with high levels of blue scattering and are absent in red-shifted waters (Reimchen 1989; Boughman 2001), in sticklebacks



**Figure 1.** Bluefin killifish color morphs. (A) Blue color morph, (B) red color morph, (C) yellow color morph, (D) yellow–blue morph, (E) Red–blue morph, (F) female. Photos A, B, C, E by A. Terceira, photo D by B. Stauffer, photo E by R.C.F. Note that blue males may also have yellow pelvic fins and/or yellow rear dorsal fins.

where blue opercle coloration increases with depth and red-shifted light (Brock et al. 2017), in the cichlid species group *Pundamilia* where blue males are found in orange-tinted waters (Seehausen et al. 2008), and in surfperch where visual pigments match the ambient light spectrum yet visual signals are outside of the color realm of the background light (Cummings and Partridge 2001; Cummings 2007). In fact, in another seminal paper “The Color of Light in Forests and Its Implications”, Endler (1993b) predicted that total



**Figure 2.** (A) The proportion of males that are blue in different populations as a function of UV/Blue transmission (Fuller 2002); each dot shows the relative abundance of blue males in a single population. (B) Swamp and spring water in white buckets. (C) The relative downwelling irradiance at 25.4 cm depth in a spring and a swamp. Curves are scaled to the maximum absolute irradiance. See Supplementary Figure 1a for absolute curves. (D) The average cone frequency in the eyes of animals from a spring and a swamp population (Fuller et al. 2003). (E) Proportional opsin expression for animals from a spring and a swamp (Fuller et al. 2004).

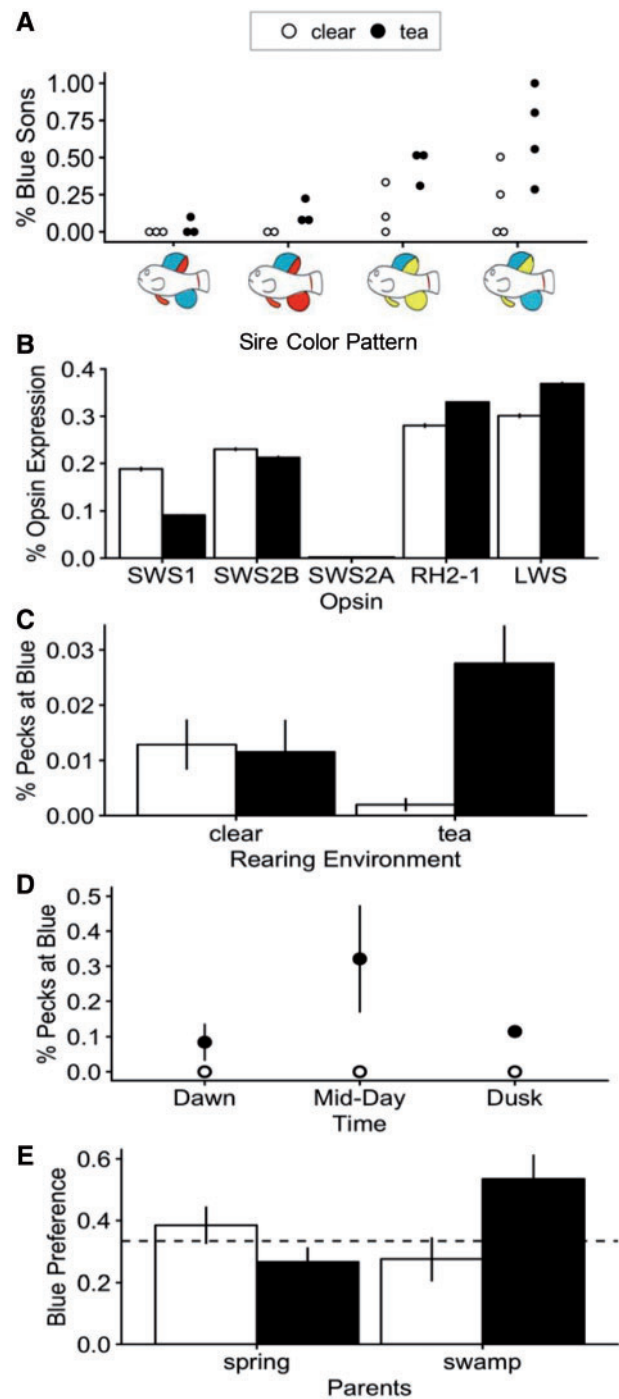
brightness will be greatest when the shape of the reflectance spectrum and the ambient light spectrum are similar but that contrasts will be greatest when the shape of the reflectance spectra differs from the shape of the ambient light spectra. In the case of bluefin

killifish, blue males most likely create high contrast against a tannin-stained water column. In addition, due to color constancy, individuals may be particularly sensitive to blue wavelengths precisely because they are rare. Color constancy is the phenomenon where the visual system differentially weights visual inputs to ensure that white is always perceived as white even under diverse illuminant (Cronin et al. 2014). In tannin-stained swamps with little UV/blue, individuals must heavily weight the UV/blue inputs (i.e., the signals from the rare wavelengths) to create color constancy. This may create a situation where swamp animals are particularly sensitive to UV/blue color signals. We note that this remains to be tested. We now turn our attention towards the effects of genetic variation and phenotypic plasticity with respect to lighting environments on male color pattern, opsin expression, foraging behavior, and mating behavior.

The variation in male coloration is attributable to genetic variation, phenotypic plasticity with respect to lighting environment, and genetic variation in phenotypic plasticity (Fuller and Travis 2004). All males have the ability to produce either yellow or red pterin, and crosses indicate that there is a locus of large effect where the yellow allele is dominant to the red allele (Fuller and Travis 2004; Johnson and Fuller 2015). When males are raised in clear water in the laboratory, they nearly all express red or yellow coloration on their anal fins, and all males, regardless of lighting environment, express red or yellow coloration on their pelvic fins (Fuller and Travis 2004). The coloration in the pelvic fins is perfectly correlated with the coloration in the anal fin. That is, males with red anal fins always have red pelvic fins, and males with yellow anal fins always have yellow pelvic fins. Johnson and Fuller (2015) extracted pigments from the anal fins and analyzed them with spectroscopy. We showed that the red pterin is likely drosopterin, and yellow pterin is likely xanthopterin. Red males express both drosopterin and xanthopterin, whereas yellow males only express xanthopterin. However, the red/yellow anal fin coloration can be masked by the expression of blue.

Blue expression has both genetic and environmental components. Fuller and Travis (2004) conducted a breeding study where they created paternal half-sib families and raised half of the offspring in clear water, which mimics springs, and half of the offspring in tea-stained water, which mimics swamps. Note that throughout this article, we use the term “tea-stained” to refer to water, where we experimentally manipulated the lighting environment by adding instant tea. We use the term “tannin-stained” water to refer to water from nature that is naturally stained due to dissolved organic materials. Fuller and Travis (2004) showed that phenotypic plasticity favored blue males in tea-stained water. Specifically, blue sons were often produced in the tea-stained treatment but were rare in the clear water treatment (Figure 3A). There was also genetic variation in phenotypic plasticity. The sons of some sires were non-responsive to the variation in lighting environment. Hence, there was genetic variation, phenotypic plasticity, and genetic variation in phenotypic plasticity. Subsequent studies have found that phenotypic plasticity is also variable among populations (L.M., C. Chang, R.C.F., unpublished result). Most important, the direction of phenotypic plasticity favors the production of blue males in tea-stained water.

Opsin expression is also influenced by both phenotypic plasticity and genetic variation, but here there is little evidence for genetic variation in phenotypic plasticity (Fuller et al. 2005a, 2010; Fuller and Claricoates 2011; Johnson et al. 2013). Lighting environments have large effects on opsin expression, where there is high proportional expression of SWS1 (the opsin responsible for UV



**Figure 3.** (A) Sons are more likely to express blue coloration when raised in tea-stained water. Genetic effects due to sires are also present (Fuller and Travis 2004). Each dot represents a clutch. (B) Opsin expression varies due to rearing environment (Fuller et al. 2005). (C) Killifish are more likely to peck at blue dots when raised and tested in tea-stained water (white bars = clear testing environment; dark bars = tea testing environment) (Fuller et al. 2010). (D) Killifish from a variable lighting population only peck at blue when tested in tea-stained water and are more likely to peck at mid-day (Johnson et al. 2013). (E) Preference for blue males is highest when swamp females are raised and tested in tea-stained water (Fuller and Noa 2010). The graph shows means from the tea-stained testing environment as a function of clear (open) and tea-stained (dark) rearing environments. The dotted line indicates the null expectation for no preference.

photopigment) in clear water and high proportional expression of RH2-1 and LWS (the opsins responsible for the green and red photopigment, respectively) in tea-stained water. The among population pattern of opsin expression (high SWS1 and SWS2B expression in springs; high RH2-1 and LWS expression in swamps; Figure 2C) can be largely recapitulated by raising animals in a greenhouse in either clear or tea-stained water (Figure 3B) (Fuller et al. 2004, 2005a). In addition, there is good evidence that diurnal rhythms have very large effects on opsin expression (Johnson et al. 2013).

Lighting environments also have multi-faceted effects on both foraging and mating preferences. Our group used a large breeding design to examine the roles of genetics (population of origin), rearing environment (clear or tea), and testing environment (clear or tea) on foraging and mating preferences (Fuller and Noa 2010; Fuller et al. 2010). This allowed us to examine genetics, developmental plasticity as a function of lighting environment, and the immediate effects of lighting environment on visually based behaviors. For foraging preferences, we dropped a clear petri dish to which we had attached different colored dots (red, orange, yellow, green, blue, black, white) into the water and counted how often the fish pecked at each dot (Fuller et al. 2010). The fish pecked at these dots as if they were pecking at food. For foraging, there were no effects of genetics, but there were large effects of rearing environments. For example, individuals were more likely to peck at red (and less likely to peck at yellow) dots when reared in tea-stained water. In addition, there were strong interactions between rearing and testing environment that suggested that lighting environments have strong effects on the development of the visual system that result in different visually based behaviors depending on the immediate testing environment. Killifish pecked more at blue dots when they were raised and tested in tea-stained water (Figure 3C; Fuller et al. 2010). Another experiment used animals from a “variable” population (a tannin-stained river adjacent to a clear spring) and tested pecking preferences in clear and tea-stained water at dawn, mid-day, and dusk (Johnson et al. 2013). That study found that killifish never pecked at blue dots in clear water. Instead, they pecked at blue dots in tea-stained water, particularly at mid-day (Figure 3D). Why these patterns emerge is unclear as bluefin killifish do not have blue food. However, it is intriguing that preferences for blue inanimate objects consistently arise in tea-stained treatments.

Finally, a complex interaction between genetics, rearing environment, and testing environment affect female mating preference for blue males (Fuller and Noa 2010). Female mating preferences were measured in no-choice spawning assays where the number of eggs laid with either blue, yellow, or red males in clear and tea-stained water was taken as a measure of preference. Overall mating preferences were weak, but the highest level of mating preferences for blue males were found in females from swamp parents that were raised and tested in tea-stained water (Figure 3E). The implication was that preference for blue males is only expressed when females have the right combination of genetics, rearing environment, and testing environment (Fuller and Noa 2010).

The patterns of genetic variation, phenotypic plasticity, and genetic variation in phenotypic plasticity across multiple traits favor the presence of blue males in swamps. However, less is known about the selective forces at play in this system. Our previous work indicated that female preference may favor blue males in swamps, but the effects were small. Little is known about the effects of lighting environments on male/male competition or predation. Below, we present the results of three new experiments that fill in these gaps.

These experiments examine the effects of lighting environments on male competition, female mating preference, and predation. Of these three experiments, the male/male competition experiment is genuinely novel. In bluefin killifish, male competition has a large influence on the outcome of mating success (Mcghee et al. 2007; Mcghee and Travis 2010, 2011; Johnson and Fuller 2015). Yet, few studies have experimentally manipulated lighting environments to mimic natural conditions and subsequently found dramatic effects on male/male competition. Our study below shows that lighting environments alter the outcome of male/male competition and favor the presence of blue males in swamps.

## Materials and Methods

To examine male/male competition in different lighting environments, we placed male bluefin killifish with blue anal fins in trials that varied in (a) lighting environment (clear versus tea-stained water) and (b) rival competitor color (males with solid yellow or red anal fins). To examine female mating preferences, we performed no-choice mating assays where we placed a single female with either a red, yellow, or blue male under clear and tea-stained water conditions and measured the number of eggs produced. To examine the roles of predators, we utilized a behavioral assay where largemouth bass could strike at different color morphs held in clear, plastic boxes in either clear or tea-stained water. We describe these three experiments below. For simplicity, we only considered males with solid blue anal fins and excluded males with combination red–blue or combination yellow–blue anal fins. Throughout the article, “tea-stained” refers to experimentally manipulated water in the lab. “Tannin-stained” refers to water in nature that is heavy in tannin levels and is typically found in swamps.

## Collection

For the male competition and female mate choice studies, we collected bluefin killifish using dipnets and seines from a swamp population (26-Mile Bend, Everglades Drainage, Broward Co., FL, USA) and a spring population (Guaranto Springs, Suwannee River Drainage, Dixie Co., FL, USA). Guaranto Springs is unique because it is a clear spring population that is connected to the Suwannee River, which is tannin-stained during wet years. Upon collection, fish were held in water from the site in coolers and immediately transported to the lab at the University of Illinois at Urbana-Champaign. In the lab prior to experimentation, fish were maintained in 114 L (29 gallon) tanks in a naturally lit, temperature controlled greenhouse and fed frozen brine shrimp daily. Killifish originating from the swamp population were kept in tea-stained water. Killifish originating from the spring populations were kept in clear water. The maximum density of fish in a tank is 1 fish per 3.8 L (1 gallon) of water. Both males and females were housed together in stock tanks to prevent females from becoming egg bound. Hence, females were housed with the male color morphs from their own populations. Stock tanks were regularly monitored, and fish had access to naturally occurring algae and invertebrates. Killifish were allowed to acclimate to the laboratory for two weeks before beginning behavioral assays.

## Male competition

Our goal was to determine the effect of male anal fin coloration on the outcome of male/male competition and whether this varied as a function of lighting environment. To do this, we allowed two males

to compete in the presence of one female in either clear water, which mimics springs, or tea-stained water, which mimics swamps, and determined the effect of anal fin coloration on male dominance in different environmental lighting conditions. We used Lipton Instant decaffeinated tea powder to create tea-stained water. We periodically added tea to the tanks so that the water mimicked the appearance of iced tea. This was necessary because bacteria degrade the tea in the water. The same phenomenon occurs in natural populations where bacteria consume dissolved organic materials. The addition of instant tea to the water mimics the natural lighting environments of swamps (see bass predation experiment below, Figure 2C, and Supplementary file 1A–B for representative irradiance spectra for quantification of irradiance). We used UV sterilizers to remove algae and bacteria from the water column to maintain our treatment effects.

We selected eight blue color morphs from each population to use as focal males. For each trial, the focal male was paired with a competitor male (either a red or yellow male) and a female from the same population. Each focal male was paired with each color morph (red and yellow) in clear and tea-stained water resulting in 4 trials per male (2 competing color morphs  $\times$  2 lighting environments = 4 trials per focal male). One focal male from the spring population died after completing only three trials. A total of 63 trials were conducted (8 males  $\times$  2 populations  $\times$  2 lighting treatments  $\times$  2 color morph competitors minus one missing trial). The order of the pairings (red or yellow competitor) and light treatments (clear or tannin-stained) were randomized for each male. Male ID was treated as a random effect in subsequent analyses.

Before beginning trials, we separated males into 38 L aquaria and visually isolated them from all other fish. Trials occurred in 114 L aquaria with naturally occurring algae and invertebrates. Each tank contained yarn mops (i.e. several  $\sim$ 12-inch pieces of yarn tied together) which served as spawning substrates. The spawning substrates were attached to either Styrofoam balls so that they floated or to small pieces of PVC pipe so that they sunk. The spawning substrates provided a place for fish to attach eggs and also provided refuge to hide from other aggressive fish.

The first author observed each set of killifish once each day for twenty minutes between the hours of 08:00 and 12:00 for 3 consecutive days and recorded the number of male aggressive behaviors during each observation period. These behaviors included: fin flares, chases, and attacks resulting in physical contact towards the competing male and stimulus female (noted as aggressive behaviors in: Johnson and Fuller 2015). We used the aggressive behavior counts to determine male dominance. The male who performed the most aggressive acts was noted as dominant. The males typically established dominance relationships within the first day. No males reverted between dominant and subordinate status during the 3 days. We also recorded courtship behaviors as the time spent within one body length of the stimulus female, the number of courting bouts (head flicks and body loops towards female), and the number of spawns. Due to low numbers of observed spawns, we did not consider this variable further. Following the completion of all behavioral assays, fish were euthanized using an overdose of buffered MS-222.

We calculated standard length (from the tip of the snout to the caudal peduncle) for every fish in every trial. Standard length did not differ among color morphs ( $F_{2,39}=2.41$ ,  $P=0.10$ ) or as an interaction between color morph and population ( $F_{2,39}=1.72$ ,  $P=0.19$ ), but it did differ between populations where the swamp fish were slightly larger than spring fish ( $F_{1,39}=5.56$ ,  $P=0.024$ ,

swamp mean = 26.8 mm, spring mean = 25.4 mm). While there was no systematic difference in size among the color morphs, we found that blue males were more likely to emerge as dominant when they were larger than their competitors. We therefore included the difference in size between the blue male and the competitor as a covariate in all of our models.

We tested whether the likelihood of blue males emerging as dominant and courting females was affected by lighting environment (clear or tea-stained), the color of the competing male (red or yellow), population of origin (spring or swamp), their interactions, or the difference in size between the two males. We analyzed four variables. We first analyzed whether the focal male was dominant or subordinate. We also considered the proportion of aggression that was performed by the blue male in each trial. We next tested whether dominance translated into mating opportunities. We analyzed (a) the proportion of courtship performed by the blue male compared to the total courtship performed by both males and (b) the proportion of time that the blue male spent within 1 body length of the female compared to the total time that either male spent within 1 body length of the female. Trials were excluded from the analysis if no courtship was performed (4 trials) or if neither male spent time close to the female (2 trials). The focal male identity (ID) was treated as a random effect in all four analyses (blue male dominance, blue male aggression, blue male courtship, and blue male percentage time near the female). For all four analyses, we initially used the following model: dependent variable  $\sim$  lighting environment (LE) + competitor color pattern (CP) + population of origin (Pop) + LE\*CP + LE\*Pop + CP\*Pop + LE\*CP\*Pop + size difference + (1|ID). However, for the analysis of male dominance status, the full model failed to converge. We then removed the interaction terms and re-ran the analysis. For the other three variables (proportion of aggression, proportion of courtship, proportion of time near the female), the full models converged. For all four analyses, we performed a type 3 analysis using the “car” package to examine the effects of each model term. The analysis of blue dominance assumed a binomial distribution and used the “glmer” function. The analyses of proportions (aggression, courtship, time) used linear models assuming normal distribution of errors. For these analyses, we used the “lmer” function in the “lme4” package in R. The advantage of performing the analyses on the proportions is that it avoids overdispersion. We visually inspected the plots of the residuals against the predicted values and normal Q–Q plots to check for heteroscedasticity. We also performed Levene’s test and found no evidence for heteroscedastic variances.

### Female preference

To determine the effect of anal fin coloration on female mate choice, we allowed one female and one male to spawn together for 5 consecutive days in either clear or tea-stained water. Each focal female was paired with all three color morphs (blue, red, yellow) in both clear and tea-stained environments across six weeks of trials. Each pairing with a male lasted 5 days. We then gave females a two-day resting period. The next week, we paired the female with another male. The order of treatments (i.e., male color and light treatments) were randomized for each female. We created tea-stained and clear treatments using the same methods used in the male competition trials.

For each weekly trial, we placed mating pairs in 34 L aquaria containing spawning substrates (i.e., yarn mops) at night and allowed them to spawn for the ensuing 5 days. We used the number of eggs produced as a measure of female preference. This is a “no-

choice” assay which we have successfully used in past studies of sexual selection and speciation (Fuller and Noa, 2010; Kozak et al. 2015; St John 2017). One of us (L.D.M.) searched the spawning substrates each day, counted the eggs, and then subsequently discarded them.

We originally selected 9 females from both the spring and swamp populations, but 1 spring female died after 3 weeks, leaving us with 8 spring females that had been paired in all 6 combinations. Hence, there were a total of 102 trials (6 treatments \* 17 females). Following the completion of all mating trials, fish were euthanized using an overdose of MS-222. This experiment ran from August 15, 2016 to September 23, 2016.

The dependent variable was the total number of eggs laid. We performed a fully factorial analysis that considered the effects of lighting environment, male color, population, and their interactions. We treated female identity as a random factor. We used the ‘lme4’ package in R with the ‘lmer’ function. We used a type 3 analysis of variance to assess the effects of our treatments. We used Levene’s test and visually inspected the residuals to check for heteroscedastic variances.

### Preliminary predation

The goal of this study was to determine if predation risk varies between blue, yellow, and red males as a function of lighting environment. We used largemouth bass as the predator. Largemouth bass are in all of our study populations in Florida (Fuller and Noa 2008), and R.C.F. has observed them preying on bluefin killifish in nature. We used bass from three populations: Florida Everglades (26 Mile-Bend, Broward Co., FL, USA), an Illinois Wild Population (Lake Shelbyville, Moultrie Co., FL, USA), and an Illinois Hatchery (Jake Wolfe Hatchery, Mason Co., FL, USA). Largemouth bass were previously considered to be one wide-ranging species with multiple subspecies, but have now been described as separate species (Kassler et al. 2002). The Florida Everglades bass were, therefore, Florida bass *Micropterus floridanus*, while the Illinois Wild and Illinois Hatchery bass were northern largemouth bass *Micropterus salmoides*. These two species do not differ in the spectral properties of their cone cells (Mitchem et al. 2018).

The Florida Everglades bass were collected with a bag seine; the Illinois Wild bass were collected via electroshocking from a boat; the Illinois Hatchery bass were provided by the Jake Wolfe Hatchery. For simplicity, we refer to these as three separate “populations”. The fish were transported back to the University of Illinois in aerated coolers. The fish were fed a mix of bass pellets and live feeder fish. We also collected male bluefin killifish of different color morphs, which served as the prey targets, from the Delk’s Bluff boat ramp (Marion Co., FL, USA) using seines and dipnets. These fish were also transported back to the University of Illinois and housed in aquaria and cattle tanks.

Two cattle tanks were established for each type of bass (4–5 bass per tank) for a total of six cattle tanks. For each population, one cattle tank was established with clear water conditions, and another with tea-stained conditions. UV sterilizers were used to prevent algae blooms in the water column and maintain the lighting treatments. Cattle tanks also had biological sponge filters connected to air pumps that removed nitrogenous waste from the tanks.

As with our male competition and female choice experiments (see above), we periodically added instant tea so that the tea-stained treatments resembled the appearance of iced-tea. This is necessary because over time the staining decreases due to bacterial decomposition of the tea. To verify that our treatments genuinely affected the light spectrum, we measured the down-welling irradiance at 25.4 cm

depth in four of our tanks (FL bass and IL-Hatchery) at noon on July 3, 2013. We did not measure light in the IL-Wild tanks because they had already been taken down when we took the data. We used an OceanOptics 2000 spectrophotometer connected to a patch cord and a cosine corrector. The spectrophotometer, patch cord, and cosine corrector had been calibrated using a calibrated using a DH2000 (Deuterium–Halogen, Ocean Optics) light source. One of us (S.S.) held the probe upward in the water at the appropriate depth (25.4 cm), while another (R.C.F.) took measurements using a laptop and SpectraSuite Software. For each tank, we calculated the relative down-welling irradiance as the absolute irradiance divided by the maximum value for a given spectrum.

For the predation assays, we placed individual killifish in clear plastic boxes in the cattle tank for two minutes and counted the number of times the bass struck the box. We tested each color morph (blue, yellow, or red) singly at three separate times (morning, mid-day, and dusk) on three separate days. Hence, each tank was tested 27 times. To analyze the data, we considered the fixed effects of color morph (blue, yellow, red), population (Florida Everglades, Illinois Wild, Illinois Hatchery), time (morning, mid-day, dusk), lighting environment (clear or tea), and their interactions on the number of strikes directed at the fish in the plastic boxes. Time of day had no significant effect, so it was removed and not considered further. There were large differences among the populations in the propensity of the bass to strike the boxes. We ran the same analysis excluding the Illinois Wild bass (which were much less likely to strike the boxes than either Illinois Hatchery or Florida Everglades), and another analysis on just the Florida Everglades bass. The latter analysis was warranted because these bass co-occur with the killifish in nature in Florida. We ran a linear model in R using the “lm” function and analyzed the treatment effects using a type 3 analysis in the “car” package. These results should be considered preliminary due to the fact that there were a limited number of tanks that were tested repeatedly. We used Levene’s test and visually inspected plots of the residuals against the predicted values to check for heteroscedasticity.

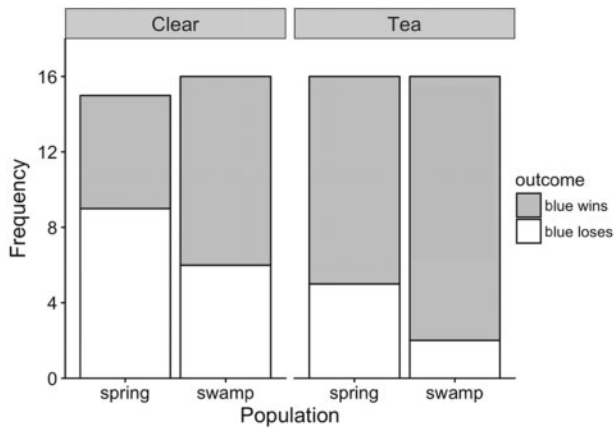
Data for all three experiments have been deposited in Dryad (doi:10.5061/dryad.3mn5rk4).

## Results

### Male competition

Blue males emerged as dominant more often in tea-stained treatments (Figure 4; Table 1,  $\chi^2_1 = 4.94$ ,  $P = 0.026$ ). In 25 out of 32 trials conducted in tea-stained water, blue males were dominant over the competitor male (binomial probability = 0.002 where the null expectation is a 50% probability of emerging as dominant). Blue males emerged as dominant in 16 out of 31 trials conducted in clear water conditions (binomial probability = 1). Differences in body size also affected the outcome where blue males were more likely to emerge as dominant when they were larger ( $\chi^2_1 = 5.82$ ,  $P = 0.016$ ). The same pattern of blue males emerging as dominant appeared to be present in both populations (Figure 4). Nearly identical results were obtained when we considered the proportion of aggressive acts performed by blue males versus their competitors (Table 2,  $F_{1,38.2} = 5.75$ ,  $P = 0.021$ ). Blue males performed a higher proportion of the aggressive acts in tea-stained water, and this was particularly so for males from the swamp population (Figure 5A). Blue males were also more likely to be more aggressive when they were larger than their competitors (Table 2,  $F_{1,46.9} = 5.73$ ,  $P = 0.021$ ).

The ability of blue males to court females and to remain in close proximity to females reflected the patterns in male dominance



**Figure 4.** The frequency with which blue males emerged as dominant (“blue wins”) versus subdominant (“blue loses”) in trials as a function of population and lighting environment. Blue males are more likely to be dominant in tea-stained treatments.

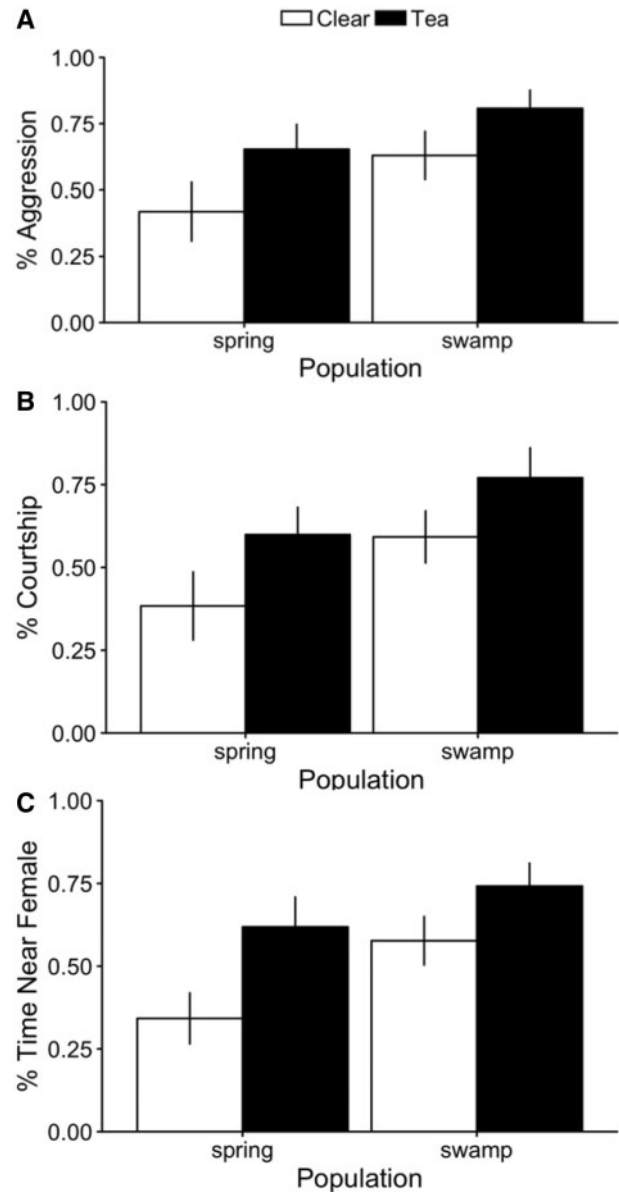
**Table 1.** Type 3 analysis of deviance (Wald  $\chi^2$  tests) on the dominance status of blue males. For each pairing, males were scored as either dominant (‘blue wins’) or subdominant (‘blue loses’). Inclusion of the interactions among the fixed terms prevented the model from converging.

Term	$\chi^2$	df	P
Intercept	1.44	1	0.230
Lighting environment	4.94	1	0.026
Population	1.90	1	0.168
Competitor color pattern	0.30	1	0.584
size difference	5.82	1	0.016

**Table 2.** Type 3 analysis of variance table (Wald F-tests with Kenward–Roger df) on the proportion of aggressive behaviors performed by blue versus competitor males

Term	F	df (num, denom)	P
Intercept	105.38	1, 17.4	0.000
Lighting environment (LE)	5.75	1, 38.2	0.021
Population (Pop)	3.52	1, 14.5	0.081
Competitor color pattern (CP)	0.22	1, 37.6	0.641
Size difference	5.73	1, 46.9	0.021
LE * CP	0.55	1, 35.1	0.465
LE * Pop	0.00	1, 38.3	0.954
CP * Pop	2.18	1, 40.6	0.147
LE*CP*Pop	2.78	1, 35.5	0.104

(Table 3A–B). Blue males were more likely to court and to remain in close proximity to females in tea-stained compared to clear water conditions (Figure 5B–C, courtship:  $F_{1,35.8} = 8.47$ ,  $P = 0.006$ ; time near female:  $F_{1,36.3} = 10.54$ ,  $P = 0.003$ ). Furthermore, blue males from swamps were particularly likely to have an advantage in courting and remaining close to females in tea-stained conditions (population effect – courtship:  $F_{1,14.3} = 6.01$ ,  $P = 0.028$ ; time near female:  $F_{1,14.4} = 5.68$ ,  $P = 0.031$ ). Likewise, blue males were also more likely to court females when they were larger than their competitors ( $F_{1,40.4} = 6.19$ ,  $P = 0.017$ ), but the effect of size was marginal for the time spent near females ( $F_{1,43.2} = 2.92$ ,  $P = 0.094$ ).



**Figure 5.** The proportion of aggression (A), courtship (B), and time near the female performed by the blue male versus the competing male as a function of lighting environment and population (C). Means  $\pm$  SE.  $N = 16$  for all means except for the clear-spring treatment combination ( $N = 15$ ).

#### Female preference

Females displayed no overall preference for any color morph between lighting treatments (Figure 6). More eggs were collected from females in tea-stained environments ( $F_{1,75} = 4.77$ ,  $P = 0.032$ ), but there were no differences in the number of eggs laid as a function of color morph nor as an interaction between color morph and lighting environment (Table 4). Similarly, there was no difference in female preference between populations.

#### Preliminary predation

The addition of instant tea dramatically decreased the amount of blue and UV light (380–550 nm) available in the tanks (Figure 7A, Supplementary Figure 1B). These results are similar to those seen in the wild (Figures 2C and 7A) with the notable exception that the UV



**Table 3.** Type 3 analysis of variance (Wald F-tests with Kenward–Roger df) for the proportion of courtship performed by the blue male versus the competitor male (A) and the proportion of time spent near the female by the blue male versus the competitor male (B).

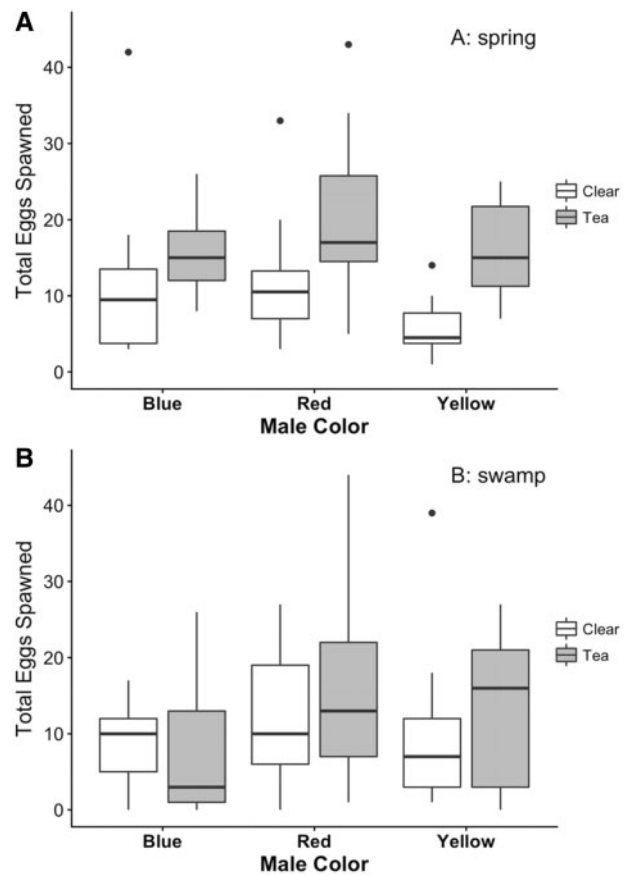
A. Blue Courtship			
Effect	<i>F</i>	<i>df</i> (num, denom)	<i>P</i>
(Intercept)	132.78	1, 16.4	0.000
Lighting environment (LE)	8.47	1, 35.8	0.006
Population (Pop)	6.01	1, 14.3	0.028
Competitor color pattern (CP)	1.82	1, 36.0	0.186
Size difference	6.19	1, 40.4	0.017
LE * Pop	0.42	1, 36.3	0.521
LE * CP	0.03	1, 35.6	0.868
CP * Pop	0.16	1, 38.9	0.695
LE*CP*Pop	1.29	1, 36.2	0.264
B. Time near female by blue male			
Effect	<i>F</i>	<i>df</i> (num, denom)	<i>P</i>
Intercept	123.77	1, 16.8	0.000
Lighting environment (LE)	10.54	1, 36.3	0.003
Population (Pop)	5.68	1, 14.4	0.031
Competitor color pattern (CP)	0.41	1, 36.6	0.526
Size difference	2.92	1, 43.2	0.094
LE * Pop	0.01	1, 36.6	0.941
LE * CP	0.17	1, 34.4	0.684
Pop * CP	0.57	1, 40.1	0.453
LE * CP * Pop	0.91	1, 34.9	0.347

wavelengths below 380 nm are absent due to the filtering properties of the greenhouse.

Preliminary evidence suggests that blue males may suffer a lower risk of predation in tea-stained water compared to clear water. The initial analysis including all three populations of bass (Florida Everglades, Illinois Hatchery, Illinois Wild) found a marginally significant interaction between lighting environment and male color morph (Table 5A,  $F_{2,144} = 5.25$ ,  $P = 0.086$ ). There was also a large overall effect due to population ( $F_{2,144} = 20.17$ ,  $P = 0.001$ ) that was caused by Illinois Wild bass striking the boxes less often than the Illinois Hatchery and Florida Everglades Bass [Table 5A,  $P < 0.001$ ; Illinois Wild:  $7.4 \pm 1.3$  (SE) strikes; Illinois Hatchery:  $16.8 \pm 1.7$  (SE) strikes, Florida Everglades:  $18.4 \pm (1.4 SE)$  strikes]. Removing the Illinois Wild Bass from the analysis resulted in a significant interaction between lighting environment and male color morph (Table 5B,  $F_{2,96} = 3.60$ ,  $P = 0.031$ ), where blue males were less likely to receive strikes in tea-stained water compared to clear water (Figure 7). An analysis restricted solely to the Florida Everglades Bass also results in a marginally significant interaction between lighting environment and male color morph (Table 5C,  $F_{2,48} = 3.14$ ,  $P = 0.052$ ). Supplementary Figure 2 shows the interaction between male color morph and lighting environment for each population.

### Discussion

Three main findings emerge from these experiments. First, the outcome of male/male competition varies depending on lighting environment where blue males are more likely to be dominant in tea-stained water. Second, preliminary studies using bass indicate that blue males may be less susceptible to predation in tea-stained water



**Figure 6.** The total eggs spawned as a function of male color and lighting environment for (A) spring and (B) swamp fish.

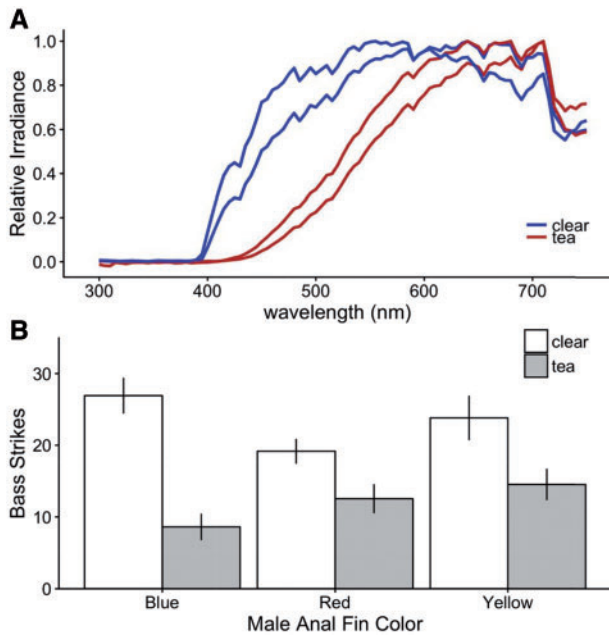
**Table 4.** Type 3 analysis of variance (Wald F-tests with Kenward–Roger df) on the number of eggs spawned by females as a function of lighting environment, population, and male coloration

Term	<i>F</i>	<i>df</i> (num, denom)	<i>P</i>
(Intercept)	161.95	1, 15	<0.001
Lighting environment (LE)	4.77	1, 75	0.032
Population (Pop)	1.37	1, 15	0.260
Male Color	2.03	2, 75	0.139
LE × Pop	1.91	1, 75	0.171
LE × Color	0.75	2, 75	0.475
Pop × Color	1.15	2, 75	0.323
LE × Pop × Color	0.08	2, 75	0.928

compared to clear water. Third, in contradiction to our previous work (Figure 3E), we found no evidence that female mating preferences favor blue males in swamps. Below, we discuss the implications of these three results and then discuss the broader importance for the bluefin killifish system.

### Male competition

Our study provides direct evidence that lighting environments alter the outcome of male/male competition where blue males are favored in tea-stained water. Previous work indicates that the outcome of male/male competition has an overwhelming influence on the actual outcome of mating (McGhee et al. 2007; McGhee and Travis 2010,



**Figure 7.** (A) Relative down-welling irradiance at 25.4 cm depth in clear and tea-stained treatments. Each curve is scaled by the maximum down-welling irradiance. See supplemental figure 1 for absolute irradiance spectra. (B) The effects of lighting environment and male color on the number of bass strikes over 2 min for the Florida Everglades and Illinois Hatchery bass pooled. Means  $\pm$  SE.  $N = 18$  for each bar.

2011). Hence, the results of this experiment suggest that blue males have a genuine fitness advantage in swamps. We see two potential explanations as to why this occurs. The first is that, like female choice, visual contrasts play an important role in male/male competition. Conspicuousness (i.e., chromatic and/or achromatic contrasts) have long been assumed, and sometimes shown, to be important to female mating preferences (Pauers et al. 2004; Gray et al. 2008; Maan and Cummings 2009; Kemp et al. 2009; Dalton et al. 2010; Morehouse and Rutowski 2010; Tanaka et al. 2011; Ronald et al. 2012). These contrasts might reduce search costs for females, advertise male health and genetic quality, or appeal to arbitrary preferences that have been shaped by evolutionary forces such as natural selection on sensory system properties, Fisherian sexual selection, or past evolutionary history (Fuller et al. 2005b; Ryan and Cummings 2013). Do these same principles apply to the signals males use in male/male competition? With competition, signals are often “put to the test”. Individuals signal to one another, but if disputes cannot be resolved via signaling, then they escalate to costly fighting (Tibbetts and Dale 2004; Searcy and Nowicki 2005; Tibbetts and Izzo 2010). Our previous work using spring fish in clear water indicates that similar phenomena occur in bluefin killifish where melanin serves as a badge of status (Johnson and Fuller 2015). Blue coloration may serve a similar function in swamp habitats. The blue anal fin may conceivably create high contrast with the water column. This explanation predicts that high contrast males should emerge as dominant more often than low contrast males and that contests between males with similar levels of contrast should be long and costly. These hypotheses are testable with detailed observations, visual detection models, and experiments that directly manipulate contrast. A second hypothesis is that blue males alter their competitive behavior in clear versus tea-stained water as a function of predation risk. This hypothesis assumes that blue males face high

**Table 5.** Type 3 analysis of variation of the effects of lighting environment (LE), population (pop), and male color (color) on the number of bass strikes at red, yellow, and blue males in clear plastic boxes. (A) All three populations. (B) Florida everglades and Illinois hatchery fish. (C) Florida everglades bass.

A. All three populations.			
Term	<i>F</i>	<i>df</i> (num, denom)	<i>P</i>
(Intercept)	342.97	1, 144	< 0.001
Lighting Environment (LE)	27.06	1, 144	< 0.001
Population (Pop)	20.17	2, 144	< 0.001
Male Color	2.00	2, 144	0.140
LE $\times$ Pop	5.25	2, 144	0.006
LE $\times$ Color	2.49	2, 144	0.086
Pop $\times$ Color	1.54	4, 144	0.195
LE $\times$ Pop $\times$ Color	0.72	4, 144	0.582
B. Illinois Hatchery and Florida Everglades Populations			
Term	<i>F</i>	<i>df</i> (num, denom)	<i>P</i>
Intercept	354.94	1, 96	< 0.001
Lighting Environment (LE)	37.23	1, 96	< 0.001
Population (Pop)	0.69	1, 96	0.407
Male color	1.07	2, 96	0.348
LE $\times$ Pop	0.48	1, 96	0.490
LE $\times$ Color	3.60	2, 96	0.031
Pop $\times$ Color	2.15	2, 96	0.122
LE $\times$ Pop $\times$ Color	0.30	2, 96	0.743
C. Florida Everglades			
Term	<i>F</i>	<i>df</i> (num, denom)	<i>P</i>
Intercept	244.77	1, 48	< 0.001
Lighting environment (LE)	18.50	1, 48	< 0.001
Color	0.89	2, 48	0.417
LE $\times$ Color	3.14	2, 48	0.052

predation risk in clear water and low predation risk in swamps (see below), and that their willingness to engage in competition reflects these effects. In this case, blue males have an advantage in swamps because they are more willing to engage in extended, conspicuous displays in tea-stained water in comparison to other color morphs. This hypothesis predicts that there should be different levels of risk taking between the color morphs as a function of the lighting environment.

**Preliminary predation**

Our preliminary evidence suggests that blue males may be less susceptible to predation in swamps than in springs. We consider these results to be preliminary because we repeatedly tested 6 cattle tanks of bass (3 populations  $\times$  2 lighting treatments). Ideally, we would have used multiple cattle tanks per treatment, but logistical constraints prevented this. Other preliminary studies examining the likelihood of bass to strike at different inanimate objects that resemble food suggest increased strike rates at blue objects in clear water (S. Feng, N. Karin, R.C.F., unpublished data). If these results hold, then they indicate that the direction of selection via predation is the same as the direction of selection via male/male competition. This is in contrast to other systems where the direction of selection via

sexual selection opposes the direction of selection via predation/natural selection (Endler, 1995).

Exactly why these effects occur is unclear. One possibility is that color patterns appear different to conspecifics than they do to predators (Siddiqi et al. 2004; Bybee et al. 2012; Crothers and Cummings 2013), creating the opportunity for animals to signal in a private channel. The visual system of the bass is quite different from that of the bluefin killifish. Largemouth and Florida bass possess only two cones in their retinas that are sensitive to longer wavelengths ( $\lambda_{\max}$  values of 535 nm and 614 nm) (Mitchem et al. 2018). In contrast, bluefin killifish have at least five cone classes with spectral sensitivity extending into the UV but less in the far-red region ( $\lambda_{\max}$  values of 359 nm, 405 nm, 455 nm, 537 nm, and 573 nm) (Fuller et al. 2003). These different visual sensitivities may set up a scenario where 'blue' signals serve as a private communication channel in tannin-stained waters. These signals might travel over the short distances required for signaling with conspecifics (~0.5 m) but attenuate or appear more cryptic to bass over the longer distances at which predators view them. Clearly, we are not the first to suggest such dynamics. Private communication channels have been suggested for many groups including swordtail fish (Cummings et al. 2003), electric fish (Arnegard et al. 2010), and moths (White et al. 2015). In all of these systems, sensory properties of the predators dictate the sensory space (i.e., range of wavelengths and frequencies) and modalities that animals use for signals.

### Female mate choice

Our previous work indicated that females had weak preferences for blue males provided that they came from swamp parents, were raised in tea-stained conditions, and were tested in tea-stained water. Our previous work also found preference for red males for females from spring parents (Fuller and Noa 2010, see also Fuller and Johnson 2009; Johnson et al. 2018). We did not repeat these patterns here. Both studies used no-choice female choice assays which are thought to be conservative (Houde 1997, but see St John 2017). However, there were some differences in methodology. The previous assays (Fuller and Noa 2010) were conducted in a fish room with good temperature control but artificial light, whereas the current study was conducted in a greenhouse with much greater thermal fluctuations, but natural light. The previous study also only considered egg production over a 4-h period, whereas the current study considered egg production over 1 week. However, we still obtain no pattern of preference even if we limit our analyses to the eggs laid on day 1. The previous study used lab-reared animals whereas the current study used field-caught individuals. Why these differences in methodology should cause these different patterns is unclear. The other explanation is that female preferences for different male color morphs are weak. While we have not demonstrated strong preferences for different color morphs, we have demonstrated strong patterns of female mating preference in our speciation work (Gregorio et al. 2012; Kozak et al. 2015; St John 2017). We have repeatedly shown that females and males that occur in sympatry with a close relative, the rainwater killifish *L. parva*, have heightened levels of preference compared to allopatric animals. Furthermore, females that co-occur with rainwater killifish also have heightened preferences for males from their own populations over foreign populations. The relevant point for this article is that we can repeatedly show patterns of preference provided that preferences are strong. We suspect that preferences for males with different color patterns are weak and are often superseded by the outcome of male/male competition (see Berglund et al. 1996 for a discussion of traits used in male competition and female choice). In fact, Mcghee et al. (2007)

compared female choice for males with different color patterns with the outcome of male/male competition and found that females mated rapidly when preferred males were dominant, but had a longer latency to mate when preferred males were subdominant. Clearly, there is good evidence that natural variation in lighting environments is correlated with changes in male coloration and that female mating preferences are associated with many of these patterns (e.g., guppies: Endler and Houde, 1995; cichlids: Seehausen et al. 2008; Maan and Seehausen 2010; Maan et al. 2010; Telemantheria: Gray et al. 2008; sticklebacks: Reimchen 1989; Mckinnon, 1995; Boughman 2001; surfperch: Cummings 2007). Yet, the roles of lighting environment on the interaction between male competition and female choice remains unclear in many systems.

### The bluefin killifish system

We contend that bluefin killifish provide some of the best evidence for Endler's theory of sensory drive (Endler 1992, 1993a). The differences in lighting environments between clear springs and tannin-stained swamps set the stage for the evolution of male color patterns, sensory system properties, non-mating behaviors (e.g., foraging), and mating behaviors (male/male competition). Furthermore, the direction of phenotypic plasticity in male color patterns (where males are more likely to express as blue when raised in tea-stained water) and foraging preferences (animals peck at blue dots in tea-stained water) suggest that the direction of phenotypic plasticity coincides with the direction of selection. Lighting environments not only affect the direction of selection and genetic differentiation among populations, they also influence the development of traits in a putatively adaptive fashion. Again, bluefin killifish provide some of the best evidence for sensory drive due, in part, to the very dramatic differences among lighting habitats.

While this system is ripe for multiple future avenues of research, we argue that there are two areas that stand out. First, while the bluefin killifish provides strong evidence for sensory bias from an ecological genetics standpoint, we have very little understanding mechanistically of why blue males are favored in swamps and disfavored in springs. The patterns presented here beg for a proper analysis via visual detection models (Vorobyev and Osorio 1998; Vorobyev et al. 1998; Kemp et al. 2015). Visual detection models will allow us to ask whether blue males possess higher contrast than red or yellow males when viewed by conspecifics in tannin-stained water and whether they are less conspicuous to bass. The bluefin killifish-bass system is excellent for constructing and testing visual detection models due to our ability to readily manipulate lighting environments, color patterns, and visual system properties. Visual detection models will also allow us to ask why blue males are absent from springs and whether they are more conspicuous to predators and/or conspecifics.

The second glaring question is what maintains the variation in male coloration? All of our study populations in Florida have multiple color morphs. Like the situation with guppies, there are staggering levels of variation in male coloration within populations, and we do not understand how these are maintained. These color morphs do not represent different alternative mating strategies. There are no sneakers and all males compete to guard females and patches of vegetation away from other males. They do not differ in body size nor in age at sexual maturation.

How can such high levels of variation be maintained? Spatial and temporal variation in lighting environments may play a role in this system. In the Suwanee, St John's, and Withlacootchee river drainages, there are spring populations that connect to tannin-stained rivers, which create dramatic spatial variation. There is also

temporal variation due to droughts and extreme rain across years. Another possibility is that there is microhabitat variation with respect to either depth or diurnal rhythms. Both theory and empirical work indicate that variation in lighting habitats may allow for the maintenance of different color morphs (Endler 1987; Endler 1993b, Schluter and Price 1993; Chunco et al. 2007; Gray et al. 2008; Hurtado-Gonzales et al. 2014). Our previous work on foraging preferences provided support for the idea that color-based preferences vary over the course of the day (Johnson et al. 2013). Whether such effects extend to male-male competition (or the ever fleeting female mating preferences) is unknown.

In conclusion, bluefin killifish provide exceedingly strong support for sensory drive. Differences between clear water and tannin-stained lighting environments affect nearly every aspect of the sensory drive process. Our review showed strong among population patterns in signals, visual systems, and visually-based behaviors attributable to variation in the lighting environment via genetic variation, phenotypic plasticity as a function of the lighting environment, and genetic variation in phenotypic plasticity. Our three new experiments provided sorely needed data concerning the direction of selection via male/male competition, female mate choice, and predation. We found strong evidence that differences in lighting environments alter the direction of competition where blue males are favored in tea-stained water but not in clear water. In contrast to previous work, we found no evidence for female mating preferences in any lighting environment. Finally, preliminary evidence suggests that blue males might experience lower predation risks in tea-stained water than they do in clear water. The emerging pattern is one where the direction of selection due to male/male competition, the direction of selection due to predation, the nature of genetic differences among populations, and the direction of phenotypic plasticity in male coloration and foraging preferences favors the presence of blue males in swamps.

## Acknowledgments

We thank M. St John, A. Bell, J. Epifanio, C.-H. Chang, R. Moran, and three anonymous reviewers for constructive comments that improved the article.

## Funding

L.D.M. was supported by an NIH SEPA Award (R25 OD020203) to B. Hug and R.C.F. and by NSF DEB (0964726) to R.C.F. S.S., N.S., and Z.T. were supported by NSF DUE (1129198) to Z. Rapti and C. Caceres. These experiments were approved by Illinois Institutional Animal Care and Use Committee (17184 and 15147).

## References

- Andersson M, 1994. *Sexual Selection*. Princeton: Princeton University Press.
- Arndt R, 1971. Ecology and Behavior of the Cyprinodont Fishes *Adinia xenica*, *Lucania parva*, *Lucania goodii*, and *Leptolucania ommata*. [PhD thesis] Cornell University.
- Arnegan ME, McIntyre PB, Harmon LJ, Zelditch ML, Crampton WGR et al., 2010. Sexual signal evolution outpaces ecological divergence during electric fish species radiation. *Am Nat* 176:335–356.
- Baube CL, 1997. Manipulations of signalling environment affect male competitive success in three-spined sticklebacks. *Animal Behav* 53:819–833.
- Berglund A, Bisazza A, Pilastro A, 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol J Linn Soc* 58:385–399.
- Boughman JW, 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature* 411:944–948.
- Braun C, Michiels NK, Siebeck UE, Sprenger D, 2014. Signalling function of long wavelength colours during agonistic male–male interactions in the wrasse *Coris Julis*. *Marine Ecol Progress Series* 504:277–286.
- Brock CD, Cummings ME, Bolnick DI, 2017. Phenotypic plasticity drives a depth gradient in male conspicuousness in threespine stickleback *Gasterosteus aculeatus*. *Evolution* 71:2022–2036.
- Buchinger TJ, Bussy U, Li K, Wang H, Huertas M et al., 2017. Phylogenetic distribution of a male pheromone that may exploit a nonsexual preference in lampreys. *J. Evol. Biol.* 30:2244–2254.
- Bybee SM, Yuan FR, Ramstetter MD, Llorente-Bousquets J, Reed RD et al., 2012. Uv photoreceptors and Uv-yellow wing pigments in *Heliconius* butterflies allow a color signal to serve both mimicry and intraspecific communication. *Am Nat* 179:38–51.
- Chunco AJ, Mckinnon JS, Servedio MR, 2007. Microhabitat variation and sexual selection can maintain male color polymorphisms. *Evolution* 61:2504–2515.
- Cronin TW, Marshall NJ, Caldwell RL, 1996. Visual pigment diversity in two genera of mantis shrimps implies rapid evolution (Crustacea: stomatopoda). *J. Comp Physiol* 179:371–384.
- Cronin TW, Caldwell RL, 2002. Tuning of photoreceptor function in three mantis shrimp species that inhabit a range of depths. II. Filter Pigments. *J. Comp. Physiol.* 188:187–197.
- Cronin TW, Johnsen S, Marshall NJ, Warrant EJ, 2014. *Visual Ecology*. Princeton: Princeton University Press.
- Crothers LR, Cummings ME, 2013. Warning signal brightness variation: sexual selection may work under the radar of natural selection in populations of a polytypic poison frog. *Am Nat* 181:E116–E124.
- Crothers LR, Cummings ME, 2015. A multifunctional warning signal behaves as an agonistic status signal in a poison frog. *Behav Ecol* 26:560–568.
- Cummings ME, Partridge JC, 2001. Visual pigments and optical habitats of surfperch (Embiotocidae) in the California kelp forest. *J. Comp. Physiol.* 187:875–889.
- Cummings ME, Rosenthal GG, Ryan MJ, 2003. A private ultraviolet channel in visual communication. *Proc. Roy. Soc. Lond B Biol Sci* 270:897–904.
- Cummings ME, 2007. Sensory trade-offs predict signal divergence in surfperch. *Evolution* 61:530–545.
- Dalton BE, Cronin TW, Marshall NJ, Carleton KL, 2010. The fish eye view: are cichlids conspicuous?. *J. Exp. Biol.* 213:2243–2255.
- De Lanuza GPI, Font E, 2016. The evolution of colour pattern complexity: selection for conspicuousness favours contrasting within-body colour combinations in lizards. *J. Evol. Biol.* 29:942–951.
- Duntley SQ, 1951. The visibility of submerged objects. *Proceedings of the Armed Forces – Natural Resource Council Vision Communication* 28.
- Ehlman SM, Sandkam BA, Breden F, Sih A, 2015. Developmental plasticity in vision and behavior may help guppies overcome increased turbidity. *J. Comp. Physiol. A* 201:1125–1135.
- Endler JA, 1987. Predation, light intensity and courtship behavior in *Poecilia Reticulata* (Pisces, Poeciliidae). *Animal Behav* 35:1376–1385.
- Endler JA, 1992. Signals, signal conditions, and the direction of evolution. *Am Nat* 139:S125–S153.
- Endler JA, 1993a. Some general comments on the evolution and design of animal communication systems. *Philos Trans Roy Soc Lond B* 340:215–225.
- Endler JA, 1993b. The color of light in forests and its implications. *Ecol Monogr* 63:1–27.
- Endler JA, Houde AE, 1995. Geographic variation in female preferences for male traits in *Poecilia Reticulata*. *Evolution* 49:456–468.
- Endler JA, 1995. Multiple trait coevolution and environmental gradients in guppies. *Trends Ecol Evol* 10:22–29.
- Endler JA, Basolo A, Glowacki S, Zerr J, 2001. Variation in response to artificial selection for light sensitivity in guppies *Poecilia Reticulata*. *Am Nat* 158:36–48.
- Escobar-Camacho D, Marshall J, Carleton KL, 2017. Behavioral color vision in a cichlid Fish: *metriacalma Benetos*. *J. Exp Biol* 220:2887–2899.
- Evans MR, Norris K, 1996. The importance of carotenoids in signaling during aggressive interactions between male firemouth cichlids *Cichlasoma Meeki*. *Behav Ecol* 7:1–6.
- Foster NR, 1967. Comparative studies on the biology of killifishes (Pisces: cyprinodontidae). *Ph.D. dissertation*, Cornell University, Ithaca, New York.

- Fuller RC, 2002. Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish *Lucania Goodei* populations. *Proc Roy Soc B Biol Sci* 269:1457–1465.
- Fuller RC, Fleishman LJ, Leal M, Travis J, Loew E, 2003. Intraspecific variation in retinal cone distribution in the bluefin killifish *Lucania Goodei*. *J Comp Physiol A* 189:609–616.
- Fuller RC, Travis J, 2004. Genetics, lighting environment, and heritable responses to lighting environment affect male color morph expression in bluefin killifish *Lucania Goodei*. *Evolution* 58:1086–1098.
- Fuller RC, Carleton KL, Fadool JM, Spady TC, Travis J, 2004. Population variation in opsin expression in the bluefin killifish *Lucania Goodei*: a real-time pcr study. *J Comp Physiol A* 190:147–154.
- Fuller RC, Carleton KL, Fadool JM, Spady TC, Travis J, 2005a. Genetic and environmental variation in the visual properties of bluefin killifish *Lucania Goodei*. *J Evol Biol* 18:516–523.
- Fuller RC, Houle D, Travis J, 2005b. Sensory bias as an explanation for the evolution of mate preferences. *Am Nat* 166:437–446.
- Fuller RC, Noa LA, 2008. Distribution and stability of sympatric populations of *Lucania goodei* and *L. parva* across Florida. *Copeia* 699–707.
- Fuller RC, Johnson AM, 2009. A test for negative frequency-dependent mating success as a function of male colour pattern in the bluefin killifish. *Biol J Linn Soc* 98:489–489.
- Fuller RC, Noa LA, Strellner RS, 2010. Teasing apart the many effects of lighting environment on opsin expression and foraging preference in bluefin killifish. *Am Nat* 176:1–13.
- Fuller RC, Noa LA, 2010. Female mating preferences, lighting environment, and a test of the sensory bias hypothesis in the bluefin killifish. *Animal Behav* 80:23–35.
- Fuller RC, Claricoates KM, 2011. Rapid light-induced shifts in opsin expression: finding new opsins, discerning mechanisms of change, and implications for visual sensitivity. *Mol Ecol* 20:3321–3335.
- Gamble S, Lindholm AK, Endler JA, Brooks R, 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecol Lett* 6: 463–472.
- Gawryszewski FM, Calero-Torralbo MA, Gillespie RG, Rodriguez-Girones MA, Herberstein ME, 2017. Correlated evolution between coloration and ambush site in predators with visual prey lures. *Evolution* 71:2010–2021.
- Gomez D, Thery M, 2007. Simultaneous crypsis and conspicuousness in color patterns: comparative analysis of a neotropical rainforest bird community. *Am Nat* 169:S42–S61.
- Gray SM, Dill LM, Tantu FY, Loew ER, Herder F et al., 2008. Environment-contingent sexual selection in a colour polymorphic fish. *Proc Roy Soc B Biol Sci* 275:1785–1791.
- Gregorio O, Berdan EL, Kozak GM, Fuller RC, 2012. Reinforcement of male mate preferences in sympatric killifish species *Lucania Goodei* and *Lucania Parva*. *Behav Ecol Sociobiol* 66:1429–1436.
- Hofmann CM, Carleton KL, 2009. Gene duplication and differential gene expression play an important role in the diversification of visual pigments in fish. *Integr Comp Biol* 49:630–643.
- Houle A, 1997. *Sex, Color, and Mate Choice in Guppies*. Princeton: Princeton University Press.
- Hurtado-Gonzales JL, Loew ER, Uy JAC, 2014. Variation in the visual habitat may mediate the maintenance of color polymorphism in a Poeciliid fish. *PLoS One* 9:e101497.
- Johnson AM, Stanis S, Fuller RC, 2013. Diurnal lighting patterns and habitat alter opsin expression and colour preferences in a killifish. *Proc Roy Soc B Biol Sci* 280:20130796.
- Johnson AM, Fuller RC, 2015. The meaning of melanin, carotenoid, and pterin pigments in the bluefin killifish *Lucania Goodei*. *Behav Ecol* 26: 158–167.
- Johnson AM, Chang C-H, Fuller RC, 2018. Testing the potential mechanisms for the maintenance of a genetic color polymorphism in bluefin killifish populations. *Curr Zool* 64, doi:10.1093/cz/zoy017.
- Kassler TW, Koppelman JB, Near TJ, Dillman CB, L, JA et al., 2002. Molecular and morphological analyses of the black basses: implications for taxonomy and conservation. In: Philipp DP, Ridgway MS, editors. *Black Bass: Ecology, Conservation, and Management*, 31:291–322.
- Kemp DJ, Reznick DN, Grether GF, Endler JA, 2009. Predicting the direction of ornament evolution in Trinidadian guppies *Poecilia reticulata*. *Proc. Roy Soc B Biol Sci* 276:4335–4343.
- Kemp DJ, Herberstein ME, Fleishman LJ, Endler JA, Bennett ATD et al. 2015. An integrative framework for the appraisal of coloration in nature. *Am Nat* 185:705–724.
- Knott B, Berg ML, Morgan ER, Buchanan KL, Bowmaker JK et al., 2010. Avian retinal oil droplets: dietary manipulation of color vision?. *Proc Roy Soc B Biol Sci* 277:953–962.
- Knott B, Berg ML, Ribot RFH, Endler JA, Bennett ATD, 2017. Intraspecific geographic variation in rod and cone visual pigment sensitivity of a parrot *Platyercus elegans*. *Sci Rep* 7, doi:10.1038/srep41445.
- Kozak GM, Roland G, Rankhorn C, Falater A, Berdan EL et al., 2015. Behavioral isolation due to cascade reinforcement in *Lucania* killifish. *Am Nat* 185:491–506.
- Kroger RHH, Fernald RD, 1994. Regulation of eye growth in the African Cichlid fish *Haplochromis burtoni*. *Vision Res* 34:1807–1814.
- Leal M, Fleishman LJ, 2002. Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. *Proc Roy Soc Lond B Biol Sci* 269:351–359.
- Long KD, Houde AE, 1989. Orange spots as a visual cue for female mate choice in the guppy *Poecilia Reticulata*. *Ethology* 82:316–324.
- Lythgoe JN, 1979. *The Ecology of Vision*. Oxford: Clarendon Press.
- Lythgoe JN, Muntz WRA, Partridge JC, Shand J, Williams DM, 1994. The ecology of the visual pigments of snappers (Lutjanidae) on the great barrier reef. *J Comp Physiol A* 174:461–467.
- Lythgoe JN, 1988. Light and vision in the aquatic environment. In: Atema J, editor. *Sensory Biology of Aquatic Animals*. New York: Springer. 57–82.
- Maan ME, Hofker KD, Van Alphen JJM, Seehausen O, 2006. Sensory drive in cichlid speciation. *Am Nat* 167:947–954.
- Maan ME, Cummings ME, 2009. Sexual dimorphism and directional sexual selection on aposomatic signals in a poison frog. *Proc Natl Acad Sci USA* 106:19072–19077.
- Maan ME, Seehausen O, Van Alphen JJM, 2010. Female mating preferences and male coloration covary with water transparency in a lake Victoria Cichlid fish. *Biol J Linn Soc* 99:398–406.
- Maan ME, Seehausen O, 2010. Mechanisms of species divergence through visual adaptation and sexual selection: perspectives from a cichlid model system. *Curr Zool* 56:285–299.
- Maan ME, Seehausen O, Groothuis TGG, 2017. Differential survival between visual environments supports a role of divergent sensory drive in cichlid fish speciation. *Am Nat* 189:78–85.
- Marchetti K, 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* 362:149–152.
- Meghee KE, Fuller RC, Travis J, 2007. Male competition and female choice interact to determine mating success in the bluefin killifish. *Behav Ecol* 18:822–830.
- Meghee KE, Travis J, 2010. Repeatable behavioural type and stable dominance rank in the bluefin killifish. *Animal Behav* 79:497–507.
- Meghee KE, Travis J, 2011. Early food and social environment affect certain behaviours but not female choice or male dominance in bluefin killifish. *Animal Behav* 82:139–147.
- Mckinnon JS, 1995. Video mate preferences of female three-spined sticklebacks from populations with divergent male coloration. *Animal Behav* 50: 1645–1655.
- Mertens LE, 1970. *In-Water Photography: Theory and Practice*. New York: Wiley-Interscience.
- Mitchem LD, Stanis S, Zhou M, Loew E, Epifanio JM et al., 2018. Seeing red: color vision in *Micropterus Salmoides* (Largemouth Bass). *Curr Zool* 65, doi:10.1093/cz/zoy019.
- Morehouse NI, Rutowski RL, 2010. In the eyes of the beholders: female choice and avian predation risk associated with an exaggerated male butterfly color. *Am Nat* 176:768–784.
- Nandamuri P, Dalton BE, Carleton KL, 2017. Determination of the genetic architecture underlying short wavelength sensitivity in Lake Malawi Cichlids. *J Heredity* 108:379–390.

- Ord TJ, Klomp DA, Garcia-Porta J, Hagman M, 2015. Repeated evolution of exaggerated dewlaps and other throat morphology in lizards. *J Evol Biol* 28:1948–1964.
- Pauers MJ, Mckinnon JS, Ehlinger TJ, 2004. Directional sexual selection on chroma and within-pattern colour contrast in *Labeotropheus fuelleborni*. *Proc Roy Soc Lond B Biol Sci* 271:S444–S447.
- Phillips JN, Derryberry EP, 2017. Equivalent effects of bandwidth and trill rate: support for a performance constraint as a competitive signal. *Animal Behav* 132:209–215.
- Reichert MS, Ronacher B, 2015. Noise affects the shape of female preference functions for acoustic signals. *Evolution* 69:381–394.
- Reimchen TE, 1989. Loss of nuptial color in threespine sticklebacks *Gasterosteus aculeatus*. *Evolution* 43:450–460.
- Reznick D, Travis J, 2001. Adaptation. In: Fox CW, Roff DA, Fairbairn DJ, editors. *Evolutionary Ecology*. New York: Oxford University Press. 44–57.
- Ronald KL, Fernandez-Juricic E, Lucas JR, 2012. Taking the sensory approach: how individual differences in sensory perception can influence mate choice. *Animal Behav* 84:1283–1294.
- Ryan MJ, Keddy-Hector A, 1992. Directional patterns of female mate choice and the role of sensory biases. *Am Nat* 139:S4–S35.
- Ryan MJ, Cummings ME, 2013. Perceptual biases and mate choice. *Annu Rev Ecol Evol Syst* 44:437–459.
- Sandkam BA, Deere-Machemer KA, Johnson AM, Grether GF, Rodd FH et al., 2016. Exploring visual plasticity: dietary carotenoids can change color vision in guppies *Poecilia reticulata*. *J Comp Physiol A* 202:527–534.
- Santos ESA, Scheck D, Nakagawa S, 2011. Dominance and plumage traits: meta-analysis and metaregression analysis. *Animal Behav* 82:3–19.
- Schluter D, Price T, 1993. Honesty, perception and population divergence in sexually selected traits. *Proc Roy Soc B Biol Sci* 253:117–122.
- Searcy W, Nowicki S, 2005. *Evolution of Animal Communication: Reliability and Deception in Signaling Systems*. Princeton: Princeton University Press.
- Seehausen O, Van Alphen JJM, 1998. The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* Complex). *Behav Ecol Sociobiol* 42:1–8.
- Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ et al., 2008. Speciation through sensory drive in cichlid fish. *Nature* 455:620–623.
- Servedio MR, Boughman JW, 2017. The role of sexual selection in local adaptation and speciation. In: Futuyma DJ, editor. *Annu Rev Ecol Evol Syst* 48:85–109.
- Siddiqi A, Cronin TW, Loew ER, Vorobyev M, Summers K, 2004. Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J Exp Biol* 207:2471–2485.
- St John ME, 2017. Reinforcement and cascade reinforcement in the *Lucania* system: the effects of experimental design, sex, and heterospecific pairings on mate preference. [M.Sc. Thesis] University of Illinois.
- Stanger-Hall KF, Lower SES, Lindberg L, Hopkins A, Pallansch J et al., 2018. The evolution of sexual signal modes and associated sensor morphology in fireflies (Lampyridae, Coleoptera). *Proc Roy Soc B Biol Sci* 285:20172384 (electronic article).
- Stieb SM, Carleton KL, Cortesi F, Marshall NJ, Salzburger W, 2016. Depth-dependent plasticity in opsin gene expression varies between damselfish (Pomacentridae) species. *Mol Ecol* 25:3645–3661.
- Strauss J, Alt JA, Ekschmitt K, Schul J, Lakes-Harlan R, 2017. Evolutionary diversification of the auditory organ sensilla in neoconocephalus katydid (Orthoptera: tettigoniidae) correlates with acoustic signal diversification over phylogenetic relatedness and life history. *J Evol Biol* 30:1094–1109.
- Stuart-Fox D, Moussalli A, Whiting MJ, 2007. Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. *Am Nat* 170:916–930.
- Tanaka KD, Morimoto G, Stevens M, Ueda K, 2011. Rethinking visual super-normal stimuli in cuckoos: visual modeling of host and parasite signals. *Behav Ecol* 22:1012–1019.
- Tibbetts EA, Dale J, 2004. A socially enforced signal of quality in a paper wasp. *Nature* 432:218–222.
- Tibbetts EA, Izzo A, 2010. Social punishment of dishonest signalers caused by mismatch between signal and behavior. *Curr Biol* 20:1637–1640.
- Tinghitella RM, Lehto WR, Minter R, 2015. The evolutionary loss of a badge of status alters male competition in three-spine stickleback. *Behav Ecol* 26:609–616.
- Travis J, Reznick D, 1998. Experimental approaches to the study of evolution. In: WJ Resitarsits, J Bernardo, editors. *Issues and Perspectives in Experimental Ecology*. New York: Oxford University Press. 437–459.
- Vorobyev M, Osorio D, 1998. Receptor noise as a determinant of colour thresholds. *Proc Roy Soc Lond B Biol Sci* 265:351–358.
- Vorobyev M, Osorio D, Bennett ATD, Marshall NJ, Cuthill IC, 1998. Tetrachromacy, oil droplets and bird plumage colours. *J Comp Physiol A* 183:621–633.
- White TE, Zeil J, Kemp DJ, 2015. Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. *Evolution* 69:14–25.
- Wright DS, Demandt N, Alkema JT, Seehausen O, Groothuis TGG et al. 2017. Developmental effects of visual environment on species-assortative mating preferences in Lake Victoria Cichlid fish. *J Evol Biol* 30:289–299.
- Wright DS, Rietveld E, Maan ME, 2018. Developmental effects of environmental light on male nuptial coloration in Lake Victoria Cichlid fish. *Peer J* 6:e4209 (electronic article).
- Zhou M, Fuller RC, 2015. Sexually asymmetric color-based species discrimination in orangethroat darters. *Animal Behavior* 106:171–179.
- Zhou MC, Fuller RC, 2016. Intrasexual competition underlies sexual selection on male breeding coloration in the orangethroat darter *Etheostoma spectabile*. *Ecol Evol* 6:3513–3522.
- Ziegler L, Arim M, Narins PM, 2011. Linking amphibian call structure to the environment: the interplay between phenotypic flexibility and individual attributes. *Behav Ecol* 22:520–526.