Mate Choice and Hybridization: Comparing the Endler's Guppy (*Poecilia wingei)* and the Common Guppy (*Poecilia reticulata)*

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Abstract

The present study addresses the question of whether or not two guppy species, the Endler's guppy *(Poecilia wingei)* and the common guppy *(Poecilia reticulata)* are able to mate and produce offspring. The question of interest is in the context of the conservation of the Endler's guppy that occurs only in a limited habitat compared to the widespread common guppy. Female mate choice, male mating behaviors, and possible hybrid offspring of these two species were assessed to determine if the two species will mate and produce offspring. Endler's guppies' females did not show a preference for males of their own species, for higher levels of carotenoid coloration, or for larger size in males. Additionally, males of these two species did not exhibit significant differences in mating behaviors. Finally, these two species were capable of producing hybrid offspring. Hybridization in their natural habitats may cause a loss of the Endler's guppy, which is discussed in the context of the relationship between biodiversity and ecosystem services.

Introduction

The Endler's guppy (*Poecilia wingei*) is a recently described species of guppy closely related to the common guppy (*Poecilia reticulata*) (Poeser *et al.* 2005). The Endler's guppy's more limited habitat could be invaded by the common guppy's more extensive one (Poeser *et al.* 2005; Schories *et al.* 2009; Lindholm *et al.* 2005; Nico 2006). Given this possibility, research questions like how Endler's and common guppies' mating preferences compare and whether these two species can hybridize and produce offspring are important to ensure that these species stay distinct. In general, hybridization of species causes a loss of biodiversity, which can have a negative impact on the ecosystem and reduces the community's ability to provide ecosystem services that humans depend on (Balmford *et al*. 2001; Ehrlich and Wilson 1991).

Background

The Endler's guppy is a relatively newly discovered species of guppy found only in the Cumaná region on Venezuela (A. Cruz *personal observation;* Poeser *et al.* 2005; Schories *et al.* 2009; Fig. 1). This species was differentiated originally from its close ancestor the common guppy (*Poecilia reticulata;* Fig. 2) on the basis of its differing coloration and behavioral patterns (Poeser *et al.* 2005).

In a follow-up study, the mitochondrial d loop (a relatively stable section of triple stranded DNA in the mitochondria) and cytochrome b (part of the electron transport chain) was used to assess the relationship between these two lines of fish, which provided molecular and genetic support that they are distinct species (Shories *et al*. 2009). Another research group, comparing a variety of stable, slowly evolving nuclear DNA and more quickly evolving mitochondrial DNA, confirmed the conclusion that the two lines were distinct species (Meredith *et al*. 2010).

The common guppy has been studied extensively (Baerends *et al.* 1955; Endler 1980; Laver and Taylor 2011; Reynolds and Gross 1992; Russell and Magurran 2006; Schartl 2008; Watson *et al.* 2011). It is known that females of the common guppy prefer males with more and brighter orange coloration on their body (Watson *et al.* 2011; Laver and Taylor 2011; Endler 1980) and preference for carotenoid based coloration in mating preferences have been seen in other fishes (Amundsen and Forsgren 2001). Females may prefer higher levels of carotenoid coloration because it means that the male has good foraging skills, which could be genetically passed on to their offspring, giving them higher fitness (an animal's success in producing offspring and therefore passing on its genes). Female common guppies have also shown preference for other visual cues in males, such as large size (Reynolds and Gross 1992). Larger size in males confers advantages such as success in intraspecific fights and in generally thought to show positive male health, generally regarded as an attractive male trait (Kodric-Brown and Brown 1984).

While the Endler's guppy is known to live in the Cumaná region in Venezuela (A. Cruz *personal observation;* Poeser *et al.* 2005; Schories *et al.* 2009), the common guppy has a much wider geographic range and is native to Brazil, Guyana, Venezuela, and the Caribbean islands (Lindholm *et al.* 2005; Nico 2006). The latter species has also been introduced widely throughout Asia, Europe, North and South America (Global Biodiversity Information Facility 2010).

If these species were able to hybridize, then the Endler's guppy, with its narrower habitat, would likely be lost as a distinct species. An understanding of how these two species arose (i.e. speciated from each other) could help determine if they would hybridize given the opportunity. A common form of speciation, allopatric (in different locations) speciation, arises from geographic isolation of two sub-populations of a species (Campbell and Reece 2002), which causes a slow build-up of different mutations in the resulting in the formation of two separate species. This mutation process can, but does not always, result in actual barriers to reproduction (reproductive isolating mechanisms). If speciation occurs within the same environment (sympatric speciation), two sub-populations specialize on different resources or microhabitats to survive. This form of evolution typically results in strong barriers to reproduction (Campbell and Reece 2002).

Natural selection favors the formation of barriers to reproduction between two coexisting groups and prevents production of offspring lacking specialization. Sub-populations that spread over a large geographic area typically evolve into separate species if the populations at the extremes of the continuum feature less genetic variation than the population as a whole (Palumbi *et al.* 1997). Within an environment, relatively rapid speciation can also occur as a result of sexual selection i.e., the females of two populations selecting for different traits in males (Williams and Mendelson 2011). Sexual selection was proposed as the driving force for the evolution of the Endler's guppy as separate species on the basis of differences reported in mating behavior and color patterns (Alexander and Breden 2004). For example, male display jumps (see below) and chasing behavior (a mating behavior) are rarely seen in the Endler's guppy because females leave promptly if uninterested in the male's advances (Poeser *et al.* 2005). Display jumps are defined as a leap away from the female in the middle of a sigmoidal display (Baerends *et al.* 1955). Sigmoidal displays are characterized by males extending their fins, creating an S curve with their bodies, and vibrating their bodies (Baerends *et al.* 1995). However, it has been pointed out (Schories *et al.* 2009) that waters in the habitat of the Endler's guppy are turbid and murky, thus challenging the proposal of mate choice by sight (sexual selection) and resulting differentiation in color and behavioral patterns. On the other hand, the increase in color may have arisen because of a need for males to be more colorful for females to detect colors in a murky environment.

While different species from the family *Poeciliidae* are known to hybridize in lab situations (Schartl 2008) and in the wild (Lampert and Schartl 2008), no information is available on the Endler's guppy. Hybridization can lead to problems even within two sub-populations of the same species group (hybridization can occur between species or varieties of species), such as those seen in hybrids of *P. reticulata* (crosses were *P. reticulata* from the Caroni and the Oropuche drainages in Trinidad) with smaller brood size and male sterility (Russell and Magurran 2006). Whether or not hybridization occurs between Endler's guppy and the common guppy would shed light on the question of whether these fish speciated via sympatric speciation (resulting in prezygotic reproductive barriers) or allopatric speciation (not involving mating and the rapid formation of mating barriers).

Before my studies began, it was already known that there are behavioral differences in the Endler's guppy in comparison to the common guppy, with the Endler's exhibiting (i) a shorter initial and a more extensive later stage of courtship, (ii) absence of display jumps, (iii) limited chasing behaviors, and (iv) fewer attempts to forcefully copulate with the female without previous display attempts (Poeser *et al.* 2005).

Objectives

A series of experiments addressing mating preferences and behavior of pure species as well as the viability and behaviors of the parent generation and F1 hybrid offspring were conducted to further assess mating behavior and hybridization outcomes beyond the differences in behavior found by previous studies (Poeser *et al.* 2005).

Mate Choice- Since species recognition and preferences are important aspects of maintaining distinct specie identity, it was assessed whether or not Endler's guppy females preferred (i) males of their own species and (ii) more colorful or larger Endler's guppy males. These experiments were conducted using either live males or filmed males.

It was hypothesized that female Endler's guppies would prefer their own species and would choose more colorful and larger males of their own species.

Hybridization and Behavior- If the two species are capable of hybridizing, survivorship of Endler's guppies as an independent species in the wild could have poor prospects. It is important to test if these two species are capable of hybridization and, if so, will they have viable (capable of successfully reproducing) offspring. Differences in behavior between the species could also serve as barriers to reproduction. Mating behaviors were therefore recorded to address these questions.

These questions are important to address in order to maintain biodiversity in this area via the continued existence of the Endler's guppy. How the mating behaviors and preferences of the common and Endler's guppies compare and knowing whether they are capable of effectively hybridizing, are vital to understanding whether geographic isolation must be maintained to keep these species isolated. Loss of the Endler's guppy as a distinct species would contribute to a loss in biodiversity for this area of Venezuela, but possible consequences of this loss would have to be further studied.

In general, a loss in biodiversity can cause a continued decrease in biodiversity, i.e., when one species is lost it is more likely that other species will also be lost (Tilman 1996). Ensuring that the Endler's guppy is not lost maintains biodiversity, possibly allows for a generally healthier ecosystem in Venezuela, although further studies would have to compare the relative importance of the common and the Endler's guppies in the ecosystem to determine the full importance. Additionally, knowledge about how different animals speciate and how their behaviors affect their ability to hybridize could be applied to different animal pairs similar to this one, aiding in the ability to predict and possibly counteract hybridization events and biodiversity loss.

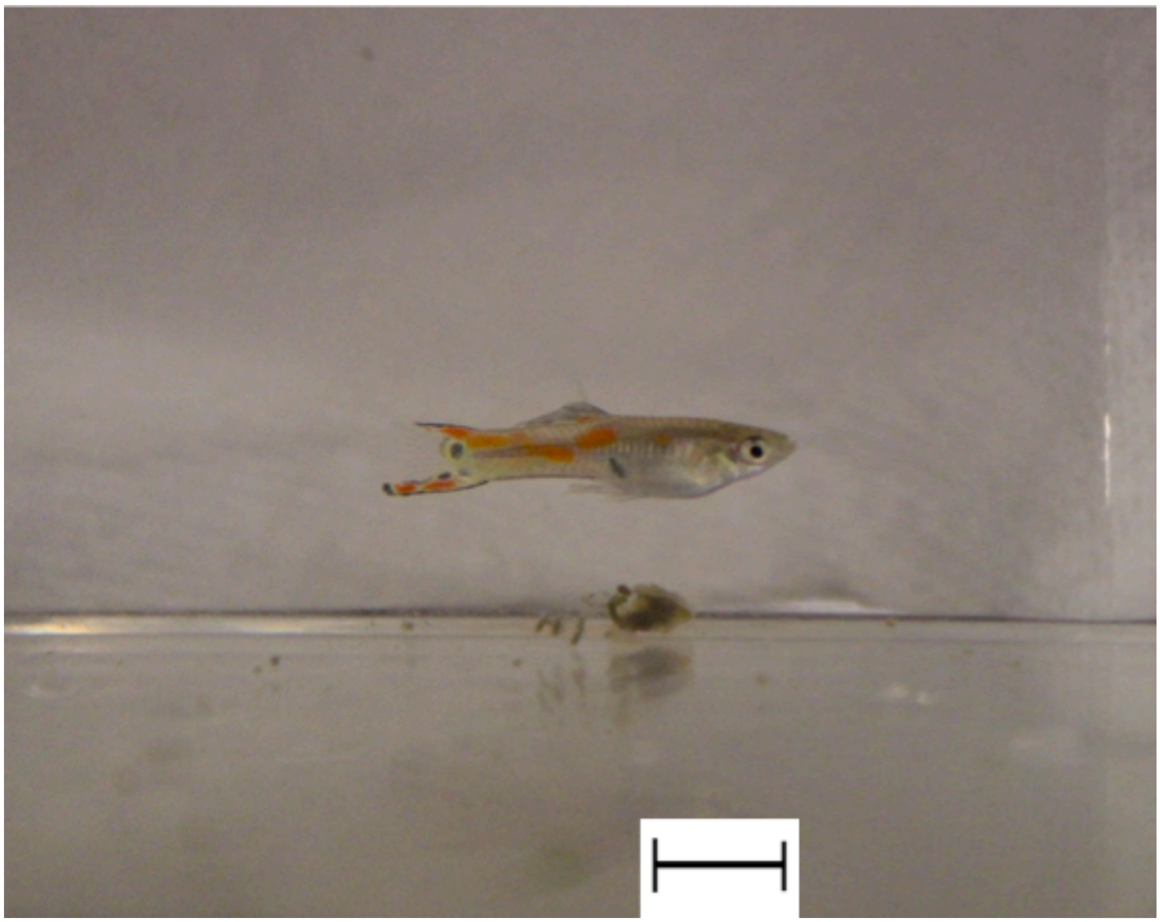

Figure 1. Photograph of the Endler's guppy. Taken February 2014 in the laboratory of Dr. Alexander Cruz, University of Colorado at Boulder. Scale bar = 1 cm.

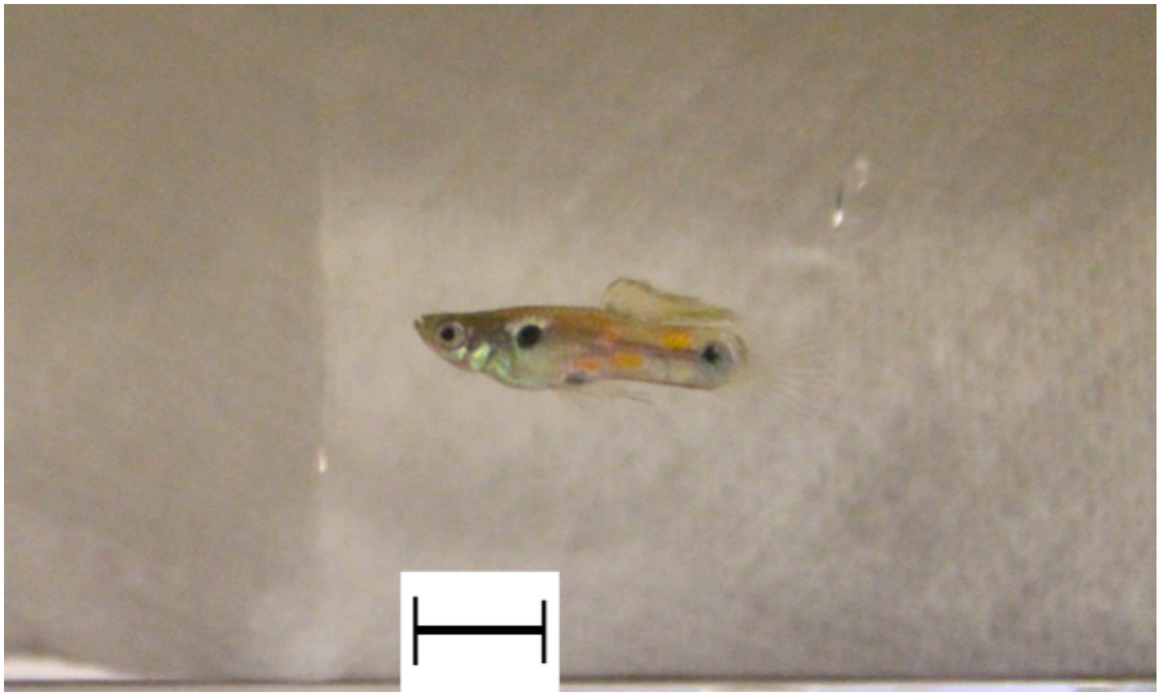

Figure 2. Photograph of the common guppy. Taken February 2014 in the laboratory of Dr. Alexander Cruz, University of Colorado at Boulder. Scale bar = 1 cm.

Methods

Female Mate Choice (for species)

Endler's and common guppies were separated by gender for three weeks (Fig. 3). Two males, one from each species, were matched for size and color and used for all trials. Ten Endler's guppy females of comparatively similar, large size were chosen (larger females are older and more likely to be sexually active). Since Endler's guppy and common guppy females are visually identical, it was assumed that the common guppy male would perform mating displays for the Endler's female (Poeser *et al*. 2005). A dichotomous (contrasting qualities) mate choice test (Fig. 4), preventing competitive aggression between males, which could influence the female's decision (Jeswiet and Godin 2011), was used to determine female interest level. This form of testing is appropriate for female mate decision in common guppies with or without a physical barrier placed in-between fish during the experiment (Jeswiet and Godin 2011). Clear, watertight dividers separated a tank into three sections, where the focal female was placed in the middle section and two males were placed in separate sections on the opposite sides of the females (Fig. 5). Before the testing period, the female was isolated visually from the males by an opaque two-litter bottle with the bottom cut out. This visual isolation was maintained for fifteen minutes before the bottle was removed and the testing period began. The female was filmed for a fifteen-minute testing period and the film used for detailed behavioral analysis. The female was considered interested in one male over the other if she spent more time in the zone of interest of one male versus the other. A male's zone of interest was an area of the tank marked by a thin strip of tape placed two average female body lengths from the watertight dividers. If the female spent time between the strip of tape and the watertight divider separating her from a male, she was considered interested in that male and time spent in that zone was recorded. If the female was in-between the two taped zones, she was considered interested in neither male. After filming had proceeded for fifteen minutes in this manner, the female was again visually secluded in a two-liter bottle and the males were switched in respect to which side of the tank they were on. The purpose of the switch was to determine if the females had a preference for one side of the tank, regardless of which male was there. After males were switched, they remained isolated to acclimate for another fifteen minutes. Post-acclimation, the experiment was repeated with the same female. Ten females were tested in this manner. To compare side bias and preference for one species versus the other, paired t-tests were used. T-tests were paired because the same female assessed both sides and both species in each trial.

Female Mate Choice (for color)

The playback approach uses film to test mate-choice preferences, with video screens displaying males placed on both sides of a tank with a female (Fig. 6). In this particular study, one Endler's guppy male film clip was modified by removing all orange coloration from a film of a male and enhancing the natural orange coloration of the same film of a male and placing it on the opposite side. Orange coloration was altered to test how the Endler's guppy preferences compare with those of the common guppy that had been previously described (Watson *et al.* 2011; Laver and Taylor 2011; Endler 1980). While playback (using filmed instead of live males) has been demonstrated to yield significant differences in female interest (Kodric-Brown and Nicoletto 1997), females do spend less time interacting with filmed versus live males (Kodric-Brown and Nicoletto 1997). On the other hand, playback allows easy color manipulation and is more reproducible (Kodric-Brown and Nicoletto 1997).

Twenty Endler's guppy females were tested, with each given ten minutes to acclimate to the tank prior to being tested for fifteen minutes using one-minute-long clips plated in a loop. To make the male fish appear live rather than filmed, playback was used to display the male in nearly the same place at the start and end of the loop to avoid breaks in the fishes' movement or placement. This procedure allows the female to focus on orange coloration and eliminates all other differences, e.g. size, other color patterns, fin size, body shape, or male response to the female. The placement of the orange or non-orange fish on the right or left side of the tank was randomly chosen to eliminate the effect of any female preference for either side of the tank.

An uncontrolled variable in this experiment was tank temperature. In the first ten trials, the tank in which females were tested was colder (20°C) than their holding tank (24°C), and in the next ten trials, the testing tank was heated to 24°C. These experiments were analyzed with multiple t-tests to independently assess female preference for color in the varying tank temperatures.

Female Mate Choice (for size)

One average-sized (2.54-cm long when displayed on testing screen) Endler's male was filmed and this clip was then altered to result in lengths of 3.175-cm ("large") or 1.905-cm ("small"), respectively. These film clips were projected as loops on either side of a tank for fifteen-minute intervals. Dichotomous mate choice procedures (see above) were used to determine female interest in the males. Each female (of a group of ten total) underwent three trials comparing small versus large, large versus average-sized, and small versus averaged-sized males. An Anova test was used to compare the frequency with which each male was chosen.

Mating Behavior & Outcome (F1 Hybrids) of Pure- & Mixed-Species Parents

Sixteen thirty-eight liter tanks, each equipped with a filter and a similar amount of algae, were divided into four tanks each for four different groups, i.e. (i) all Endler's guppies, (ii) all common guppies of the Rio Piedras region of Puerto Rico, (iii) Endler's guppy females and common guppy males, and (iv) common guppy females and Endler's guppy males. Before being placed in the experimental tanks, males and females were kept in isolation tanks with about twenty-five fish of their own gender and species for three weeks. This was to ensure that females were not gravid (pregnant) and were thus receptive to mating and also that all fry (offspring) birthed in tanks were fathered by a male within that tank. The males were isolated for consistency with the treatment of the females and to increase male interest in mating. After this isolation period, the testing tanks were set up. Three males were measured for length (measured with and without including their tail length) and three females were measured similarly before being placed in a tank. This resulted in tanks with three pairs for each species and each mixture of species.

Mating behavior was observed for two minutes each day. Two minutes was chosen due to previous observations, which concluded a consistency of behavior over a five-minute period. Gonopodial thrusts, sigmoidal displays, copulation, female interest, chasing, nipping and following behaviors were recorded. These behaviors have been used and described in various studies done on the common guppy (Baerends *et al* 1955; Liley 1996). Any fry produced by the group were promptly removed and placed in tanks with other offspring of their own experimental group. After sixty days of observation regularly seen behaviors were analyzed using various tests. The most commonly seen behaviors, gonopodial thrusts and sigmoidal displays, as well as the number of offspring produced were analyzed using Anova tests to account for the variation in the data.

Mating Behavior & Outcome (F2 Hybrids) of F1 Hybrids and Pure Species Parents

Sixteen tanks were set up in an identical manner to that described above for four experimental groups of four tanks each, i.e. (i) offspring of the all-Endler's-guppy tanks, (ii) offspring of the all-common-guppy tanks, (iii) offspring of the Endler's females/common guppy males, and (iv) the offspring of the common guppy females/Endler's guppy males. The offspring were isolated by gender between the ages of three- to five-months. Larger, older females were chosen for the experiment. Each tank had three males and three females from their experimental group.

Behaviors were observed for two minutes per day for the reasons given above. Gonopodial thrusts, sigmoidal displays, and chasing behavior were recorded. Any fry produced by the fish were removed and counted. Behavior was watched for sixty days and only fry produced within that timespan were counted. Analysis was done to compare how often the males of each experimental group performed each behavior and to compare the number of offspring produced by each experimental group. Anova tests were used to analyze results. The data for sigmoidal displays was analyzed with a linear and non-linear mixed effects model. Since the data on offspring did not exhibit a normal distribution, data were normalized via log transformation before using an Anova test.

Figure 3. Each isolation tank held one gender of one species.

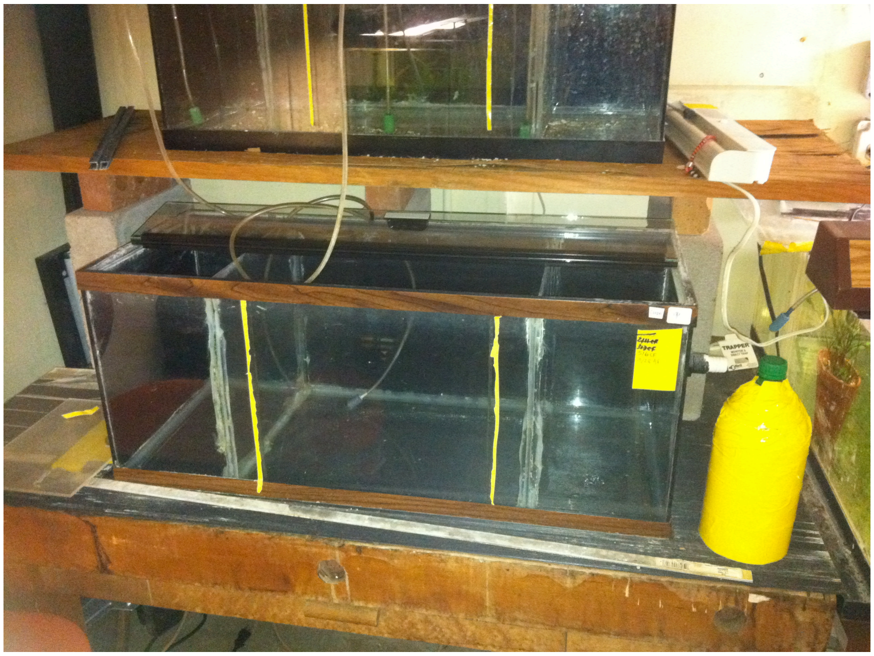

Figure 4. The dichotomous mate choice testing tank

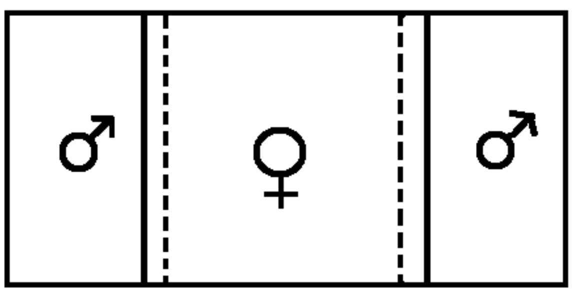

Figure 5. Dichotomous mate choice testing tank set-up. The tank is partitioned into three sections; the males were placed in the outer two sections. The dotted lines mark of zones of interest for the females.

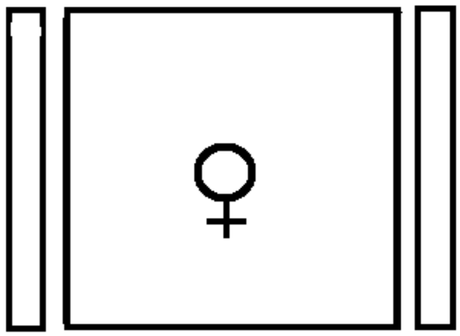

Figure 6. Playback mate choice set-up. Female in center tank and screens on both sides.

Results

Female Mate Choice (for species)

For experiments using live males, neither the time spent on each side of the tank (Fig. 7) nor the time spent with the common guppy versus the Endler's guppy males varied significantly for Endler's guppy females (Fig. 8).

Female Mate Choice (for color)

In experiments using film, females did not exhibit and preference for orange versus non-orange males, regardless of water temperature (Fig. 9-11). While none of the pairings resulted in significant differences, a (non-significant) trend for a higher mean in female preference for orange males was seen at experimental water temperatures of 24°C (same as holding-tank temperature; Fig. 10).

Female Mate Choice (for size)

In experiments based on film, Endler's guppy females showed no preference between larger versus smaller males, large versus average-sized males, or average-sized versus smaller males (Fig. 12).

Mating Behavior & Outcome (F1 Hybrids) of Pure- and Mixed-Species Parents

The first generation of hybrid and non-hybrid groupings showed a significant difference in the number of gonopodial thrusts (Fig. 13). There was no significant difference in the number of sigmoidal displays performed by the males in the first generation (Fig. 14) or in the average number of offspring produced by the respective groups (Fig. 15).

Mating Behavior & Outcome (F2 Hybrids) of F1 Hybrids and Pure Species Parents

There was no significant difference between in chasing behavior (Fig. 16), gonopodial thrust frequency (Fig. 17), or in the number of sigmoidal displays (Fig. 18) between pure species and F1 hybrid-mating groups. Furthermore, the number of offspring produced by the different groups did not differ significantly (Fig. 19).

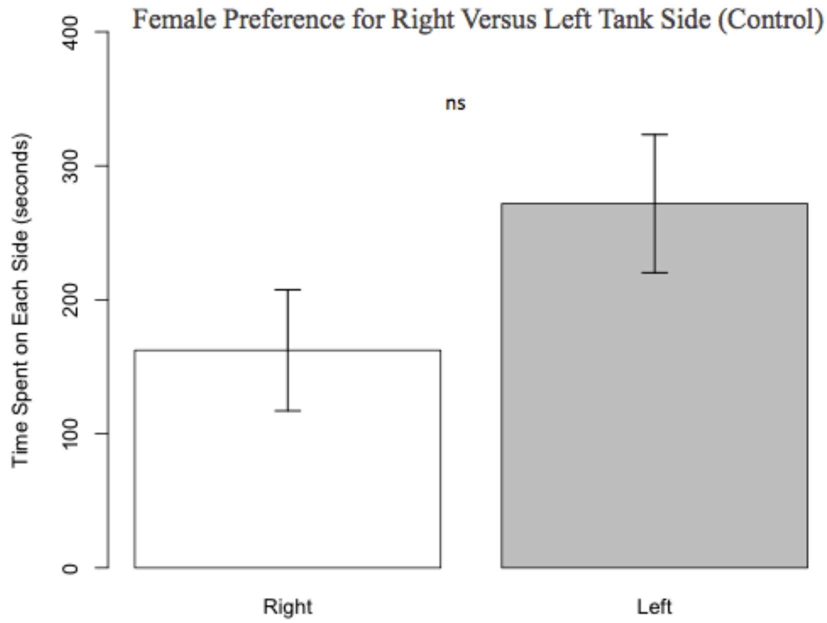

Figure 7. Time spend by females on each side of the tank (t_{40}=-1.60, p=0.1188).

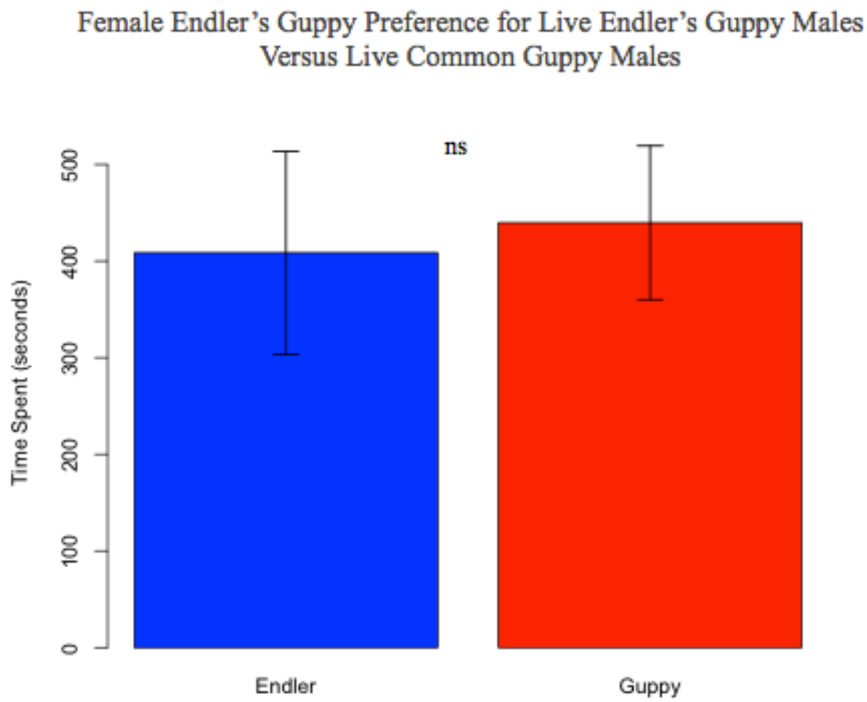

Figure 8. Mean time spent by females with the common guppy male versus the Endler's guppy male (t_9=-0.19, p=0.85).

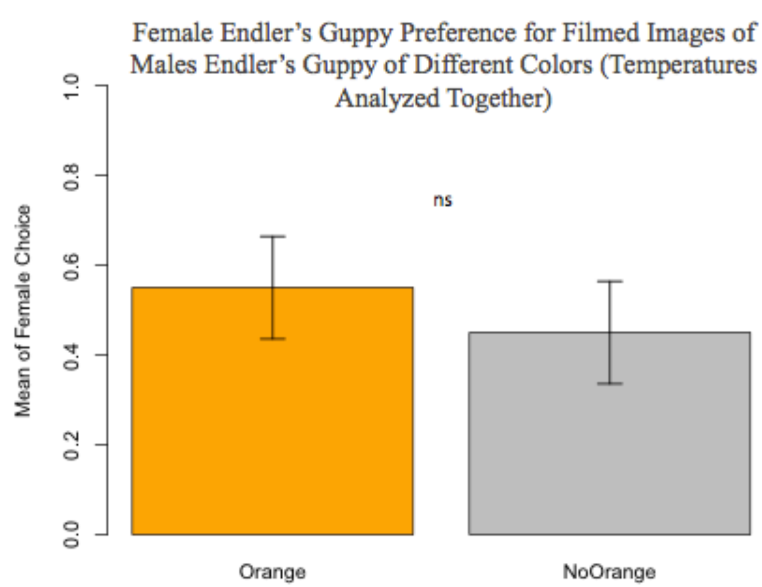

Figure 9. Female preference for males with or without orange coloration (t_{19}=0.44, p=0.67). This test did not take into account the variation in water temperature in the testing tank.

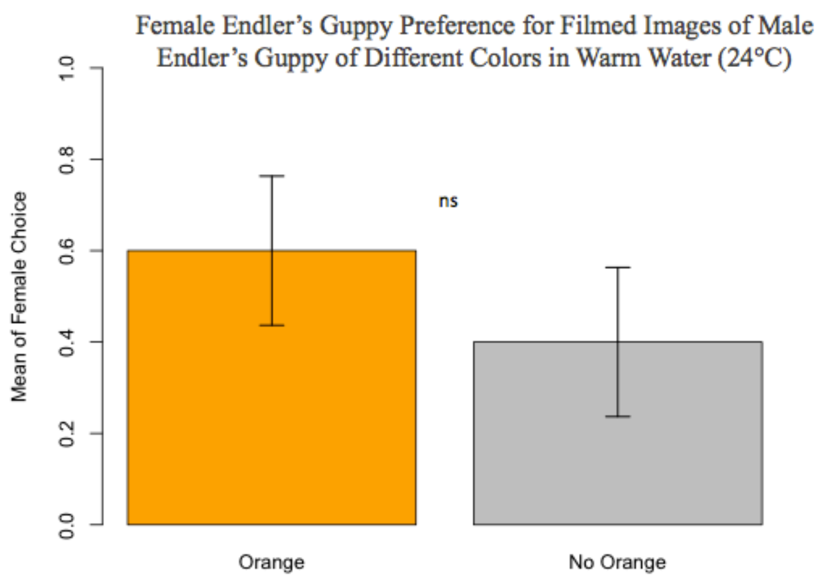

Figure 10. Female's preference for orange or non-orange males in water of 24°C (t_9=0.62, p=0.56).

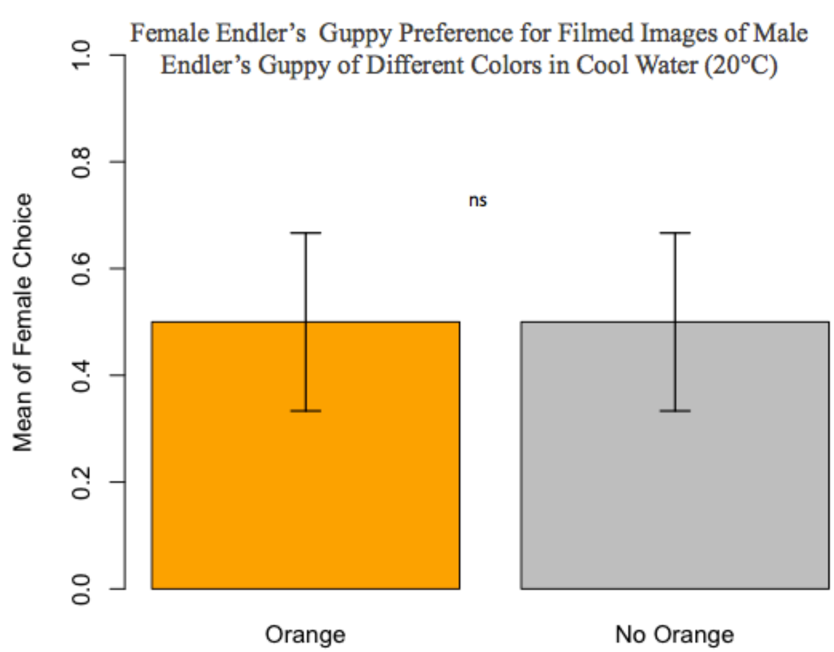

Figure 11. Female preference for male color in a testing tank that was cooler (20°C) than their holding tank (t_9=0, p=1).

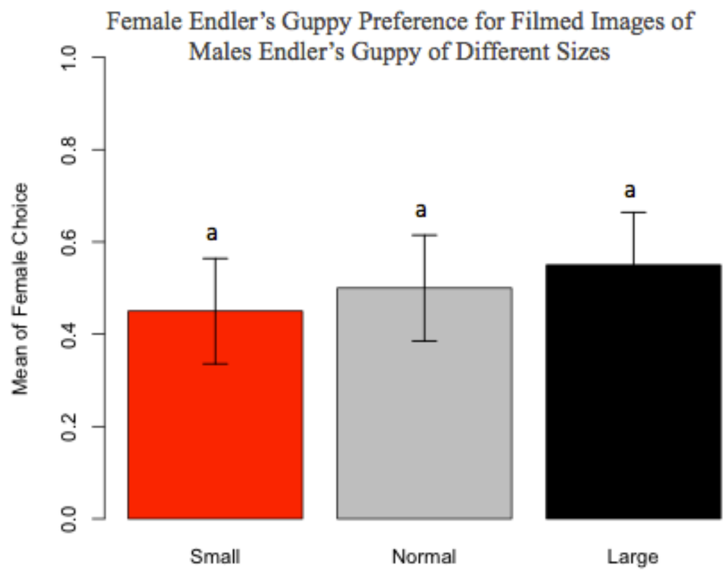

Figure 12. Female preference for male size, small and average-sized males (T_{59}= 0.309, p=0.758), small and large sized males (T_{59}=0.619, p=0.539), or average and large sized males (T_{59}=0.309, p=0.758).

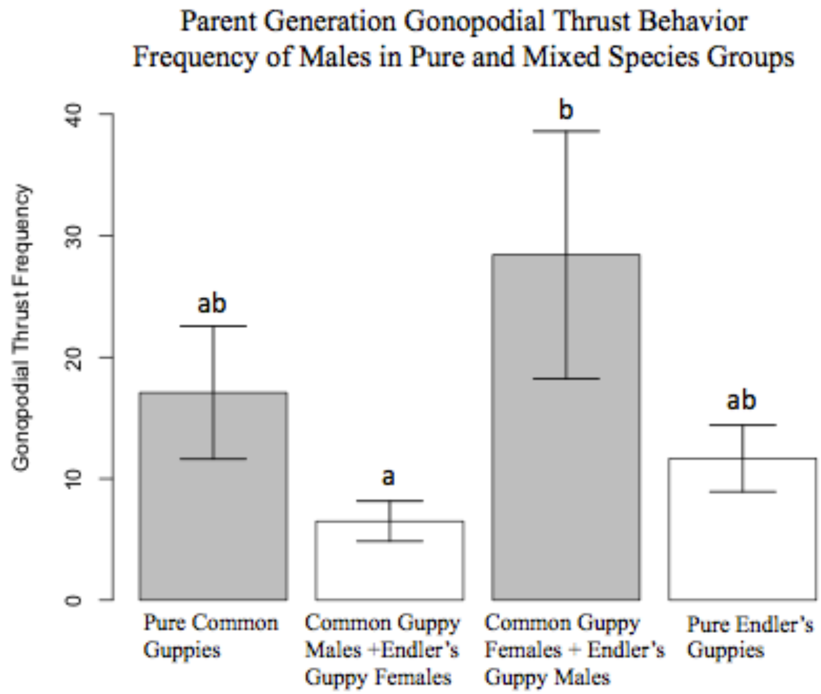

Figure 13. Gonopodial thrust frequency for four groups of pure and mixed species; Common tanks with only the common guppy, common guppy males and Endler's guppy females, Endler's guppy males and common guppy females, and Endler's guppy tanks showed an overall statistical outcome (F_3=42.177, p=0.0399). The pairwise comparisons of the 1st and 2nd mixed species group the only significant pairwise comparison.

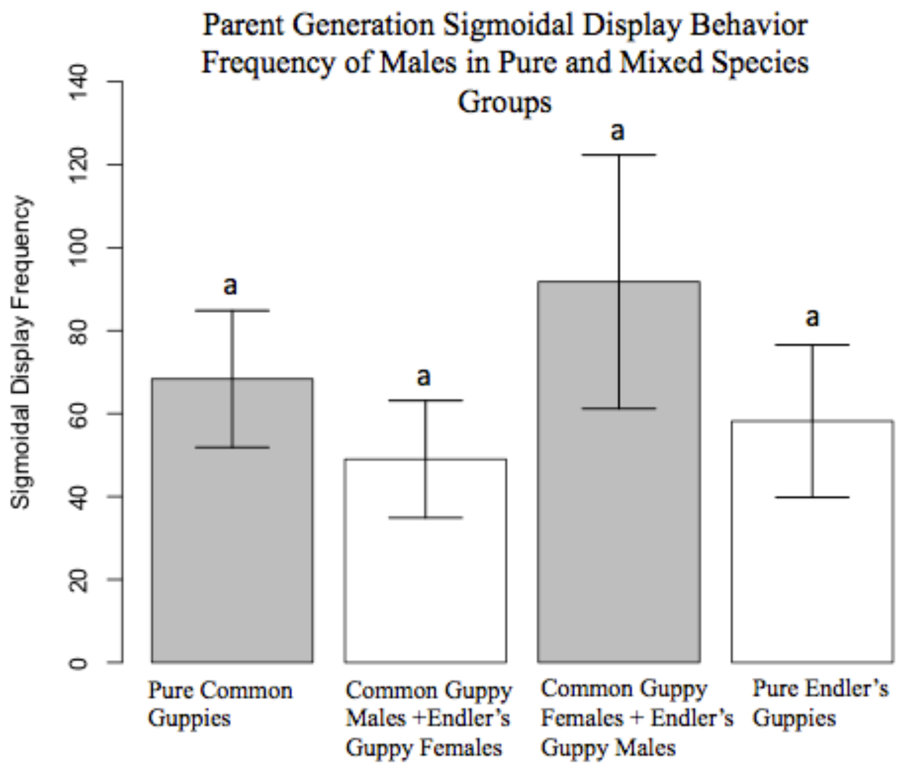

Figure 14. Sigmoidal display frequency for the four groups of pure and mixed species; common guppy tanks, common guppy male and Endler's guppy females, common guppy females and Endler's guppy males, Endler's-guppy tanks showed no significant difference in sigmoidal display frequency (F_3=0.3526, p=0.7874).

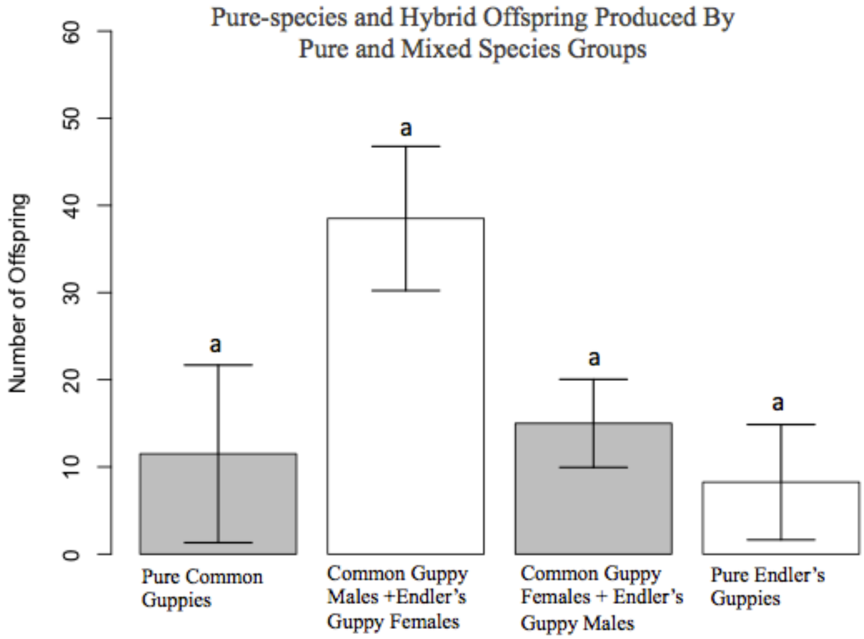

Figure 15. Number of offspring of pure and mixed species groups; common guppy tanks, common guppy males and Endler's guppy females, common guppy females and Endler's guppy males, and Endler's guppies tanks showed no significant differences between groups (F_{12}=3.059, p=0.06948).

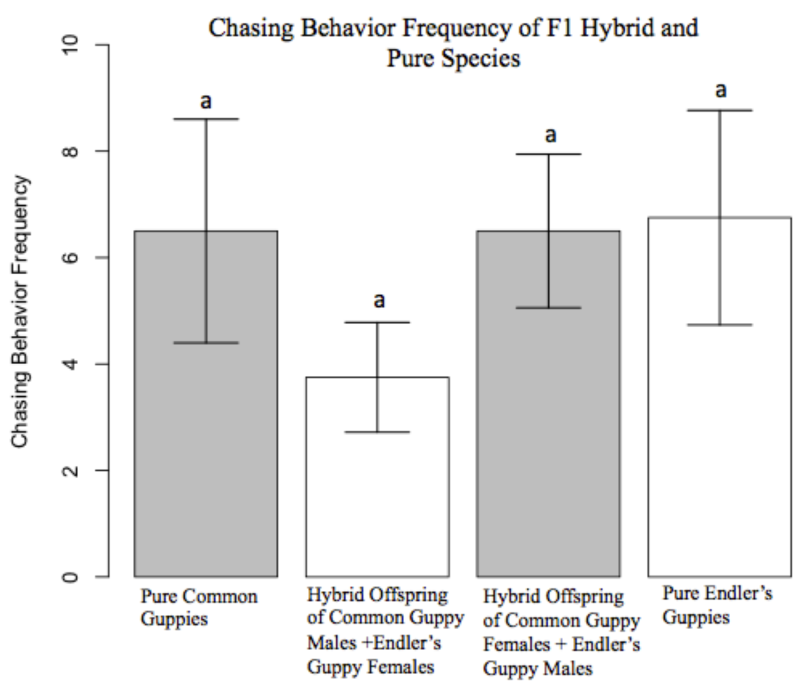

Figure 16. Chasing behavior frequency for offspring of pure and hybrid species groups; common guppy tanks, hybrids of common guppy males and Endler's guppy females, hybrids of common guppy females and Endler's guppy males, or Endler's guppy tanks showed no significant difference in any of the comparisons above (F_{12}=0.695, p=0.5725).

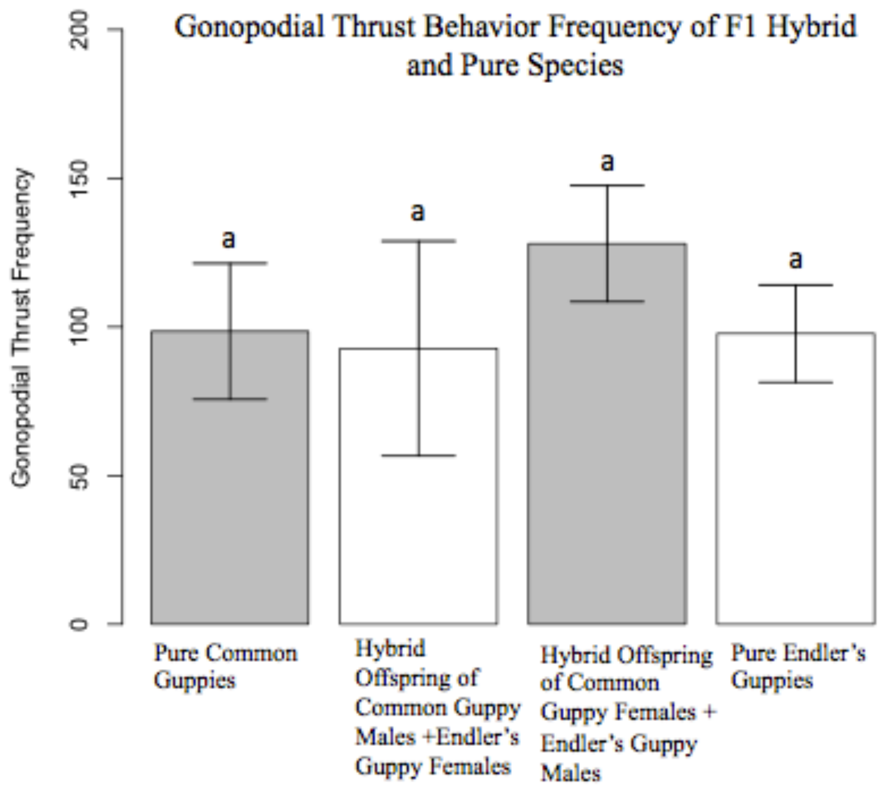

Figure 17. Gonopodial thrust frequency for offspring of pure and hybrid species groups; common guppy tanks, hybrids from common guppy males and Endler's guppy females, hybrids from common guppy males and Endler's guppy females, or Endler's guppies tanks showed no significant differences between groups (F_{12}=0.42, p=0.745).

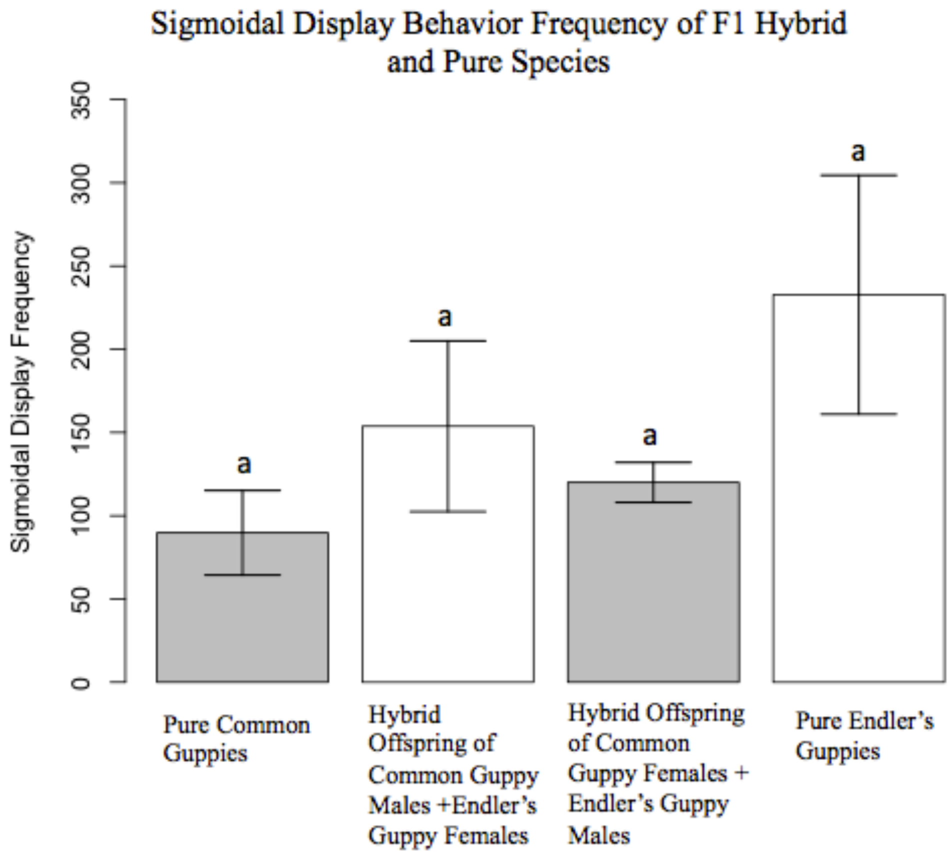

Figure 18. Sigmoidal display frequency for the offspring of pure and hybrid species groups; common guppy tanks, hybrids from common guppy males and Endler's guppy females, hybrids from common guppy females and Endler's guppy males, or Endler's guppy tanks showed no significant differences between groups (F_{12}=127.57, p=0.2814).

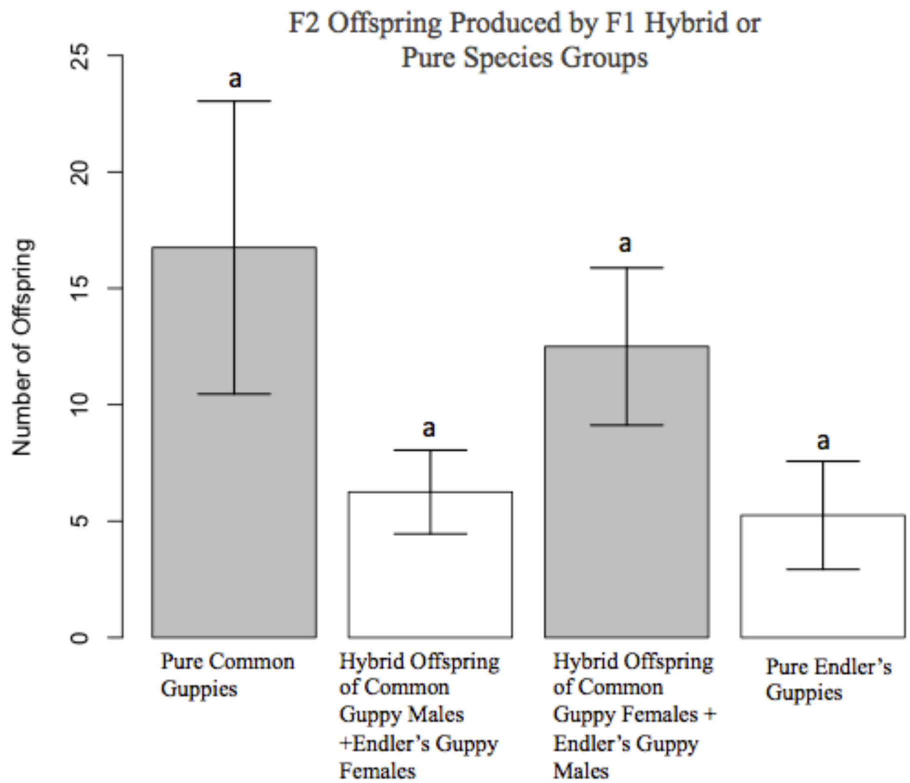

Figure 19. Number off offspring from pure and hybrid mating groups; common guppy tanks, hybrids from common guppy males and Endler's guppy females, hybrids from common guppy females and Endler's guppy males, or Endler's guppy tanks showed no significant differences in the comparisons between the groups (F_{12}=1.84, p=0.1928).

Discussion

Female Mate Choice (for species)

Female Endler's guppies neither showed a significant preference for one side of the tank versus the other nor for males of their own species (the Endler's guppy) versus the common guppy. This finding (of absence of preference for their own species) makes it unlikely that increase in carotenoid coloration are a product of sexual selection and that, unlike previously suggested, a mating preference of female Endler's guppies for specific carotenoid-based colorations was involved in driving speciation (Alexander and Breden 2004).

A limitation of the present study was that only a single pair of males was used for the present mate choice experiment, leaving open the possibility of confounding factors, i.e. individual characteristics unrelated to species like coloration or size, contributing to the females' decisions. More studies should thus be done, randomizing the use of males for each female choice and increasing the number of females tested. Additionally, the males tested should be given longer breaks, than the 15 minutes where the females became accustomed to the tanks, between tests to be better rested for each displaying attempt. In order to determine how long of a break is appropriate the breaks could be increased in length until the male displays continuously throughout the trial. Finally, different tank partitions should be used to acclimate the female rather than the bottle used here, which presented an apparent disturbance preventing the females from immediately responding to mating attempts.

Female Mate Choice (for color)

While no significant results were obtained regarding a female preference for more colorful males, there was a trend towards preference for males with more carotenoid coloration, especially when the testing tanks were adjusted to a water temperature similar to that of the tanks in which fish were raised. Further studies should be conducted with more replicates to assess whether trends are or are not significant. If the absence of significant preference based on color were to be confirmed, this would be consistent with the fact that the Endler's guppy's native habitat is turbid and polluted (A. Cruz *personal observation*; Schories *et al.* 2009). It is possible that this low-visibility native habitat has, in fact, limited the Endler's guppy's ability to make mating decisions based on visual cues. Mating decisions can be made with a range of other senses. It has been shown that many fish (Giaquinto *et al.* 2010; Rosenthal *et al*. 2011), including guppies (Guevara-Fiore *et al.* 2010), use pheromones (volatile chemicals) of possible mates to help in their mate-selection process. Common guppies find groups of females receptive to mating based on pheromones released by the females as well as on some visual cues (Guevara-Fiore *et al.* 2010). It is possible that the Endler's guppy evolved away from visual cues and toward phenomenal cues for finding mates in their murky environments. This could explain the Endler's guppies' lack of significant mate preference based on color.

Previous findings that Endler's guppies reside in areas containing a major guppy predator, *Astyanax bimaculatus*, making their bright carotenoid coloration a risk factor in predation, have been used to speculate that this coloration must be a product of sexual selection (Alexander and Breden 2004). While such a preference for bright carotenoid coloration in the common-guppy (Watson *et al.* 2011; Laver and Taylor 2011; Endler 1980) and other fish species (Amundsen and Forsgren 2001) has been seen, the present study did not confirm a preference for overall bright carotenoid coloration in the Endler's guppy.

However, it may be important that Endler's guppies were previously not only found to possess high levels of carotenoid coloration, as was found to be important in one study, but specifically a higher number of carotenoid spots than the common guppy (Alexander and Breden 2004). A future test could investigate increasing the number of carotenoid spots and the carotenoid color of the males used for female mating preference. Future studies should also investigate the roles of the number of carotenoid spots versus overall intensity of the carotenoid color of males used for female mating preference.

These future studies should all be conducted in the warmer testing tank water to eliminate the variation seen as dependent on water temperature.

Female Mate Choice (for size)

Unlike female common guppies (Reynolds and Gross 1992), females Endler's guppies did not show a significant preference for males of larger size, despite the generalization that larger size is a good indication of male health (Kodric-Brown and Brown 1984). This difference could be due to females not using visual cues for mating, as discussed above. Conversely, the slight trend shown toward preference of males of larger size may be strengthened by a higher sample size of Endler's guppy females in future studies.

Mating Behavior & Outcome (F1 Hybrids) of Pure- and Mixed-Species Parents

Mating Behavior – The trend for male Endler's guppies placed with female common guppies to display more, and perform more gonopodial thrusts, than male common guppies placed with female Endler's guppies. These observations may be the result of common guppy females recognizing the Endler's guppy males as separate species and the females not responding to their sigmoidal displays; while Endler's guppy females fail to make this distinction and mate with common guppy males. In other words, if common guppy males and females (but not Endler's guppy males and females) were capable of differentiating their species from another, it would makes sense for them to make less mating attempts or show less interest in the other species. There was no difference in behavior between the species when paired conspecifically, indicating that the two species have not evolved separate mating behavior trends.

Offspring (F1 Hybrids)- The number of offspring produced by the pure or mixed species parent groups were not significantly different. Despite this lack of significance, the highest number of F1 (hybrid) offspring was observed in the mixed mating group consisting of male common guppies and female Endler's guppies. This mixed-species parent group was also the group that displayed the lowest amount of mating behaviors. Female guppies are receptive to mating for 1-2 days after giving birth (Evans 2012). Male mating attempts outside of this receptive period can cause negative fitness effects across generations (Gasparini *et al.* 2009) and a 25% decrease in time spent foraging for common guppy females (Magurran and Seghers 1994). Female *Poeciliids* perform various behaviors to limit these negative effects such as shoaling behaviors with other females (Pilastro *et al.* 2003) and association with larger males who are less aggressive in their sexual behaviors (Pilastro *et al.* 2003). The males of the mixed-species group where the females produced the most offspring, displayed the least number of mating behaviors. This could be because females have developed behaviors to limit harassment while not sexually receptive i.e. pregnant, as described above. Presumably these females spent more of the duration of the experiment in this state than other groups because they produced more offspring.

Mating Behavior & Outcome (F2 Hybrids) of F1 Hybrids and Pure Species Parents

Mating Behavior – The F1 groups and the pure species groups exhibited no significant difference in chasing, gonopodial thrusts, or sigmoidal display behaviors. F1 hybrids from the Endler's guppy females and common guppy males parent group was the group exhibiting the least chasing behavior. In previous studies looking at Endler's guppies behavior and comparing it to that of the common guppy found that Endler's guppy males chased less frequently than common guppy males (Alexander and Breden 2004). My study found no significant chasing behavior difference between the two pure species groups. Future studies should confirm whether or not the trends seen in the present study could be significant with a higher number of replicates. Since there was no evidence for reduced mating efforts in F1 hybrid versus non-hybrid offspring, it can be concluded that the hybrid can survive to maturity and exhibit the same behaviors as their parent species. Differences in mating behavior can be beneficial for female species differentiation. Since there are no differences in behavior seen the females will not be able to use this as a way to distinguish their own species from another.

Offspring (F2 Hybrids) - The number of F2 offspring produced by the F1 groups did not differ from the number of offspring produced by pure species groups. There was a trend toward F1 hybrids from the Endler's guppy male/common guppy female group producing more offspring than the other groups.

Implications of Hybridization

In summary of the above-mentioned results, the two species studied here mated, and showed no significant differences in mating behavior not only in the parent generation, but also in the F1 hybrid generation. The differences in gonopodial thrusts seen in the parent generation between mixed species groups could mean that common guppies are capable of differentiating between species, but that the Endler's guppies are not, suggesting the possibility that Endler's guppies are relying less strongly on visual cues; perhaps in response to their turbid native habitat. While differentiation in mating behaviors can help some species tell apart members of their own species from those of another species, the two species studied displayed similar mating behaviors, hybridized, and produced viable offspring. Despite the significant genetic (Shories *et al*. 2009; Meredith *et al*. 2010), behavioral, and coloration differences (Poeser *et al*. 2005), these two species are therefore not separated by reproductive barriers and would presumably mate in the wild if given the opportunity. Additionally, there is a possibility that the hybrids may actually produce more offspring than the Endler's guppy, thus presumably expediting the process of species loss. This lack of barriers to reproduction suggests that the Endler's guppy was a result an allopatric speciation processes, as has been shown for other species groups, such as birds (Cade 1983), or fish (Stelkens and Seehausen 2009), that are more likely to hybridize if sub-populations diverge allopatrically. In another species pair capable of hybridizing, i.e. as the barred and spotted owls, additional barriers to reproduction occurring in the wild, such as possible feeding preferences, roosting preferences, etc., have kept the two species from hybridizing at a frequent rate (Hamer *et al.* 1994). Mitochondrial DNA has been used to determine which of these birds were barred versus spotted owls and which were hybrids (Haig *et al.* 2004). Raising and hybridizing Endler's and common guppies in a more natural setting, where both species are capable of mating with either species, and then using mitochondrial DNA testing to determine hybridization rates could help determine how this mating scenario would play out in the wild.

Future Studies

Many of the trends seen in the present study were not significant. Future studies should increase the sample size of females used for the mate choice experiments and increase the number of tanks used in the hybridization and behavior experiments.

Additional time spent watching the fish for behaviors could also have possibly allowed for identification of other significant differences in behavior.

The DNA differences between the species (Shories *et al*. 2009; Meredith et al. 2010) are not in vital locations for mating or they would have inhibited the hybridization process. Nevertheless, further investigation into how these actual differences in DNA influence hybrid development could shed light on possible differences of hybrid fitness. Further studies should address possible differences in hybrid fitness such as growth rate.

Additionally, gonopodia differ between species and such differences are found in the common guppy and Endler's guppy (Poeser *et al.* 2005; Fig. 20) Hybrid guppies could have some intermediate form of gonopodium that serves as a barrier to reproduction between the hybrid and the original species (although not between the hybrids as demonstrated here). More experiments, such as attempting to breed the hybrid guppies with the pure species guppies, would be of interest.

Further insight is needed into Endler's guppies' mating preferences and hybridization capabilities. In order to preserve this group as a distinct species, habitat changes such as novel dam or waterway creation, should be avoided as such manipulations could allow for common guppy and Endler's guppy habitat to come into contact. Any movement of either of these species should also be avoided.

<u>Conclusions</u>

Maintaining the Endler's guppy as a distinct species would maintain higher levels of biodiversity in this area. In general, higher levels of biodiversity typically stabilize ecosystems, decrease extinction rates, (Tilman 1996), maintain ecosystem function

(Hooper *et al.* 2005), and have a positive effect on ecosystem services the environment can provide (Balvanera *et al.* 2006), such as including maintenance of soil composition, atmospheric and climatic stability, nutrient cycling, and a larger pool of species to draw from for pharmaceuticals, food, and fuel from (Balmford *et al*. 2001; Ehrlich and Wilson 1991). Other services, such as reduction of disease transmission, have shown conflicting positive and negative effects with increased biodiversity (Wood and Lafferty 2013). Importance of certain species for ecosystem function is species specific and depends on many variables i.e. redundancy of species performing one ecosystem function and strength of interactions one species has with another (Hooper *et al*. 2005). Maintenance of the Endler's guppy as a distinct species would therefore have presumably mainly positive effects on the ecosystem, but the relative importance of the Endler's guppy in this ecosystem should be further studied.

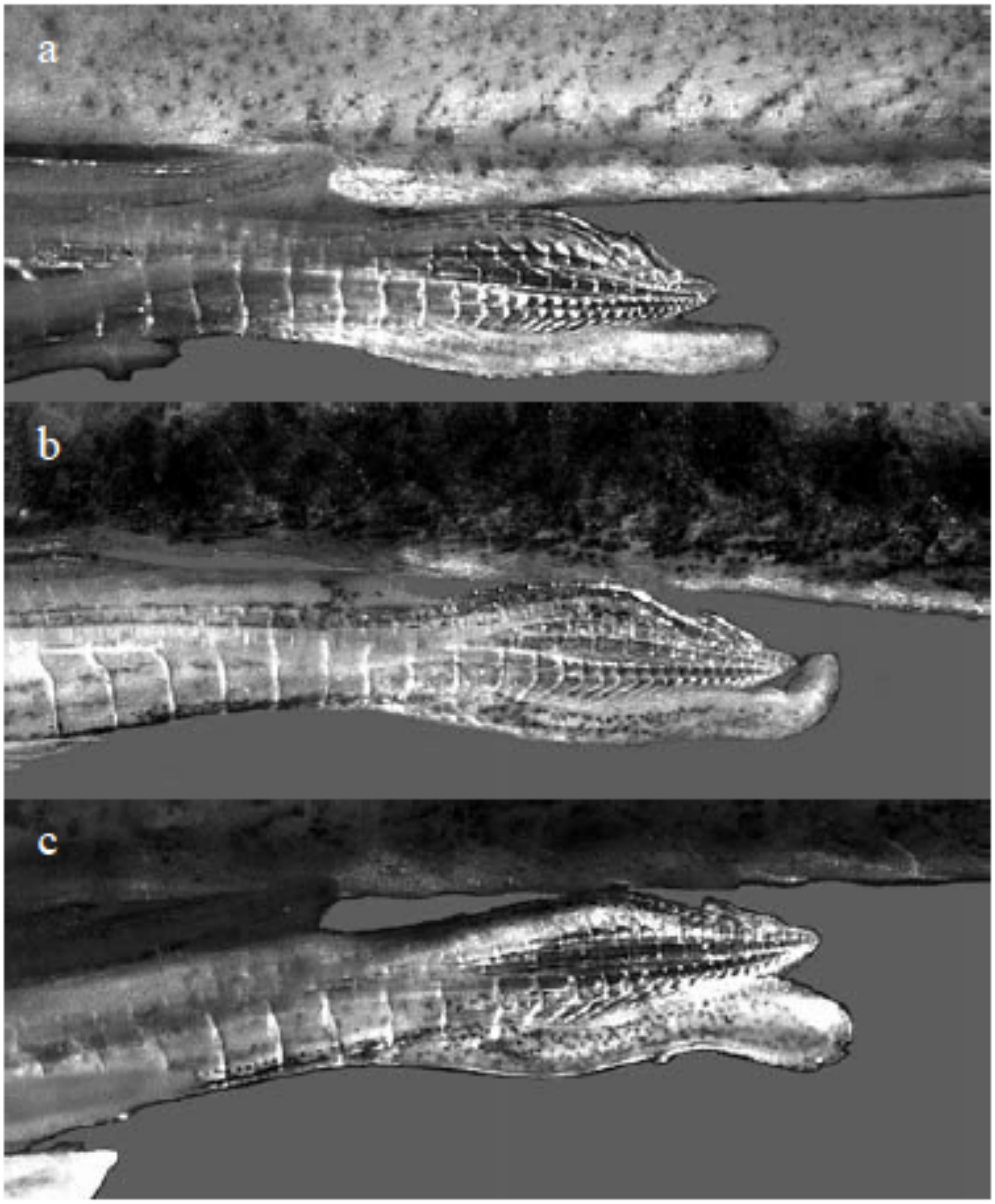

Figure 20. Photographs of guppy gonopodia: (a) Common guppy, (b-c) Endler's guppy (Poeser *et al.* 2005)

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