

How does sexual selection and breeding biology play a role in the conservation of the threatened *Xenotoca eiseni*?

By
William Ryan Currier
University of Colorado at Boulder

A thesis submitted to the
University of Colorado at Boulder
in partial fulfillment
of the requirements to receive
Honors designation in
Environmental Studies
May 2013

Thesis Advisors

Dr. Alexander Cruz, Ecology and Evolutionary Biology, Committee Chair
Dale Miller, Environmental Studies
Dr. Andrew Martin, Ecology and Evolutionary Biology

© 2013 by William Ryan Currier
All Rights Reserved

Table of Contents

Preface	iii
Abstract	vi
Introduction.....	1
Background and Literature Review.....	3
Locality	3
Description	8
External Description	8
Reproductive Characteristics.....	9
Systematics.....	10
Sexual Selection	13
Breeding Biology	17
Developmental Biology	24
Conservation.....	27
Materials and Methods.....	35
Open Aquarium.....	36
Open Aquarium Data Analysis.....	37
Dichotomous Choice.....	38
Video Playback.....	40
Video Playback & Dichotomous Choice Data Analysis.....	41
Results	43
Open Aquarium.....	43
Dichotomous Choice.....	45
Video Playback.....	47
Discussion.....	50
Open Aquarium.....	50
Dichotomous Choice.....	51
Video Playback.....	51
Conclusion	55
Bibliography	58
Appendix.....	63
A. Open Aquarium Data:.....	63
B. Dichotomous Choice Experiment Raw data:	64
C. Video Playback Raw Data:	65

Preface

I first became interested in fish conservation when I moved out to Colorado from Upstate New York. The cool mountain streams of Colorado and their aesthetic beauty lured me in immediately. It did not take long before I became very interested in the hidden world within these streams. Species such as the Greenback Cutthroat Trout, the state fish of Colorado, fascinated me as I found them beautiful and became aware of how they are nearly extinct. While taking courses such as Ecology, Fish Biology, Evolutionary Biology, Lake and Stream Ecology etc. my interest in fish conservation grew. I became increasingly interested in how humans have altered the water supply and chemical nature of these rivers. In addition, I became interested in how humans were capable of easily moving around vast quantities of fish and how this dramatically altered the trophic dynamics within this hidden world.

Later on in my career I met Dale Miller, who suggested that I try to get involved in Dr. Alexander Cruz's fish laboratory. Here, Dr. Cruz introduced me to a species commonly referred to as the Redtail Splitfin. He shared with me how they were similar to humans in that they are viviparous and have a placenta like structure. I found these characteristics intriguing and from that point on, along with the help of many generous people, this project was born.

I want to thank Dr. Cruz for letting my work take place in his facility at the University of Colorado, Boulder and for all the help he has provided me throughout the past year. Thank you for guiding me throughout the process and sitting down with me to discuss

my project. This allowed for new insight and clarification each time. I consider this unbelievably generous on his part.

I would also like to thank Dale Miller for his guidance throughout the entire process. Dale Miller recommended I meet with Dr. Alexander Cruz and therefore served as the catalyst to this project. He also provided an unparalleled amount of advice throughout the writing process. His persistence in helping me as well as many other students should be noted as he gave many of us Honors Students the confidence to keep working when there was doubt we could complete the project.

I would also like to thank Dr. Andrew Martin for serving as a committee member. Dr. Martin was my Evolutionary Biology Professor and his work involving the Devils Hole pupfish (*Cyprinodon diabolis*) in Death Valley National Park and the Greenback Cutthroat Trout in Colorado was inspiring to me. His work and instruction in the classroom allowed me to obtain a better grasp of my own work and to think about things from a new perspective, something that Dr. Martin encourages many of his students to do.

Marcus Cohen should also be acknowledged for being the first to meet with me and introduce me to the fish facility. Furthermore, I want to acknowledge Marcus Cohen for his help, along with the IT department, on the video playback portion of this study. They were both a tremendous help. Chloe Ramsay and Joseph Mihaljevic should also be acknowledged for helping me in my statistical analysis, particularly with writing code in the program “R”. In addition I want to acknowledge everyone who works/volunteers in the Fish Lab for feeding the fish and their consistent laboratory maintenance.

Lastly, I want to thank my parents because without them none of this would have been possible. Their tremendous support and wise words throughout my entire

undergraduate career here at the University of Colorado, Boulder is incomparable. It allowed me to persevere at an unprecedented level. Thank you everyone for your support. It is truly and greatly appreciated.

Abstract

The subfamily Goodeinae consists of thirty-six matrotrophic viviparous species, all of which are endemic to Mexico (Webb *et al.* 2004). *Xenotoca eiseni*, a species within the subfamily Goodeinae, is sexually dimorphic in hue and varies geographically in its coloration (Fitzsimons 1970). Currently, six or seven natural populations remain (Lyons 2011). Fortunately, *X. eiseni* is raised in many aquariums and is commonly traded amongst hobbyists. Therefore populations of *X. eiseni* can be reintroduced into extant populations (Dominguez-Dominguez *et al.* 2005). Natural populations suffer from habitat degradation and invasive species (Lyons 2011, Domínguez-Domínguez *et al.* 2005). They are currently not listed as endangered as they have little economic value and have not been assessed by the International Union for Conservation of Nature (IUCN 2012). However, many national and international researches use *X. eiseni* and other Goodeidae as model organisms for evolutionary, biogeographic and livebearer studies (Domínguez-Domínguez *et al.* 2005). This study is designed to use a variety of methods to see how sexual selection and breeding biology play a role in the conservation of the threatened *X. eiseni*. Male Goodeinae cannot successfully mate with females without the female possessing an interest in the male. This is because the males do not possess a true gonopodium. *X. eiseni* males therefore possess an elaborate courtship behavior when seeking a mate (Kingston 1970). This study showed that large females show a disinterest in small males and males that exhibit relatively less coloration than the more colorful males. Therefore when reestablishing populations of *X. eiseni* males should be closely matched in color to the extant populations to ensure successful copulation and sustain populations of this unique Mexican treasure in the future.

Introduction

The unique fish biota within Mexico has been decreasing at unprecedented rates due to a variety of anthropogenic factors (Lyons 1998, Kenway-Lynch 2010). Mexico contains around 2,130 species of fish, 500 of which reside in freshwater (Domínguez-Domínguez *et al.* 2005). Thirty-six of these 500 species are in the subfamily Goodeinae (Family Goodeidae) and are endemic to the Mesa Central region of Mexico (Ritchie *et al.* 2007). These thirty-six species give live birth and therefore show significant insights into fish reproduction and evolution (Mendoza 1965, Domínguez-Domínguez *et al.* 2005)

Unfortunately, species, such as the endemic *Xenotoca eiseni*, within the unique subfamily Goodeinae have been decreasing in population abundance at alarming rates without much recognition (Domínguez-Domínguez *et al.* 2005, Lyons 2011). The Mexican government only considers eight of the thirty-six species of Goodeinae endangered in the wild, while the work of three biologists, who have eighteen years of field research coupled with an immense study of the literature, considers twenty-four species to be threatened, i.e. critically endangered, endangered or vulnerable (Domínguez-Domínguez *et al.* 2005, Lyons 2011). The majority of this paper will specifically focus on the species *X. eiseni* but will also include more general information involving the family Goodeidae.

Currently, *X. eiseni* along with the majority of Goodeidae receive no governmental protection (Kenway-Lynch *et al.* 2010, Lyons 2011). Furthermore, *X. eiseni* has not been assessed by the International Union for Conservation of Nature (IUCN) and therefore is not on their red list (IUCN 2012). However, *X. eiseni* is one of the species that is considered to

be endangered by Lyons, Domínguez-Domínguez and their colleagues due to them persisting in small and localized populations (Domínguez-Domínguez *et al.* 2005, Jelks 2008, Lyons 2011). Their long-term viability is uncertain and are therefore in danger of becoming extinct, making their attention just as paramount as any other organism.

Behavioral studies involving *X. eiseni* can provide insight on how this species interacts in the wild. I therefore provided information on the species breeding biology and examine the species sexual selection. A thorough literature review, which focuses on the family's taxonomic history and previous behavioral/sexual selection studies, is also included. Furthermore, the literature review discusses previous findings involving Goodeidae conservation. In addition, general information about sexual selection theory and unique characteristics to the species and family are provided in the background section of this paper for those that are not experts in ecological and fish behavioral studies.

Lastly, this document provides primary data from the examination of *X. eiseni*'s sexual selection so that an understanding of the species behavior within a controlled environment is understood. This is done through three different methodologies, which will be discussed in the methods section, and provides recommendations as to what kind of habitats *X. eiseni* can successfully be restored. Similarly, this paper seeks to understand how anthropogenic factors may alter their behavior and ultimately their reproductive success within the wild.

Background and Literature Review

A fish is an aquatic vertebrate that has gills at one point throughout its life and if it has limbs they are in the shape of fins. From this definition fish are therefore a paraphyletic group, meaning that all species have ancestral characters such as gills, which are characterized by sympleisomorphs. A sympleisomorph is a shared primitive character, which contrasts from a synapamorph, or a shared derived character. Therefore, the evolution of all fish is not studied as a single event because fish are characterized on the basis of sympleisomorphs. However, certain phylogenetic trees within the twenty six thousand species of fish can be described by synapamorphies and are thus termed monophyletic. A monophyletic group will include the most recent common ancestor and all of its descendants.

This paper will be looking at a group of species in the order Cyprinodontiformes. Cyprinodontiformes are characterized by a variety of synapamorphies, such as the caudal fin being rounded or truncate. These synapamorphies distinguish Cyprinodontiformes from other members within the Superorder Acanthopterygii. The order Cyprinodontiformes has been broken up into five families some of which are oviparous, while others are viviparous. This paper will be focusing on the viviparous *Xenotca eiseni*, which is in the family Goodeidae, one of the five families in the order Cyprinodontiformes.

Locality

The family Goodeidae contains two subfamilies. The subfamily, Empetrichyinae, is located in isolated spring pools throughout southern Nevada. This is 2,414 kilometers away

from the more diverse subfamily, Goodeinae, which is endemic to Mexico (Webb *et al.* 2004).

The Empetrichyinae contain two genera, the *Empetrichthys* and the *Crenichthys*. The *Empetrichthys* only contains one extant species and is currently considered endangered due to the introduction of non-native species (USFWS Federal Register 2004). Other subspecies of *Empetrichthys* have historically been threatened by groundwater pumping, which resulted in the *Empetrichthys latos* being relocated from its historical habitat in the Pahump Valley to three different springs (USFWS Federal Register 2004). Unfortunately, the species *Empetrichthys latos* has been recently subjected to floods, invasive species and vandalism, which shut off the species water source (USFWS Federal Register 2004). This has resulted in just one population of *Empetrichthys latos* remaining. They now exist only in the isolated spring pools within the Shoshone Ponds Natural Area, which is within White Pine County, NV (USFWS Federal Register 2004).

The genus *Crenichthys* contains two species, which are not as critically endangered as the genus *Empetrichthys*. However, both species are considered vulnerable by the IUCN (Gimenez 1996). Based on the IUCN assessment the extant species have a high risk of extinction within the medium-term future (IUCN 1994). *Crenichthys nevade* is also threatened due to habitat depletion and invasive species such as the *Ictalurus punctatus*. They therefore have been relocated into a various springs throughout Mineral County, NV (Goodeid Working Group). *Crenichthys baileyi*, is also threatened by invasive species and is subject to protection by the Nevada Department of Wildlife (Goodeid Working Group).

The second, more species rich family, the Goodeinae are located in the shallow freshwaters of the Mexican Plateau. Webb *et al.* (2004) recently verified the relationship

between Empetrichyinae and Goodeinae through molecular work and the fossil record. These two subfamilies diverged in the middle Miocene era, which was about 16.8 million years ago (Webb *et al.* 2004). The main difference between the Goodeinae and the Empetrichyinae is that the Goodeinae are viviparous while the Empetrichyinae lay eggs and are thus oviparous. This paper will be more focused on the viviparous Goodeinae, found in Mexico, despite both subfamilies having similar threats.

Goodeinae occupy shallow freshwaters at elevations that range from 1000 to 2300m despite one species being located at 2800 m (Webb *et al.* 2004). They can only be found in Pacific drainages within the biogeographical region known as the Mesa Central (Miller *et al.* 2005). The range of elevations that Goodeinae have adapted to, can be explained by a complex geomorphological history of the Mesa Central. The Mexican Plateau has experienced volcanism and block faulting leading to an isolated highland surrounded by vast escarpments (Miller *et al.* 2005). This geologic activity has promoted isolated endorheic basins and a diversity of drainage systems (Miller *et al.* 2005). This has led to a tremendous amount of vicariance within the population of Goodeinae and the habitat is in stark contrasts to a relatively homogenous habitat of the subfamily Empetrichyinae (Webb *et al.* 2004). Therefore, the greater magnitude of vicariance within the Goodeinae produces a greater probability for allopatric speciation and is thus is a likely explanation as to why the Goodeinae subfamily has a greater species richness (Webb *et al.* 2004). Domínguez-Domínguez *et al.* (2005) provides a map showing the various river basins within Mesa Central Mexico, a result of this complex geologic history. (Fig. 1) (Miller *et al.* 2005).

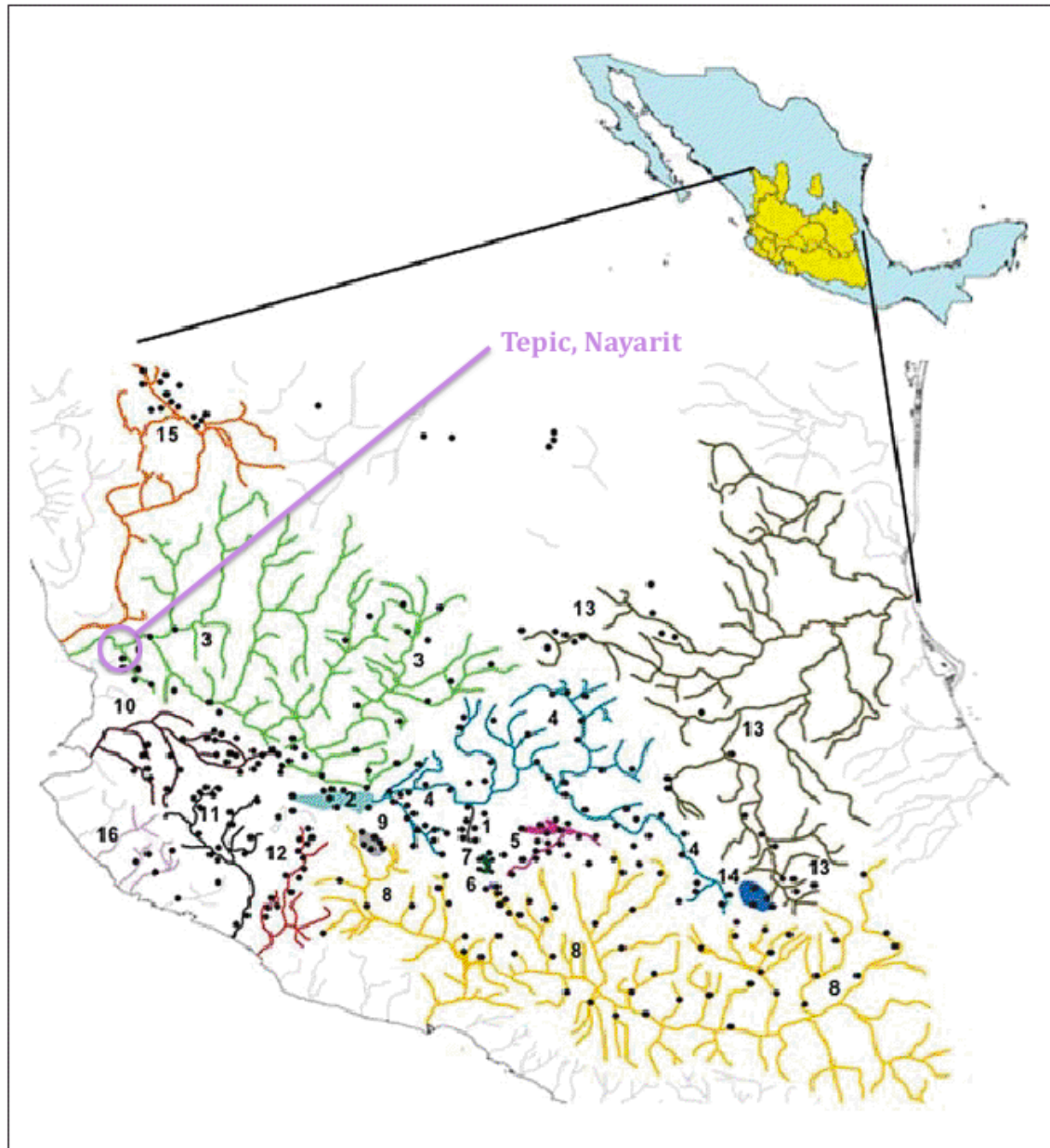


Fig. 1; A Map of the Mesa Central Mexico Drainage Basins. Map has been modified to show the location of Tepic Nayarit. (Domínguez-Domínguez *et al.* 2005)

X. eiseni was first collected in 1894 by Dr. Gustav Eisen in a branch of the Río Grande de Santiago near Tepic, Nayarit (Rutter 1896). Following his discovery, it was described by Rutter in a publication and given the species name *Characodon eiseni* based on Dr. Gustav Eisen being the first to collect it (Rutter 1896). It was then placed in the *Characodon* genus based on a description of the four collected specimens, that most closely represented *Characodon variatus* (Rutter 1896, Durham 1979). The species is no longer a member of the genus *Characodon* based on its reproductive biology and genetic studies (Fitzsimons 1970, Webb et al. 2004). Furthermore, the species no longer persists in its original habitat near Tepic Nayarit as the springs it was originally found in no longer exist (Domínguez-Domínguez et al. 2005)

X. eiseni is still present within the tributaries of the Rios Grande de Santiago, Ameca , Amería-Ayuquila, Compostela, Coahuayana-Tamazula River and the endorheic basin Lago de Magdalena (Kenway-Lynch 2010, Domínguez-Domínguez 2006, Fitzsimons 1972). However, their abundance has become threatened and many populations have vanished or become small and localized (Kenway-Lynch et al. 2010). The Río Compostela and Ríos Ameca basins have experienced the greatest decrease in *X. eiseni* population size (Kenway-Lynch et al. 2010). In contrast, *X. eiseni* were most abundant in the Rios Grande de Santiago basin and Magdalena basins, where they were commonly found in spring-fed pools, streams or lakes that had depths of one meter (Kenway-Lynch et al. 2010). Furthermore, Fitzsimons (1972) notes that species of *X. eiseni* have been collected from turbid roadside streams that are heavily polluted from sugarcane factory effluent.

X. eiseni is tolerant to a variety of benthic environments, as stream beds can range from muddy bottom to a cobble bottom (Fitzsimons 1972). On occasion macrophytes can

form extensive floating mats while at other localities it is possible for minute amounts of algae and other vascular plants to be largely absent (Fitzsimons 1972, Kenway-Lynch *et al.* 2010). These diverse habitats demonstrate that *X. eiseni* has an omnivorous feeding nature (Fitzsimons 1972).

Description

All fish differ in their morphology and based on natural selection each species contains particular characters that serve a function. Each character increases their survival and fitness. For the purpose of this paper *X. eiseni*'s reproductive and external characteristics should be well understood. A picture of a male *X. eiseni* is shown below in Figure 2 to help explain their external morphology.



Fig. 2 Male *X. eiseni*, Photo: William Ryan Currier

External Description

The following is largely based on my own observations, which has been checked with Fitzsimons (1970) description to maintain accuracy.

The portion of *X. eiseni* where the blue color convenes with the orange color is known as the caudal peduncle while the orange portion, posterior to the caudal peduncle is the caudal fin. Females differ from the males because they are olive-brown colored and do not express vivid life colors. These colors include the bluish colorations around the caudal peduncle and the orange caudal fin. Therefore, *X. eiseni* are sexually dimorphic in hue. The coloration that the males exhibit will be described as ornaments throughout the rest of this paper. Females, while mostly olive-brown contain a black blotch, which lies posterior and lateral to the belly (Fitzsimons 1970). This is referred to as the “pregnancy spot” because it enlarges during gestation. This mark can be seen in both gravid and non-gravid females. It is caused by a dark pigment, which lines the abdominal organs and can be seen in some Poeciliidae fishes as well (Fitzsimons 1970).

Both sexes contain similar positions of their paired fins, which are the pectoral and pelvic fins. The pelvic fin is located ventrally and posterior to the pectoral fin and are thus termed abdominal. The Goodeidae also contain a terminal mouth because the mouth opens at the front end of the head and both jaws are located as far forward as the other.

Unlike, their related family, Poeciliidae, the Goodeidae does not have a gonopodium. A gonopodium is a modified anal fin and intromittent organ that specializes in delivering sperm during copulation. Instead of a gonopodium, male goodeids have developed other ways to transfer spermatzeugmata.

Reproductive Characteristics

The *X. eiseni* has a unique split anal fin and therefore is commonly referred to as the Red Tail Splitfin. In 1978 Grier and others described the notch in the anal fin as the spermatopodium, which facilitates the process of transferring spermatzeugmata. These

are best described as sperm bundles with the tail oriented on the outside (as cited in Miller *et al.* 2005, p. 265). Mohsen (1965) showed that internally there is a highly muscular tube that surrounds the dilated vas deferens, which helps facilitate the transfer of spermatozeugmata. During copulation the spermatozeugmata is projected into the female's genital opening by the aid of the muscular tube, which surrounds the vas deferens.

The females within the subfamily Goodeinae are unique because they contain a placenta like structure within their belly. This is called a trophotaeniae. The trophotaeniae provides nutrients and respiration so that the young can be developed within the mother and give live birth. For this reason the *X. eiseni* is commonly referred to as a livebearer but this specific form should be referred to as matrotrophic viviparity because the mother is providing parental care and it is giving birth to live young.

Systematics

In the early 1900's after *X. eiseni* was first described by Rutter (1896), the Goodeinae were believed to be members of the families Cyprinodontidae and Poeciliidae (Webb *et al.* 2004). However, Carl Hubbs (1924) was the first to recognize the Goodeidae as a natural group. In 1939, fifteen years after Hubbs suggested that Goodeids were their own natural group, Hubbs and C. L. Turner published the first comprehensive study of the Goodeidae, which was largely based on the species morphology, ovarian structure and trophotaeniae (Hubbs and Turner 1939). The comprehensive study by Hubbs and Turner (1939) has provided the basis of the Goodeinae taxonomy until Webb's *et al.* (2004) study. Webb's *et al.* study (2004) used biogeographic patterns, sequence data from two mitochondrial DNA loci and a calibrated "clock" to help clarify the Goodeidae phylogeny (Fig. 3).

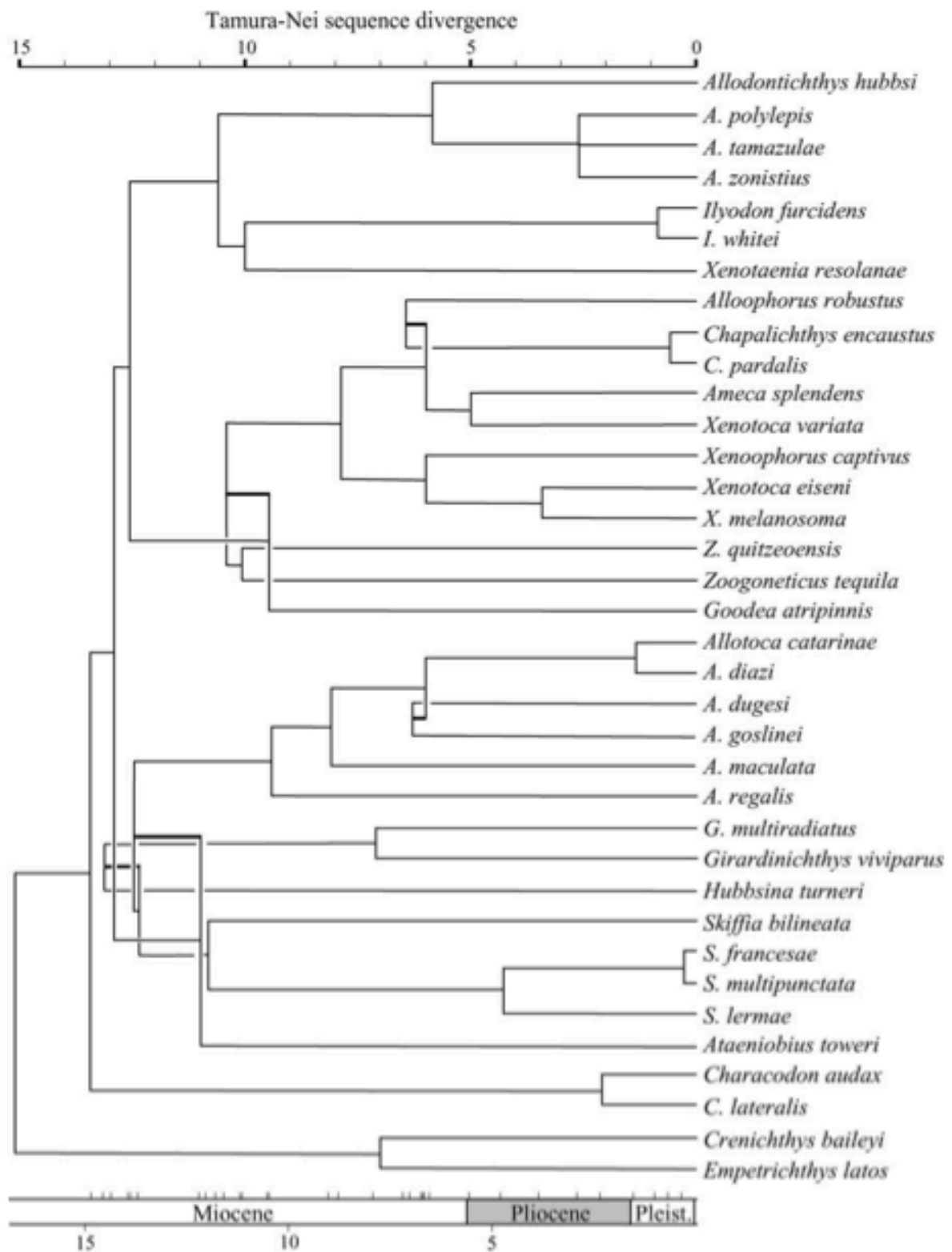


Fig. 3 Phylogenetic Tree (Webb *et al.* 2004) Tick marks are associated to summarize the divergence date of the species.

Webb and others (2004) found that the majority of the speciation within the family Goodeidae is a result of allopatric speciation, meaning that the population has experienced vicariance and therefore the original population can no longer interchange genes. This can be caused by river piracy, which is when a river no longer flows down its original path but instead flows into an adjacent stream, or volcanism (Webb *et al.* 2004). However, *X. eiseni* and *Xenotoca melanosoma* were found to have undergone sympatric speciation through Webb and others (2004) genetic analysis. Sympatric speciation is when a new species is derived within the same biogeographic region. It is typically a result of reproductive isolation further emphasizing the importance of the species behavior and sexual selection.

Webb (1998) suggested that the close relatives, *X. eiseni* and *X. melanosoma* be in their own genus labeled “*Xenotichthys*” based on genetic and morphological evidence that compared these two species to *X. variata*. Furthermore, Fitzsimons (1970) and Mendoza (1965) showed that *X. eiseni* and *X. melanosoma*’s respective trophotaeniae and ovarian structures differ from that of *X. variata*, concluding that these two species should be withdrawn from synonymy with *X. variata*. Therefore, it is common to find these two species labeled as “*Xenotoca*” *eiseni* and “*Xenotoca*” *melanosoma* because it is believed that the name of their genera will eventually change (Lyons 2011). However, this genus has not been formally published and therefore until this new genus has been described and diagnosed in a scientific journal it cannot be used as an official genus name in publications (Lyons 2011).

Diverse ecological niches, feeding modes and sexual selection likely resulted in the sympatric speciation of the Goodeidae (Webb *et al.* 2004). Margaret Ptacek (2005) notes that divergent female mating preferences, which are based on sexual preferences, leads to

reproductive isolation and speciation. Furthermore, Ritchie (2007) concluded that sexual selection results in divergence at a greater rate when a species is sexually dimorphic, such as *X. eiseni*.

Recently, Populations of *X. eiseni* have shown high genetic divergence. For instance populations of *X. eiseni* in the Santaigao, Magdalena/Ameca and Coahuayana basins may represent distinct taxa (Doadrio and Dominguez-Dominguez 2004). Furthermore, Kenway-Lynch (2010) has recently studied the genetic differentiation found between the population of *X. eiseni* in the endorheic Lago de Magdalena basin and compared it to all other basins. By analyzing mitochondrial (cytochrome b) and nuclear (ITS-1) DNA sequences along with microsatellite data, Kenway-Lynch (2010) found that species showed a nucleotide difference of 2.51% and a cytochrome b difference of 0.88% amongst the two populations. This led Kenway-Lynch (2010) to believe that there may be distinct species amongst the population within the endorheic basin compared to those that are outside of the basin. As a result of this variation Kenway-Lynch (2010) suggested that each clade should receive its own conservation attention and protection.

Sexual Selection

Sexual Selection was first introduced and made famous by Charles Darwin in 1859 when he published his book *On the Origin of Species*. Sexual selection is distinct from natural selection in that sexual selection concentrates on the species fitness in relation to its secondary sex characteristics, which are characters that differentiate between sexes but do not relate to genitalia. These characters, which differentiate from the naturally selected optimum become sustained in the population because it improves the female's ability to

detect quality males, as these males are capable of expending energy to develop and maintain these characteristics (Zahavi 1975). As a result, males with a certain ornamentation become preferred by females (Zahavi 1975).

Charles Darwin in the *On the Origin of Species* states how sexual selection, "depends, not on a struggle for existence, but on a struggle between the males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring." This expresses the importance for a species to have a high fitness, or reproductive rate. Therefore, if a female *X. eiseni* is not attracted to the male then the female will not reproduce with that male because the male is not desirable. Furthermore, the male is not capable of circumventing the female based on the species genitalia and within small populations this could be detrimental (Arellano-Aguilar & Marcías Garcia 2008).

Ritchie (2007) states that within the Goodeinae some female species have driven males to become sexually dimorphic as the result of the females sexual preference. Therefore, the sexual dimorphic coloration of *X. eiseni* is likely to have derived from female mate choice. Female *X. eiseni* gained a preference for male coloration once the allele that expressed the sexually dimorphic coloration was selected for by the female. If the female did not select a male that expresses this phenotype then it would be eliminated from the population.

Males are more desired based on the magnitude of their ornament expression (Arellano-Aguilar & Marcías Garcia 2008). Zahavi (1975) notes that organisms with a greater magnitude of ornamentation are of greater quality. Thus, they can provide direct benefits to the female's offspring and are consequently more desired (Marcias Garcia 2003). An individual that expresses the greatest ornamentation has invested energy and resources,

which allow for its ornaments to be generated and maintained. Based on female response, a positive feedback loop involving the expression of ornaments may develop because individuals contain an additional advantage than those that do not express the desired phenotype (Zahavi 1975). These individuals contain a high quality genotype, which is essential in the struggle for existence. They also contain an exaggerated phenotype, which allows for males to better advertise themselves to females. This gives males that contain ornamentation a distinct advantage over those that do not contain this characteristic

Sexual selection will drive the male to produce secondary sex characteristics but this creates a paradox. A species that contains exaggerated secondary sex characteristics has characteristics that are not within the naturally selected optimum and will therefore increase the species fitness but decrease the species ability to survive. As Arellano-Aguilar and Marcías Garcia (2008) note, the larger an ornament is the more costly the ornament is to the species. This may skew the availability of males towards a less preferred phenotype.

In the case of *X. eiseni*, the male is more colorful and thus has a lower chance for survival because it is more susceptible to predators. Therefore, based on the number of predators in a given region, ornamentation may be detrimental because those with higher ornamentation will not survive. Natural selection and sexual selection undergo an evolutionary arms race and continue to inflict costs on the species until generating an ornament is no longer viable or a medium can be found.

Moyaho (2004) studied *Xenotoca variatus* to look at mate choice and the expression of a sexually dimorphic trait. *X. variatus* is similar to *X. eiseni* in that both species are sexually dimorphic and vary geographically (Moyaho 2004, Fitzsimons 1970). Fitzsimons (1970) found that some populations of *X. eiseni* contain vivid life colors while other

populations are drab and often colorless. This is the result of the geological history, which fragmented the populations (Webb *et al.* 2004).

X. variatus' difference in ornamentation magnitude between location is thought to be the result of either predation or sexual selection. In contrast to males, female *X. variatus* do not contain speckles or a yellow terminal band on their caudal fin. The shiny scales referred to as speckles can reflect a silvery violet to silver green light. Moyaho's study (2004) found that females, while in clear water, exhibited a preference for males that contained the greatest number of speckles. At the same time *Thamnophis melanogaster*, a freshwater snake and common predator to many Goodeidae species was found to target the *X. variatus* that contain more speckles. Moyaho's publication (2004) shows the evolutionary arms race between sexual selection and natural selection. The male is able to advertise his ornamentation to the female but has an increased risk of predation because he is more conspicuous to predators.

Depending on the severity of female preference within a population of Goodeidae, males may need to express their secondary sex characteristics to successfully reproduce. If males do not possess secondary sex characteristics when introduced to a population, copulation may not occur due to the Goodeinae's breeding biology and therefore non-effective populations sizes may result. For instance, female *Girardinichthys multiradiatus*, a species of Goodeinae, has a stringent mate choice selection and it is possible that they will refuse to mate with suboptimal mates (Arellano-Aguilar & Marcías Garcia 2008).

This 2008 study by Arellano-Aguilar & Marcías Garcia shows a case of anthropogenic contamination adversely affecting the natural sexual selection process of *G. multiradiatus*. It showed that an increased concentration of sub lethal methyl parathion, an

agrochemical, significantly lowers the yellow chroma saturation of the *G. multiradiatus*' sexually distinct fins. Exposure to methyl parathion results in a notable color and size difference in the offspring that are exposed to it as the pesticide is able to bioaccumulate (De la Vega-Salazar and Macías-Garcia 2005, Arellano-Aguilar & Marcías Garcia 2008). Due to the embryos being bathed in ovarian fluid and taking up nutrients through a trophotaeniae the Goodeidae embryo's are at high risk to take up pollutants ingested by the mother throughout gestation (De la Vega-Salazar and Macías-Garcia 2005). The result of Arellano-Aguilar & Marcías Garcia's (2008) study showed that female mating probability decreased therefore reducing the chance of successful copulation within contaminated populations. This stringent sexual selection process displayed by *G. multiradiatus* and other species of Goodeidae may lead to reproductive isolation between species based on female preference (Zuarth and Macias Garcia 2005).

As a result of these two studies (Arellano-Aguilar & Marcías Garcia 2008, Moyaho 2004) and Kenyway-Lynch's study (2010) I infer that populations of *X. eiseni*, which vary in coloration from one population to another (Fitzsimons 1970) may differ in female's sexual preference. Therefore, an understanding of *X. eiseni*'s breeding biology, sexual preference and its conservation threats should be well understood to maintain *X. eiseni*'s viability in the wild.

Breeding Biology

Males display conspicuous courtship behavior while in pursuit of the female making them vulnerable to snake predation (Zuarth & Marcías Garcia 2005). However this conspicuous behavior may be essential for Goodeinae to successfully copulate. The stringent selection process that takes place in some species of Goodeinae, such as *G.*

multiradiatus, must be taken into consideration when attempting to successfully restore and maintain a population. Sexual selection has thought to be strong in Goodeids because females tend to avoid breeding when confined with a single male and because of the need for female cooperation (Marcias Garcia & Sobario 2004; Gonzalez Zuarth & Marcias Garcia 2006).

Females do not store sperm during pregnancy and remain receptive for about 1 week after being inseminated (Marcias Garcia and Saborio 2004, Bisazza 1997). Therefore for females to produce offspring they need to copulate with a male before they can begin reproduction. In contrast, the family Poeciliidae produces offspring much more frequently as females are capable of storing sperm and delivering young at a rate of about one month (Marcias Garcia & Valero 2010). Male Goodeinae remain sexually active and exert energy by attempting to mate with females that are undergoing gestation despite females having a swollen abdomen (Kingston 1979). It is thought that this behavior would have been eliminated through the process of natural selection, as it is an unnecessary use of energy because it not possible for males to pass along their genetic material with a female that is undergoing gestation.

Furthermore, the subfamily Goodeinae differs from the family Poeciliidae because males do not posses a gonopodium and therefore are not capable of circumventing an unreceptive female (Marcias Garcia & Valero 2010). As discussed earlier, males must therefore depend on female cooperation and have a way of gaining the females attention. This is the result of male Goodeinae's lacking a specialized copulatory organ, however, they are capable of transferring sperm to the female cloaca through the assistance of specialized pelvic muscles (Marcias Garcia & Valero 2010). The use of what is referred to as a

“spermatopodium” can lead to epigamic characters, such as complex courtship events, which can result in rapid population differentiation (Marcias Garcia & Valero 2010).

Kingston (1979) completed her dissertation work at the University of Michigan on the behavior of the Goodeidae genus *Ilyodon*. She compared the *Ilyodon* courtship behavior to other members in the family Goodeidae. In her work she found that *X. eiseni* undergo a multitude of behaviors before courtship proceeds. These behaviors include an introductory behavior, four types of head flicking behaviors, four quiver behaviors, a zig zag dance, and a headwag behavior. The following is a summary of the *X. eiseni*'s courtship behavior as described by Kingston (1979)

The introductory behavior performed by *X. eiseni* is referred to as parallel swimming. The male will begin to swim next to the female at the same speed. The male's fins may become erect and he may spontaneously dart on alongside his potential mate. This action can be dependent on the size of the species habitat and therefore it may not occur if confined to a small aquarium. After the male performs an introductory behavior the female will initiate a response, which notifies the male to proceed to the next step in Goodeid courtship behavior. This response is referred to as a headwag. During a headwag a female's fins are folded and the female undergoes an anterior undulation that is relatively slow but large in amplitude. A headwag is similar to a headflick and can be done as a female remains in place or while the two individuals are moving.

This next step involves headflicking behavior. Headflicking behavior can be categorized by inconsistent undulations that travel throughout the male's body. These undulations vary in intensity, sometimes hardly noticeable. The male may be positioned alongside or parallel to the female, directly in front of the female, looking at the female or

off at an oblique angle to the female facing toward or away from her. These four positions typically occur within the same plane as the female and are within the female's site. Figure 4 shows the frontal headflicking behavior. Other headflicking behaviors differ in orientation but are consistent in the type of orientation performed.

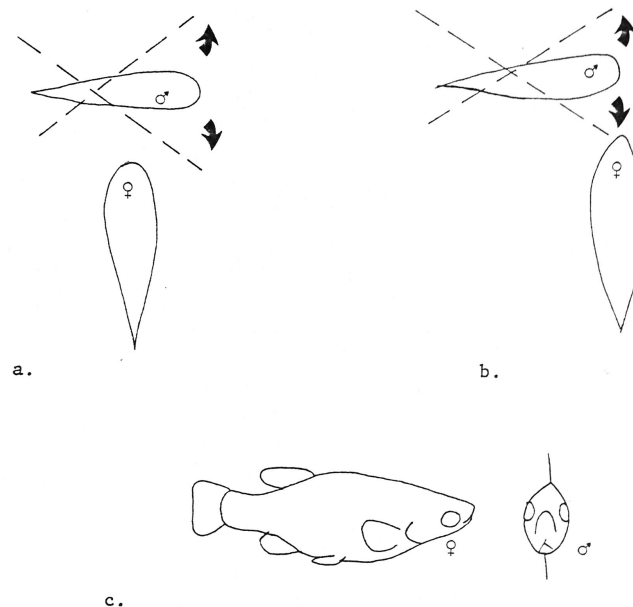


Fig. 4 Frontal Headflick behavior a) and b) are dorsal views while c) is a lateral view (Kingston 1979)

In contrast to headflicking behavior males tend to proceed with their courtship behavior by carrying out a quiver behavior. This is similar to the headflicking behavior but the fins are now fully erect and the undulations are more consistent and rapid than headflicking behavior. The quiver behavior may take place in a stationary position or while they are darting alongside a female, in the same positions as described in the headflicking behaviors.

While headflicking and quiver behaviors are taking place the males tend to swim back and forth in front of and behind the female in what is referred to as the zig-zag dance. This is designed to keep the female in place or to maintain his position in front of the

female. While zig zaging in front of the female it is common for the male to turn in and move towards the female at an acute angle. This behavior can have a range from 1-10 continuous turns. This also may be limited by the amount of space in the individual's habitat. It should be noted that rarely are all of these behaviors exhibited before a copulation attempt. Figure 5 helps explain a typical zig-zag dance.

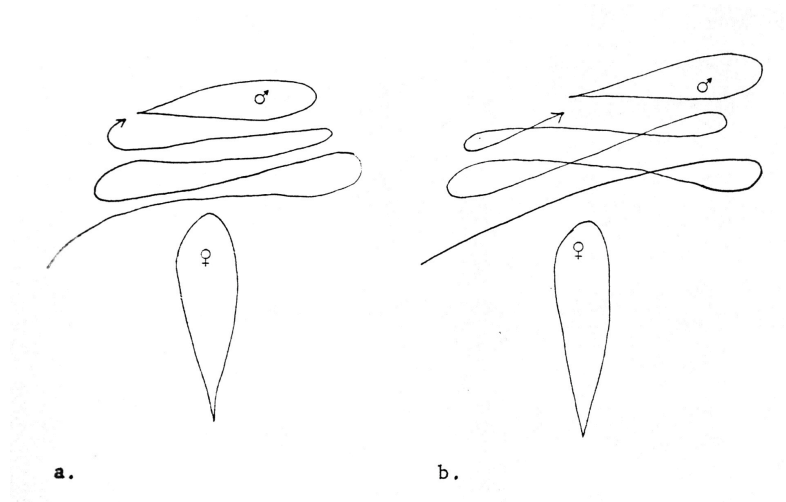


Fig. 5 Zig-zag dance. The amount of turns and the way the male turns is variable. a) shows a male turning to the opposite end of the female at the end of the zig-zag dance while b) shows the male turned toward the female at the end of each loop (Kingston 1979)

Typically male quivering behavior is followed by a copulation attempt. Within *X. eiseni* the males move towards the female so that the two sexes are parallel to each other. The male then begins to quiver. The female may flee, remain impassive or quiver in unison. If the female decides to quiver alongside the male then the male will surround the “spermatopodium” over the female’s genital opening. Both sexes will then quiver in unison for several seconds and a successful copulation attempt will be complete. Figure 6 helps explain the orientation of the male and female during courtship.

Throughout courtship there are many behaviors that commonly take place and are believed to be unrelated to courtship behavior. It has been noticed that throughout

courtship the male tend to frequently bite the female if the male has initiated an introductory behavior but the female does not respond. Males and females also commonly circle around members of the opposite sex but this usually does not lead to further courtship behavior. Lastly, males normally chase females for long periods of time within aquaria leading to no further courtship behaviors such as a headwag from the female.

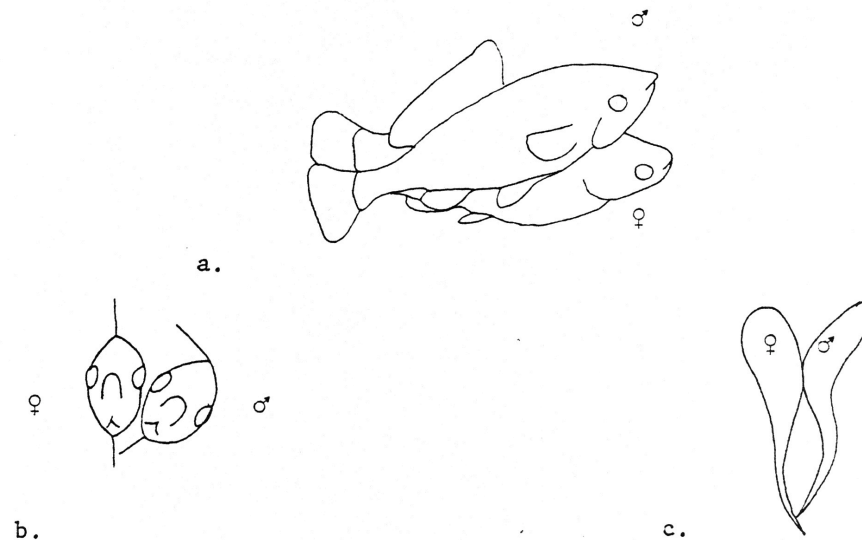


Fig. 6 Drawing of a male and female Goodeidae copulating
a.) lateral view, b.) frontal view c.) dorsal view (Kingston 1979)

Males also tend to exhibit aggressive behavior against conspecific males. In the field it was noticed that only males exhibited aggressive behavior before courtship. It is typical for the larger male to displace the smaller male but if the smaller fish remains or is relatively the same size as its new found competitor then the fish may undergo a behavior known as tailbeating. During tailbeating the fins remain fully erect and slow undulations are performed as water is forced toward the opponent. Throughout this bout it is common for males to also undergo a “v” formation so that the two species heads remain head to

head while continuing their tailbeating behavior. Figure 7 is a diagram that exhibits the Goodeidae's aggressive behavior.

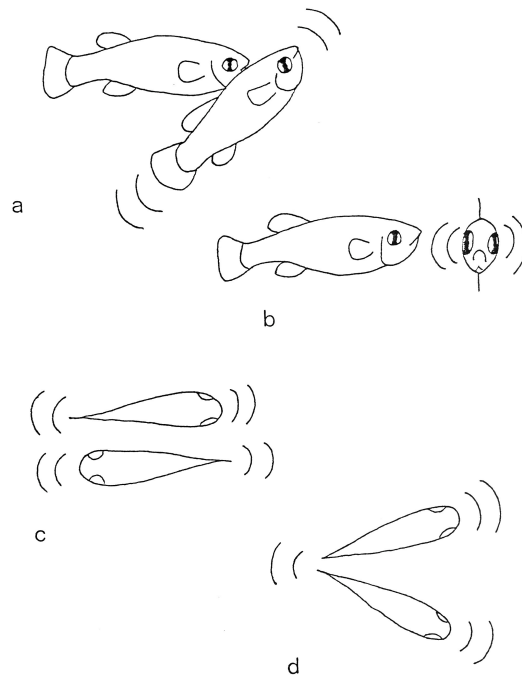


Fig. 7 The Goodeidae's aggressive behavior a) and b) are lateral views of the tail beating behavior while c) and d) are dorsal views and show the "v" formation (Kingston 1979)

Throughout this aggressive behavior it is common for the males' color patterns to become increasingly vibrant and for the males' eyes to develop a black bar. The male that loses the bout commonly has the black bar completely darken its iris as he folds his fins and leaves the competitor. However, if neither fish is dominant then both fish may develop a black iris.

A study by Kelley *et al.* (2006) noted that captive bred butterfly split fins (*Ameca splendens*) exhibits a greater magnitude of aggression based on density and habitat structure. The study compared the behavior of fish that were bred in the London Zoo, UK for 40 years to those that were caught in their natural habitat. The study showed that the captive bred males were more aggressive when observed in a structured habitat rather

than an unstructured habitat and that aggression was positively correlated with density. Kelley *et al.* (2006) suggests that this may affect their suitability for reintroduction into wild habitats as attacks may be targeted towards females limiting their chances at reproductive success. Furthermore, captive-bred *A. splendens* may be less aware of predators attacking. Similarly, their aggression may make them more conspicuous to predators (Kelley *et al.* 2006). It should be noted though that throughout time this behavior may change the aggressive males die off leaving only the non-aggressive males to breed successfully. Similarly male behavior may change as they adapt to their new habitats.

Developmental Biology

Goodeidae's gestation period last about two months and fecundity is positively correlated with female size (Marcias Garcia and Saborio 2004). Goodeiane are matrotrophic and therefore the offspring are dependent on their mother for providing the embryo nutrition throughout development. While the young are developing throughout gestation they undergo significant growth (Hollenberg and J.P. Worums 1995). The young receive their nutrition from their mother through a particular organ, referred to as the trophotaeniae, or feeding ribbons. Nutrients are either secreted or synthesized through the ovarian epithelium and into the ovarian fluid where the embryos lie bathed (Hollenberg and J.P. Worums 1995).

The trophotaeniae or "growth ribbons" vary amongst different species in their size and type (Turner 1937). Mendoza (1965) provides a ventral drawing of how a typical *X. eiseni* trophotaeniae appears (Fig. 8).



Fig. 8 Mendoza (1965) 306 Pl. II fig. 9 - Drawing of the *X. Eiseni* Trophotaeniae shape/branching characteristics (ventral view)

Embryos developing with a trophotaeniae have a connection to the intra-ovarian cavity of their mother. This allows for secure access to nutrition and oxygen throughout their development. The trophotaeniae is then attached to the abdomen of each embryo, with the number and length of each branch varying from species to species (Turner 1937). Mendoza (1965) provides a trace of a photograph to help visualize how the trophotaeniae is connected to the developing embryo (Fig. 9).

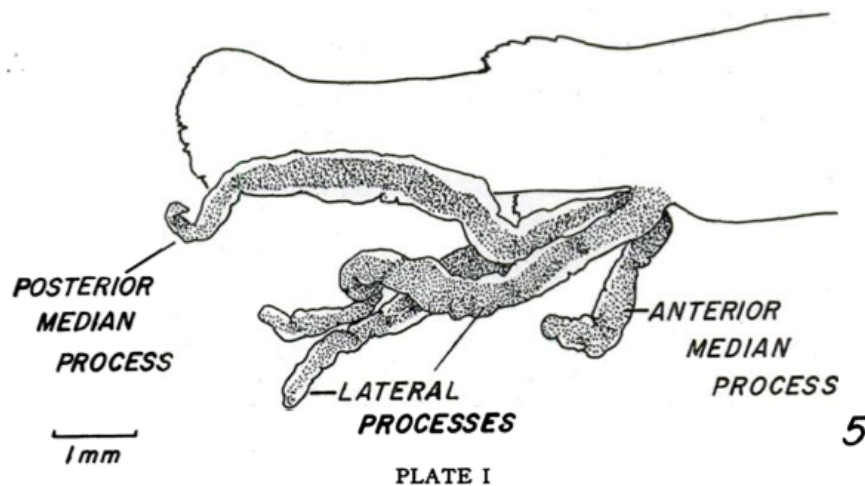


Fig. 9 Mendoza (1965) 304 Pl. I Fig. 5 - Drawing of *X. eiseni* Trophotaeniae Connection to the gut of a developing embryo.

Typically the anterior ribbons are much shorter than the posterior ribbons (Mendoza 1965). These ribbons increase in length throughout the gestation period to reach a maximum size but then retract slightly just before birth. During development many trophotaeniae are sheathed meaning that it is encased in a protective dermal layer. When the fry are released the trophotaeniae can lose this protective layer (Mendoza 1965). The

description by Mendoza (1965) and a confirmation by Fitzsimons (1970) have been used in the past to determine anatomical taxonomy within the family Goodeidae.

A drawing of a dissected Mexican Goodeid ovary, *Ameca splendens* shows how well developed embryos are oriented in conjunction with the trophotaeniae. The drawing of the posterior two thirds of an embryo (Fig. 10) provided by Julian Lombardi, from Wourms *et al.* (1988) helps visualize how well developed embryos are oriented and interacting with the trophotaeniae while in the mother's ovary.

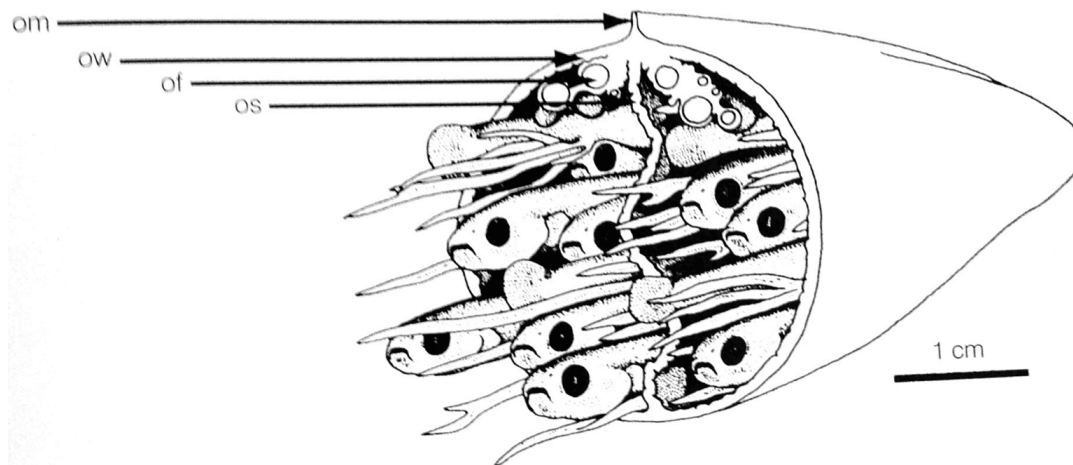


Fig. 10 Drawing by Julian Lombardi from Wourms *et al.* (1988) – Cross section of the posterior two-thirds of *A. splendens* ovary. Fingerlike projections represent the Trophotaeniae. om: ovarian mesentery, os: ovarian septum, ow: ovarian wall, of: oocytes. (as cited in Helfman *et al.* 2009)

Once born the fry do not have any attachment to their mother and are capable of standard swimming patterns once their swim bladder is full (Marcias Garcia and Alejandra Valero 2010). *X. eiseni* then begin to undergo postnatal sexual dimorphism. Around five to six weeks after birth the males color begins to develop and the anal fins start getting thicker (Durham 1979).

Turner *et al.* (1962) explains the dichotomous development between male and female anal fin rays. Males and females both contain fifteen anal fin rays after birth and for

sixty days both sexes undergo the same growth rates of their anal fin rays. However, sixty days after birth *X. eiseni* develops sexually distinct anal fins. The female develops branched rays from rays three to fourteen, while the males remain unbranched. Once sexually mature, females undergo a notable difference in the growth rate of their anal fin rays. Anal fin rays three to ten become longer than the others. In contrast, male anal fin rays two through seven, have a significant decrease in their growth rate. Finally once the males become ninety-eight days old they show a marked difference between the length of anal fin rays two through seven.

After four months of postnatal development it is believed that the *X. eiseni* have become large enough to begin courtship and produce offspring (Durham 1979). However, Durham notes a more successful copulation event takes place if the female is at least six months. This is likely to be a result of the energy involved with matrotrophic viviparity and Marcias and Sobario's (2004) indication that fecundity and female size has a positive correlation.

Conservation

Robert Rush Miller (1961) was the first to call attention to the conservation of freshwater fish in Mexico (as cited in Contreras-Balderas 2003). At the time of his publication in the Papers of the Michigan Academy of Science, 11 species were listed as threatened, while today the scientific community is aware of 168 species at risk in Mexico (Contreras-Balderas 2003). This is significant because Mexico has 163 endemic freshwater fish species (Lyons 1998, Dominguez-Dominguez *et al.* 2008). About 70 of these freshwater fish species are endemic to the Mesa Central part of Mexico (Dominguez-Dominguez *et al.*

2005). 36 of these species are members of the subfamily Goodeinae, twelve of which are classified by the Mexican government as being threatened (Ritchie 2007, Lyons 2011).

Goodeids have little economic benefit and have thus been largely ignored by many conservation efforts (De la Vega-Salazar and Macías-Garcia 2005). However, Goodeidae play important ecological roles and many national and international researchers use them as model organisms for evolutionary, biogeographic and livebearer studies (Domínguez-Domínguez *et al.* 2005). Furthermore, Goodeidae can be seen as national treasures as they are endemic and possess a unique reproductive system. Lastly, they are important in maintaining freshwater fish biodiversity in Mexico.

Many fish biologists are concerned for their future because habitat destruction could lead to their extinction. The Goodeinae are at a high risk due to an increase in urbanization and agriculture throughout the Mesa Central biogeographic region. Seventy-three percent of Mexico's population lives within the Mesa Central region and as a result sixty-seven percent of the industrial activity takes place within this region (Dominguez-Dominguez *et al.* 2008). Similarly, the population within the Mexican Plateau has drastically increased from 14 million to 104 million people from 1910 to 2000 (Miller *et al.* 2005) and has continued to increase.

As a result of Mexico's increased population, ecosystems in Central Mexico rank among the most heavily disturbed by human activities in the country (Domínguez-Domínguez 2006). As a result, the Goodeinae have undergone significant and unprecedented habitat degradation within the past two decades (Guzmán-Arroyo 1990, Lyons 1991, Lyons 2011, De la Vega-Salazar and Macías-Garcia 2005). Despite their habitat

degradation, *X. eiseni* does not receive any governmental protections and is not recognized by the IUCN as being endangered (IUCN 2012, Kenway-Lynch *et al.* 2010).

The IUCN Standards and Petition committee released a revised guideline involving species classification in 2013. The guideline provides nomenclature, which explains how the IUCN classifies a threatened species i.e. Critically Endangered, Endangered, or Vulnerable (IUCN Standards and Petitions Subcommittee 2013). The IUCN considers a species to be endangered if it meets any one of the five criteria (A-E) listed below (IUCN Standards and Petitions Subcommittee 2013). For a complete explanation of each threatened category see the “Guidelines for Using the IUCN Red List Categories and Criteria” Version 10.

- A. Declining population (past, present and/or projected)
 - a. Population size is 50% less or equal to historical abundance and reduction may not have ceased, be understood or be reversible
 - b. OR Population size is 70% less or equal to historical abundance and the causes of the reduction are clearly reversible, understood and ceased.
- B. Geographic range size, and fragmentation, decline or fluctuations
 - a. The geographic range is less than 500 km²
 - b. Less than 5 locations
 - c. Continued decline in their occupancy, occurrence
- C. Small population size and fragmentation, decline, or fluctuations
 - a. Less than 2,500 mature individuals
 - b. AND an estimated 20% decline in projected populations
- D. Very small population or very restricted distribution
 - a. Less than 250 mature individuals
- E. Quantitative analysis of extinction risk (e.g., Population Viability Analysis)
 - a. Probability of extinction in 20 years is less greater than 20% in 20 years

Five quantitative criteria that are used to determine whether the taxon is threatened (Critically Endangered, Endangered, or Vulnerable) (IUCN Standards and Petitions Subcommittee 2013)

Unfortunately, the *X. eiseni* has not been assessed by the IUCN to see if these species meet the criteria but many biologists use these guidelines when making suggestions about a species conservation status (Lyons 2011, Domínguez-Domínguez 2005). Furthermore,

priority for conservation action is not dictated by the IUCN red list, as many species not assessed by the IUCN still need immediate conservation action. Other factors that are taken into account when considering conservation actions are costs, logistics and chances of success (IUCN Standards and Petitions Subcommittee 2013). Therefore many research and conservation policies in freshwater systems throughout Mexico have had their focus on species that have had high economic value (Domínguez-Domínguez *et al.* 2005)

Dr. Lyons, an honorary member of the Mexican Ichthyological Society in 2010 and a professor at the University of Wisconsin, Madison, believes that many Goodeids are rare enough to be internationally and federally listed as endangered based on their respective definitions of an endangered fish. Yet the exhaustive bureaucratic process, which is mandatory for any species to be listed as endangered by either the Normas Oficiales Mexicanas (NOM) or the IUCN is not worth the trouble because Mexico does not provide the level of protection or funding that the United States does (J. Lyons, personal communication March 06, 2013). He suggests that within the Goodeinae two species are extinct, three species are extinct in the wild, sixteen species are critically endangered, seven are endangered, nine are vulnerable, none are threatened and only three are of least concern (Lyons 2011). In contrast, the Mexican government classified only two as extinct, eight as endangered and four as threatened (Lyons 2011).

Since the 1990's Lyons and his colleagues have found that many species of Goodeinae have been declining in their range or abundance (Lyons 2011). Domínguez-Domínguez *et al.* (2006) found that *X. eiseni* is extinct from 67.86% of its historical locations and that 51.4% of the surrounding watershed has been converted to non-natural environments. The change in the surround landscape is best shown in Figure 11.

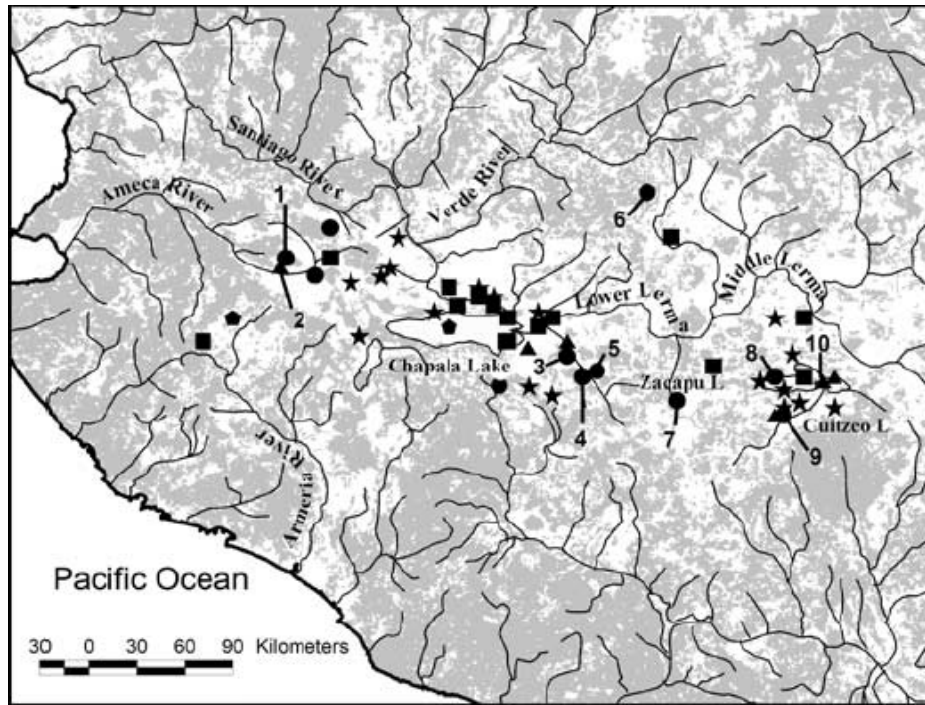


Fig. 11 Map of the Western Mesa Central of Mexico (Domínguez-Domínguez *et al.* 2006) showing Pacific Drainages, White area represents land completely changed. Dark area shows natural habitat.

The dramatic decline of *X. eiseni* within the past twenty-five years is due to stream channelization, water diversions, pollution and the introduction of non-native species (Domínguez-Domínguez *et al.* 2005, Lyons 2011, De la Vega-Salazar and Macías-García 2005). Based on a 2003 survey, populations within the Santiago River basin have been extirpated due to water pollution, invasive species and springs drying up (Domínguez-Domínguez *et al.* 2005). One small population that has remained within the Santiago River but is currently threatened by the development of the town, Seis de Enero, which lies along the river (Domínguez-Domínguez *et al.* 2005).

A population in the Compostela River has survived despite heavy pollution from the wastewaters of Compostela City (Domínguez-Domínguez *et al.* 2005). Furthermore, the populations in the Ameca and Coahuylana basin are very small and almost all of these

habitats remain polluted and infested with parasites (Dominguez-Dominguez *et al.* 2005).

Figure 12 shows the distribution of *X. eiseni*'s historical distribution and its current distribution in the Santiago River basins, Ameca River and the Coahuylana Basin based on a 2003 survey conducted by Domínguez-Domínguez and others (2005).



Fig. 12 *X. eiseni* distribution: Red Box indicates locality where *X. eiseni* has not been seen in past six years. Black circle indicates where *X. eiseni* has been found in the past six years (Dominguez-Dominguez *et al.* 2005)

Populations of *X. eiseni* in the Ameca River have suffered from an increase in organic pollutants that are derived from the sugar cane industry (Domínguez-Domínguez *et al.* 2006). Unfortunately, concentrations of pollutants can be magnitudes greater in maternal issues (Webb *et al.* 2004, De la Vega-Salazar and Macías-García 2005).

A change in the surrounding habitat for human development yields an increased demand for water. The endorheic Magdalena Lake, where *X. eiseni* currently reside, was drained in 1926 for agricultural purposes (Domínguez- Domínguez *et al.* 2006). The *X. eiseni* population in the Magdalena are now threatened by organic pollution and exotic species such as the Common Carp (*Cyprinus carpio*), Grass Carp (*Ctenopharyngodon idella*)

and Tilapia (*Oreochromis* spp.) (Domínguez-Domínguez *et al.* 2006). These species outcompete the native species for food, change the habitat or prey on the native species. As a result, the invasive species are better equipped to survive in the altered environment than species of *X. eiseni* and other Goodeinae (De la Vega-Salazar and Macías-García 2005). These invasive species can also bring parasites with them that adversely affect fish populations (Dominguez-Dominguez *et al.* 2006).

Furthermore, an isolated spring, El Moloya, within the Ameca drainage, has been threatened due to the development of the spring as a recreational swimming area along with the introduction of species of *Oreochromis*. These populations are in decline along with their genetic variability leading to recent bottleneck events in this area (Domínguez-Domínguez *et al.* 2006). The bottleneck effect gets rid of the genetic diversity and the development for unique phenotypes.

As a result of these alterations, many populations of *X. eiseni* within the Santiago, Ameca and Coahuylana basins have been eliminated and the species currently persists at six or seven sites, all of which are small and localized (Lyons 2011. Domínguez-Domínguez *et al.* 2005).

Omar Dominguez-Dominguez and his colleagues plan to ultimately use stocks from the Laboratory of Aquatic Biology of the School of Biology at the Michoacán University of San Nicolás de Hidalgo (UMSNH), which contains 40 Goodeidae species (Domínguez-Domínguez *et al.* 2005). It is important though that Domínguez-Domínguez and his colleagues are able to provide habitats that are suitable for their survival and reestablishment based off of the species needs (Domínguez-Domínguez *et al.* 2005). Domínguez-Domínguez and the UMSNH are seeking further studies in various scientific

disciplines such as the biology and ecology of the family as a whole, which can be an aid when designing future conservation strategies (Domínguez-Domínguez *et al.* 2005). However, it is suspected that fish species at risk will continue to decline in abundance until Mexico develops a way to use their water and aquatic resources more sustainably (Contreras-Balderas 2002). Fish that are endemic with small population sizes and near major urban areas, transportation corridors or ranges that are truncated by large impoundments are most likely to be more vulnerable to future extinction because of exposure to multiple stressors (Burkhead 2012). Therefore it is critical that we minimize or reverse the effect of habitat degradation by integrating species biology and social dynamics to reach effective solutions immediately.

Lastly, when a population is small as the result of a bottleneck event or when a species in general is endangered, a population can go extinct for a variety of reasons but sometimes this is the result of their behavior (Sutherland 1998). Models based on behavior allow for us to see how changes in water quality, habitat loss or fragmentation affect species mortality (Sutherland 1998). The following methodologies seek to contribute to model development and Domínguez-Domínguez's work. The goal is to help establish healthy populations of *X. eiseni* back in the wild. Their sexual selection should be well understood as populations, which differ in ornamentation may not successfully breed.

Materials and Methods

Male and female *X. eiseni* were obtained from the University of Colorado's fish laboratory, which is located in the Ramaley Biology building. The males were separated from the females for three to four months to ensure that the females were not gravid and that both sexes were ready for copulation.

Sexual selection experiments were run to see the female's preference for a mate. This was done by looking directly at the behavior of the female in the absence of male-male competition or in the presence of male-male competition. Three different experimental methods were used: Open Aquarium, Dichotomous Choice and Video Playback. This study was designed to gain an overall understanding of *X. eiseni*'s breeding behavior within a controlled environment.

Methodologies differed in the physical structure of the aquarium. There were differences within social groupings, which lead to different types of data. Video playback was similar to the dichotomous choice experiment but the females viewed a videotaped male rather than a living male in the video playback experiment.

The benefit of using live fish versus video playback was that there was a true interaction amongst the fish. The fish were allowed to display to the female and in some cases compete with other males. One uncertainty was that not every variable could be controlled. This was the benefit of Video Playback. However, video playback also had its own flaws and thus the following three methods were obtained to try and grasp a better understanding of *X. eiseni*'s true behavior.

Open Aquarium

The open aquarium design involved making observations of *X. eiseni* while they interacted in a group setting. This was believed to be the most realistic setting to observe their natural social interaction. The purpose was to conduct mate choice experiments and observations about the female's sexual preference. In this method males were allowed to interact and successfully copulate.

The experiment took place in a 76-liter long aquarium. The aquarium contained only a gravel layer with nothing else i.e. plant material. This allowed for normal swimming and foraging activity. Males and females could be individually identified by their color patterns. Taking notes of their relative size and other unique characteristics made them easy to identify. These notes were created into an identification chart so that each of the eight individuals could be identified in the aquarium when reviewing the videotape. The males and females interaction were videotape for 15 minutes. After each trial the males were removed from the tank, isolated, and the video was reviewed. The open aquarium technique was video taped because the video allowed for the behavior to be looked at more than once. The video also ensured that all interactions were taken into account and scored correctly.

Each time the male displayed, a score was recorded, which was indicated by the female response. Response takes into account how much of the courtship sequence was completed. A zero showed that the female did not react to the male's courtship behavior and a score of five indicated that the female maintained her response throughout the courtship and a copulation attempt occurred. The table below (Table 1) shows the scoring

system, which was used to assess female responses to male displays. Females were matched as close in size, age and prior experience as possible.

Score	Female and Male Behavior
0	No response; female ignores male
1	Female orients towards male but does not move closer
2	Female glides toward male
3	Male and female circle around each other
4	Copulation attempt; male thrusts and makes contact
5	Copulation; spermatopodium contact and male jerking

Table 1: Method for Scoring Female Response to Male Courtship Behavior

Source: A. E. Houde 1997

Note: Each stage includes behavior from all previous stages, plus the behavior noted. Males sometimes omit stage 3 and attempt copulation as soon as the female responds

Open Aquarium Data Analysis

The data were collected by measuring the size of each fish, male and female, and then placing a numerical value of 0-5 based on their interactions. The size of each fish was then judged to be either large, medium large, medium small and small based on its relative size throughout each trial. A generalized linear model was run in the program “R” along with a nested anova. A zero-inflated distribution was used because the data showed a disproportionate number of zero’s. It was common for the female to either ignore the male or not respond to him. Therefore male size was nested within the female size because females look at one male individually and then judge that particular male, or interact based on female preference.

The same setup was done for color as well. However, instead of the males being labeled either large, medium large, medium small and small based on its relative size the

experiment use the amount of color or ornamentation. For these results a zero-inflated generalized linear model was also used, testing the relative magnitude of male color instead of relative male size.

Dichotomous Choice

The dichotomous choice experiment was designed to look at female choice when a female is offered a choice between two different males. The assumption was made that the amount of time a female spent in proximity to a male with certain characteristics inferred female preference for a certain phenotype.

A 76-liter long tank is divided into three separate aquariums (fig. 13). Two black dividers were placed in the aquarium on the left and right side of the middle compartment. This inhibited the vision of the female's and the two male's while they were acclimating. The female that was placed in the center had the choice of either male. Thin pieces of yellow tape were placed on the outside of the fish tank to create a "zone of preference". The length of the zone of preference was dictated by twice the female's length and was used to determine whether the female had a preference for a particular male. Once the female entered the zone of preference the timer started and conversely when the female left the zone of preference, the timer ended.

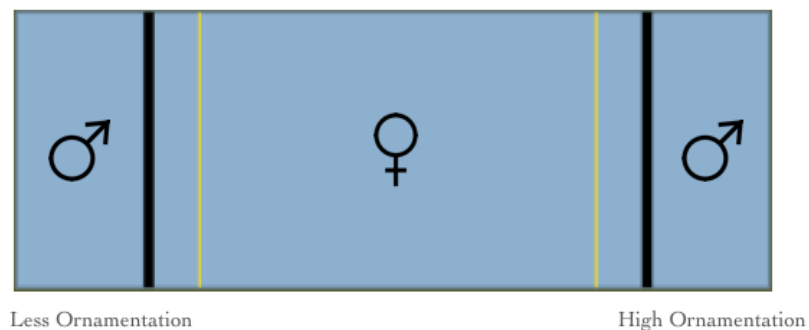


Fig. 13 Design of the Dichotomous Choice Experiment – Lateral View

A female was selected at random from the isolated *X. eiseni* group and its total length was recorded with a metric caliper. The female was placed in the center compartment of the aquarium. Two males were then selected at random from the collection of isolated male *X. eiseni*. They were then measured and judged as to which male was more colorful. Color was decided by taking out the male, holding it in a hand under the same light each time and making a judgment as to which male was more colorful i.e. possessed a greater magnitude of blue around its caudal peduncle or contained more orange coloration on its caudal fin. Smaller males were always placed with a slightly larger female to obtain consistency between each trial. The fish were then allowed a fifteen-minute acclimation period in their isolated compartments.

At the onset of the trial the black dividers were pulled up simultaneously and the female was then free to move from one side of the tank or the other. The amount of time the female spent in the zone of preference on either side was recorded. Similar to the open aquarium experiment the trials were filmed so that an accurate time could be obtained. Time was measured by using time stamps, which appeared while watching the video. Each trial went for 30 minutes, with the first part running 15 minutes and the second part running another 15 minutes. There was a fifteen-minute acclimation period between each part of the trial. During the acclimation period the males switched sides with the black dividers in place so that the female was unaware that they had switched sides. Switching the males to different sides of the tank was done to ensure that the female did not have a side preference. Once the fish were acclimated, the female was exposed to each male again and the video recording continued.

Video Playback

A single highly ornamented male was placed in a 38-liter aquarium and filmed in isolation from other fish. After the male was filmed with an HD camcorder, the video was uploaded into Final Cut Pro 7. A ten second video of the male swimming normally was created. The male fish swam back and forth in the frame till he returned back to where he first appeared in the frame. This was done to ensure that the loop was as true as possible. The video was then checked to make sure that the size of the male on the video was 45 mm in size when displayed on the computer monitor.

The video was then exported from Final Cut Pro 7 into Color. Here the video was altered. The blue on the male's caudal peduncle was increased in saturation along with the orange around its caudal fin. This was accomplished by adjusting the secondary colors of the video. In another video, the same colors that were increased in saturation were also decreased so that no ornamentation appeared. This resulted in two different videos, one saturated and one with no saturation. The benefit of using the program Color was that the secondary colors could be adjusted while all other colors in the frame stayed true. For instance, the anterior olive brown color of the fish stayed the same while the orange caudal fin increased in saturation.

After the colors were altered the video was rendered and sent back to Final Cut Pro 7 where a loop was created. The two videos were then displayed to a female of similar size on two separate screens. Figure 14 helps explain the design of the experiment.

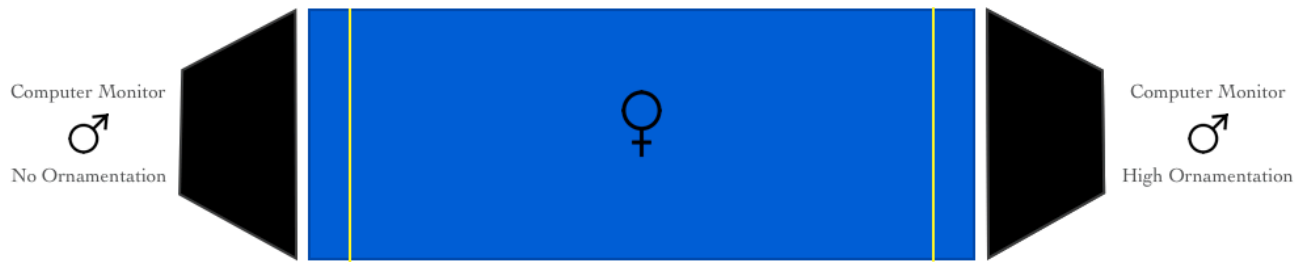


Fig. 14. Design of the Video Playback Experiment – Lateral View

A female was selected at random. Her total length was then recorded to make sure that it was a similar size to the 45 mm male. She was then placed into the fish tank prior to the experiment for fifteen minutes to make sure that she had acclimated to the water temperature, quality and general space.

One video screen showed the highly ornamented male while the other video screen projected the unsaturated male. The two videos were viewed for ten minutes while a video camera recorded the female's preference. Her preference was determined by the amount of time she spent in the "zone of interest", which was determined by taking twice the size of the female's length. Two lines on the video screen were taped vertically on the tank to determine the distance from the edge of the tank to the point where the "zone of interest" begins. The video of the female's preference was then reviewed to determine the time the female spent in each zone. This process was repeated with the video displays switching locations to ensure that the female does not have a specific preference to a particular side of the tank. Two black dividers were used again to ensure that the female was unaware the videotaped males had switched to different monitors.

Video Playback & Dichotomous Choice Data Analysis

The data analysis for both the dichotomous choice experiment and the video playback experiment was the same. First, the difference in the time stamps from the

recorded video was taken to ensure an accurate time measurement. At the end of the trial the sum was taken to see the total amount of time the female spent in a particular zone of preference.

The data were imported into the statistical program "R". In "R" both means were calculated along with the standard error. A paired t-test was then run because each female was able to look at two males, a more colorful one and a less colorful one at the same time, which yielded our t-statistic and p-value. This was then used to create the two bar graphs showing whether the female in general preferred a highly ornamented male versus a less ornamented male. These are shown in the results section of this paper. Furthermore, a graph representing the sums of each trial, part 1 and part 2, is shown in the results section to assess potential side biases.

Results

Open Aquarium

The results based on size showed a distribution with a mode, after negating the zeros, of two, which is when a female orients towards male but does not move closer (fig. 15). A successful copulation attempt was observed in six out of the twenty experiments. After nesting the male size with female size the results showed that compared to large males, large females preferred small males significantly less ($z = -3.62$, $P=0.00029$). Similarly the medium large females preferred small males significantly less and large females preferred medium small males significantly less, when small, medium small and medium large males are compared to large males ($z = -2.14$, $P=0.032$; $z = -3.42$, $P=0.0063$). For a complete table of the z-statistics, p-values, estimates and standard errors refer to Appendix A. To summarize, the results show that large or medium large females preferred to mate with larger males rather than small males.

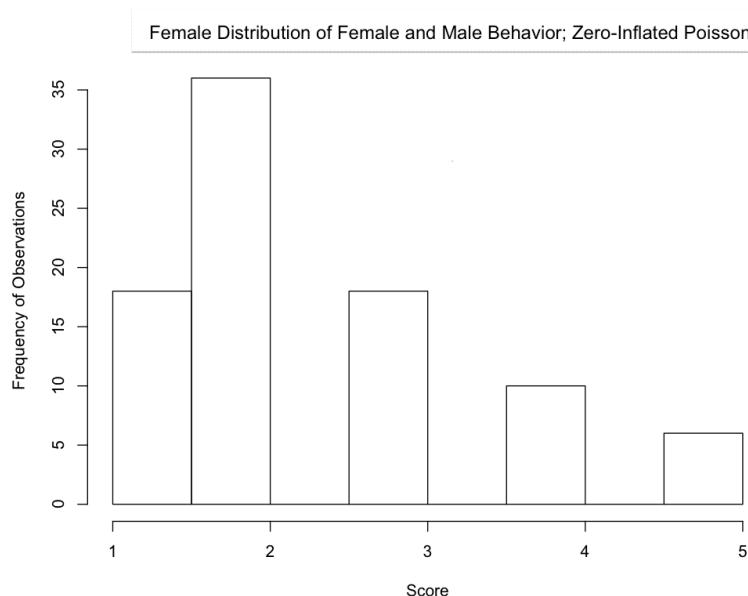


Fig. 15 Histogram showing the distribution of how females interact with males based on size (n=20)

The results based on color showed a distribution with a mode of two and a total of six successful copulation attempts, which is consistent with the size distribution (fig. 16). One thing that can be noted when looking at the original data table is that the third most colorful male was sometimes the male that successfully copulated and that it was not always the most colorful male that resulted in a successful copulation attempt. One significant finding was that large females were significantly less interested in mating with the least colorful males ($z = -3.17$, $p = .0015$). Similarly, medium large females were significantly less interested in mating with the least colorful males ($z = -3.08$, $p = 0.0021$). For a complete table of the z-statistics, p-values, estimates and standard errors refer to Appendix A.



Fig. 16 Histogram showing the distribution of how females interact with males based on magnitude of ornamentation/color (n=20)

Dichotomous Choice

The dichotomous choice experiment showed that the female had a preference for a male that possessed high ornamentation ($T_{14}=4.0636$, $p=0.001$) (Fig. 17).

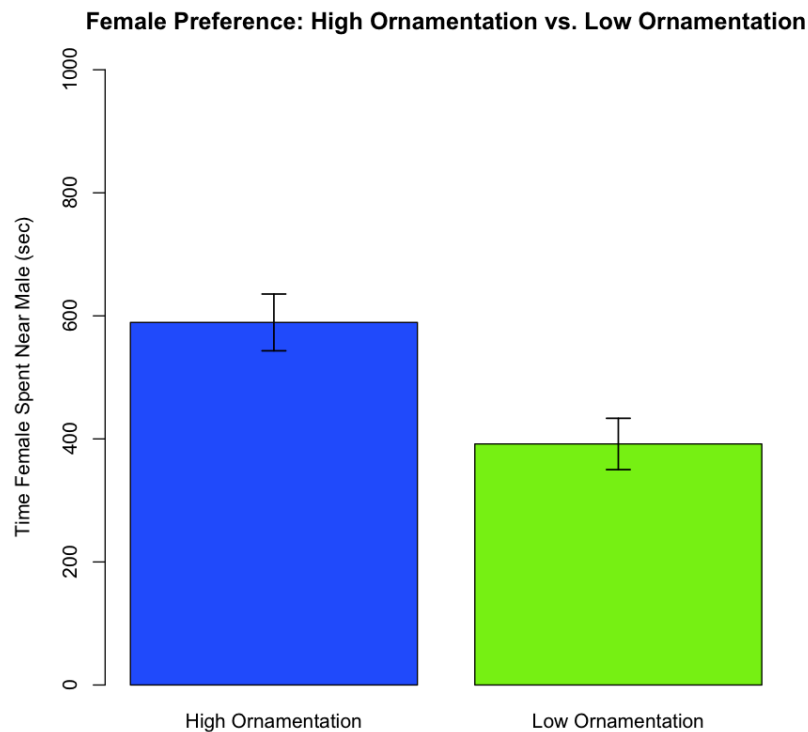


Fig. 17: Female Preference: Saturated Male vs. Unsaturated Male, Female fish spent more time near the male that possessed high ornamentation than the male that possessed less ornamentation ($n=15$)

The female spent significantly more time with the highly ornamented male. The bar graphs (Fig. 18 & 19) indicated that there was much less side bias amongst the fishes. This may be a result of male courtship displays. Side biases were only found in five out of the fifteen trials (trials 6, 7, 8, 13, 14). Despite the suspected bias, the female spent more time with the highly ornamented male in trials 6, 7, 13 & 14 when the highly ornamented male was on the side that she preferred. In these specific trials when the highly ornamented male was on the side the female preferred she spent the majority of her time on that side

and very little time visiting the other male. Trial number 8 was a side bias because the female spent the majority of her time on one side even after the males were switched.

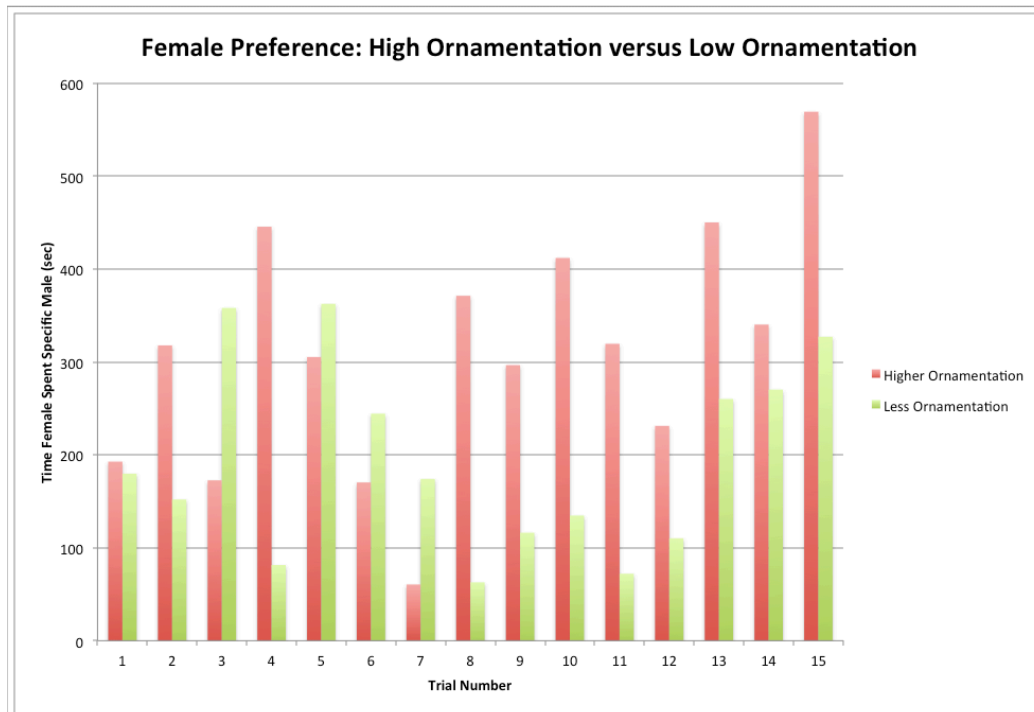


Fig. 18: Female Preference: High Ornamentation versus Low Ornamentation, Trial numbers 6 and 7 indicate that the female preferred the side with the less ornamented male

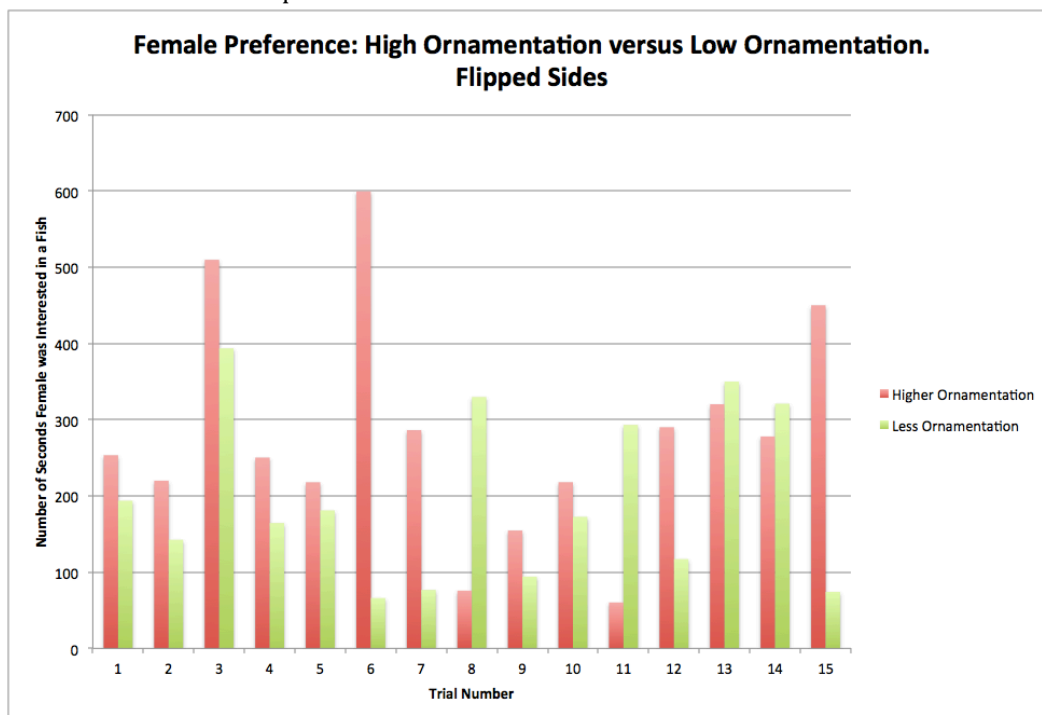


Fig. 19: Female Preference: High Ornamentation versus Low Ornamentation, Trial numbers 8, 13 & 14 indicate that the female preferred the side with the less ornamented male

Video Playback

In all the video playback trials there was a side bias (Fig. 20 & 21), which involved the female spending more time on one side of the tank in both trials. In trials one, two and five the female spent the majority of its time on the left side where the saturated fish was located. Once the two video screens were switched the female once again spent the majority of its time on the left side where the unsaturated fish was displayed. This behavior was repeated in trials three and four but the preference was instead for the right side of the aquarium.

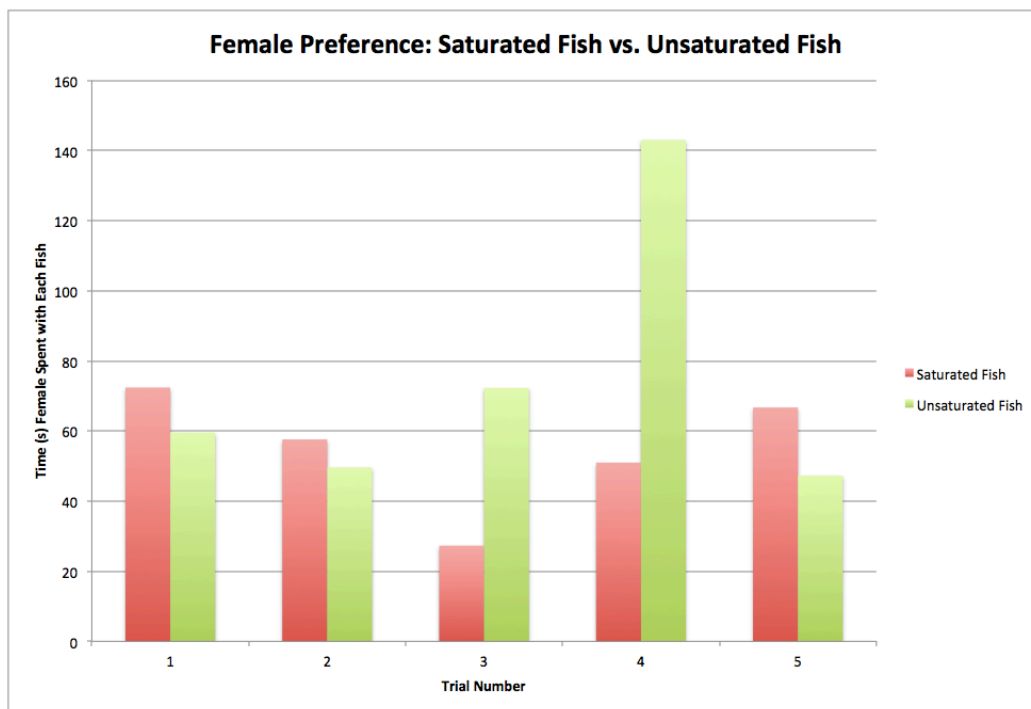


Fig. 20: Female Preference for a Saturated Fish or an Unsaturated Fish using Video Playback

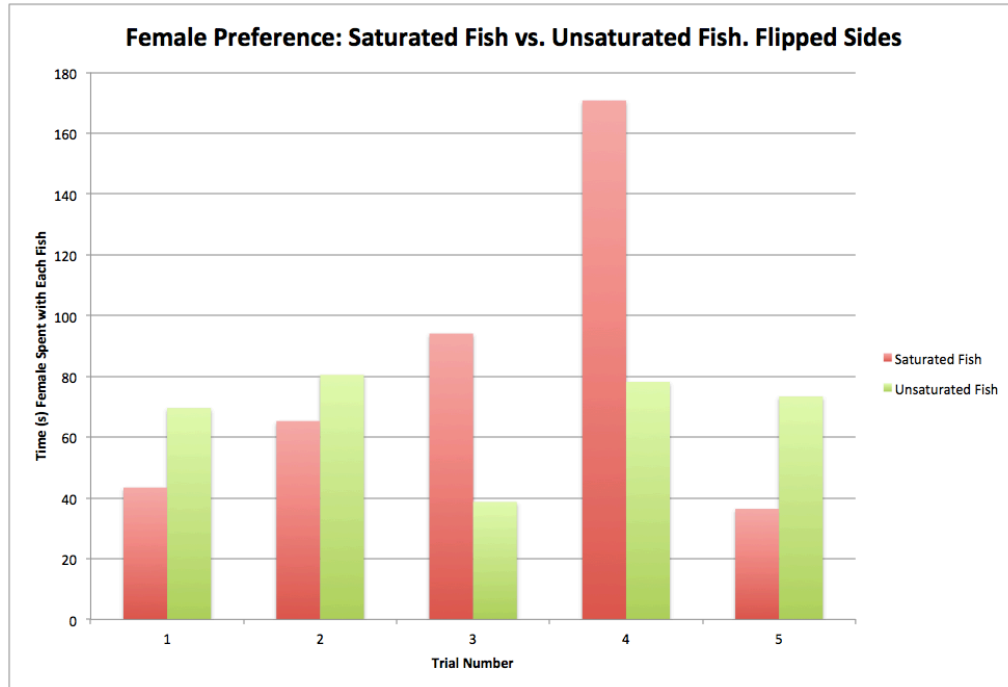


Fig. 21: Female Preference for a Saturated Fish or an Unsaturated Fish using Video Playback, Each trial represents the same trial number as in Fig. 2 but shows the results of a ten minute observation period after the video screens had been switched

The results also indicated that after five trials of thirty minutes each the female spent more time with the unsaturated fish (Fig. 22). However, the data were not significant ($T_4 = -1.0861$, $p = 0.3385$; Figure 22).

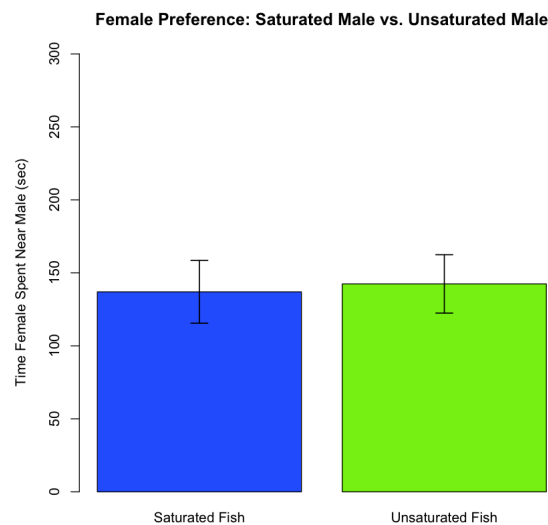


Fig. 22: Female Preference: Saturated Male vs. Unsaturated Male, Female fish within two and a half hours spent more time near the video screen that displayed the unsaturated fish then the saturated fish

It was also found that females spent much more time in the middle of the tank during each thirty-minute trial than they did near a particular video screen. Therefore, despite being able to control many of the male's characteristics the female did not appear to have a preference for a highly saturated male or unsaturated male throughout the video playback experiment.

Discussion

Open Aquarium

The open aquarium results showed that large females did not prefer small males or medium small males but rather preferred larger males. This is similar to Bisazza's (1997) study, which showed that for there to be a successful copulation amongst *X. eiseni* there must be two individuals of similar size because males do not possess a true gonopodium. Without the two mates being synchronized in undulations during sperm transfer the copulation attempt may be unsuccessful (Bisazza 1997).

The open aquarium also showed that large females preferred to mate with more colorful males. In future studies more variability in male ornamentation should be considered. For instance, populations from the Ameca River differ from other population's ornamentation and these different populations should be analyzed to see whether females prefer males that are similar in ornamentation to their own population. Furthermore, the amount of color should be quantified to make the results more accurate. However, this study was limited in its ability to do both of these suggestions as the University of Colorado only contains one population of *X. eiseni*. Kodric-Brown (1997) was able to quantify the carotenoid and iridescent spots of guppies (*Poecilia reiculata*) by anaesthetizing the fish with MS222 and then photographing the fish with a 50-mm macro lens and Kodachrome 64 DX film. These images were then projected onto a computer-digitizing tablet and the total area of the carotenoids and iridescent spots were taken into account.

Lastly, in future experiments virgin females should be used so that females have had no prior experience, which may result in the fish having a certain preference for a

particular male. Again this study was limited and could not ensure that all of the females used in this experiment were virgins because the *X. eiseni* had been kept in a fish run at the University of Colorado's fish laboratory with both males and females freely interacting for an unknown amount of time. However, it is important to note that *X. eiseni* were guaranteed to be non-gravid when paired with males by being placed in isolation for three to four months before the experiment begun.

Dichotomous Choice

X. eiseni females had a preference for males that had higher ornamentation. In future studies, courtship frequency and duration should be taken into account. It is suspected that males that display longer and more often may be able to gain the females' attention for a longer period of time. Lastly, size should be taken into account. In this experiment females and males were measured to ensure that they were similar in size. In future studies the same method could be performed but instead of choosing males that differ in ornamentation one could choose males that differed in size. It was shown in the open aquarium methodology of this paper that large females are more likely to mate with large males rather than small males.

Video Playback

In previous studies this methodology had produced significant results and therefore it was thought that a similar methodology would work with *X. eiseni*. Amanda Daniel, a former student and the University of Colorado had used this methodology to study *Limia Perugiae*, a species of livebearers (Family Poeciliidae) found in Hispanola. Two populations were compared because their habitats varied in turbidity and as a result they varied in

their secondary sex characteristics. For instance, males contained a brighter caudal fin and black dorsal fins in the Asufrada spring population while the population from Laguna Oviedo was more turbid. Therefore, the males did not display as much coloration. The results from Daniels' experiment showed that there was not a significant difference between the live males and video taped males. Furthermore, the male that was more colorful within the Asufrada population was more likely to be chosen while the Laguna Oviedo population had a propensity to choose the dull population.

However, the video playback experiment in this paper focused on a different species of fish, from a different family. I found that there was a more natural interaction among the fish when live fish were used because the fish were allowed to display to the female and they were able to interact visually with each other. However, not every variable such as size, courtship duration and frequency could be controlled in the experiment. This is the benefit of video playback. Variables such as size and color are consistent and could be controlled. However, video playback is not perfect either. Given the conditions, I was not able to film an isolated male while he was displaying a courtship dance or displaying the black bar on the lower portion of his dorsal fin and therefore there was not a true interaction between the males and the females during the video playback portion of this experiment. These two factors seemed to be critical in keeping the females attention and reiterated the significance of male courtship behavior.

Throughout the video playback experiment I found that the females spent the majority of their time outside the zone of interest. The female seemed to have a lack of interest in the males that were being displayed and it is likely that this resulted in a side

bias, which can occur because the females may have a preference for a particular side of the tank due to differences in the amount of light or the placement of the aquarium's filter.

In this experiment the female was very uninterested in the males as they "swam" by on the computer screen. Only for a few seconds did the female try to interact with the saturated fish during each trial period. Also the time they spent inside the zone of interest was much less than in the Dichotomous Choice experiment.

One factor to consider is that fish may not be able to process visual signals displayed by video monitors as these video monitors are designed for human visual systems. Fish may distinguish the color, brightness, depth, size and temporal resolution that a video monitor displays as irregular.

One major difference between the human visual systems and a fish's visual system is that a fish's eye is designed to see things underwater and therefore there is a difference amongst the rods and cones within our eyes (Fleishman *et al.* 1998). These rods and cones receive photopigments differently between the two species and therefore the color and brightness may be perceived differently between the different vertebrates (Fleishman *et al.* 1998). Further research is needed to understand the visual receptors in the fish's eye compared to those in our eyes, as the video monitors may need to be altered so that the fish correctly views the color.

Also, the video playback method does not allow for a three-dimensional object and is limited to projecting a two dimensional object. Also, the frequency of how often a video monitor displays an image is designed for the human eye. Computer monitors typically have a critical flicker fusion frequency of 50 to 60 Hz, which is just above the level needed for the human eye, while fish have higher critical flicker frequencies i.e. 14 to 67 Hz (D'Eath

1998). Therefore if fish have a higher critical flicker frequency then it is possible that they see the monitor as a series of images that flicker rather than one continuous image.

Furthermore, I suggest that in future studies a male is filmed so that the male is isolated but able to interact with females. This can be obtained by filming a male in an isolated tank with two smaller tanks at oblique angles to the male's tank. These two tanks at oblique angles would have females inside of them. In between the two tanks a camera would be placed so that a more accurate representation of the female interacting with the male. See figure 23 for an example of how future experiments should be set up.

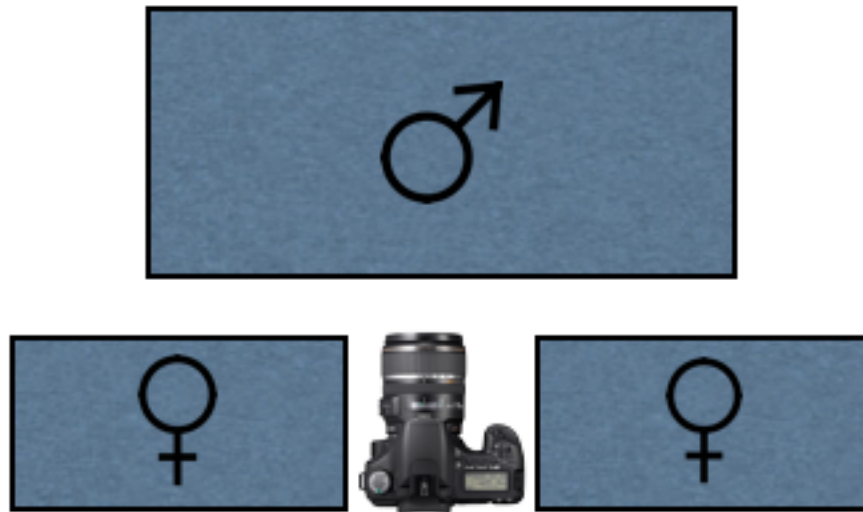


Fig. 23 Future design for video playback experiment

The design in figure 23 is described in Kodric-Brown's (1997) study with guppies i.e. *Poecilia reiculata* and was found to be effective way to allow for guppies to display courtship behavior. However, future studies involving Goodeinae perception of color and light should also further be studied to see if these results are accurate.

Unfortunately the video playback methodology did not yield significant results and cannot be used to help explain the question of how sexual selection plays a role in conservation. However, both the open aquarium and the dichotomous choice experiment

helped play a role in answering the question: How does sexual selection and breeding biology play a role in the conservation of the threatened *Xenotoca eiseni*?

Conclusion

It was found that in both the dichotomous choice experiment and the open aquarium methodology that male ornamentation played a role in the sexual selection of females. When female *X. eiseni* were looking for a mate they preferred to mate with a male that possessed a greater magnitude of ornamentation. This is what one would expect to happen as it coincides with sexual selection theory. However, this had never been looked at before with *X. eiseni*. It is typical for species that have elaborate courtship behavior and are sexually dimorphic to have females prefer the male with higher ornamentation as their offspring will likely be of higher genetic quality (Arellano-Aguilar & Marcías Garcia 2008).

Males that possess a greater amount of ornamentation deviate further from the optimum value and therefore male ornamentation is very costly to produce (Arellano-Aguilar & Marcías Garcia 2008). Therefore there must be an advantage to the species fitness for it to be maintained. When Domínguez-Domínguez, his colleagues or other scientists are considering locations for possible fish reintroductions, the particular ornamentation of the extant population should be considered as species of *X. eiseni* can vary in ornamentation from population to population (Fitzimsons 1970, Goodeid Working Group, Moyaho 2004).

Based on the results of this study it is shown that females choose males that possess a high level of ornamentation and therefore when these aquaria bred populations are reintroduced into the wild the extant population needs to be considered so that the two species can successfully interact and reproduce. Due to the stringent sexual selection

process hinted at throughout this study and the study done by Zuarth and Marcías Garcia (2005), it is believed that *X. eiseni*, *G. multiradiatus* and other species of Goodeinae may become reproductively isolated as a result of female preference, once species that differ from the norm are introduced into the wild.

Therefore, further studies should look at the success rate of reintroducing Goodeinae into the wild at small and controlled sites. This study should also take populations that differ by their locality and ornamentation and see whether females from one population will mate with the other population and vice versa.

In addition, Kenway-Lynch and others (2010) suggest that researchers seek additional information on population sizes and enact a monitoring program to make certain that extant populations are maintained. This will allow for researchers to become aware of when populations sizes are increasing or decreasing. John Ransier (2005) has showed that coded wire tags last for about 56 days in *X. eiseni* and show great promise to ensure that an introduced stock of aquarium bred fish is surviving in the wild. It will also be useful to note the male's ornamentation within each population while undergoing stock reclamation or stock enhancement so that captive bred females can be matched to the female's preference.

Furthermore, I suggest that the habitat and watershed area of each population be better studied. For instance, if there is agricultural development or industrial development near the species habitat then the amount of methyl parathion and other pesticides, that leach into the *X. eiseni*'s habitat, should be noted as these pollutants can drastically alter the development of their sexually dimorphic traits (Arellano-Aguilar & Marcías Garcia 2007). Similarly, if the area is receiving more dissolved solids and is therefore becoming

more turbid, then the female's vision may be reduced and successful copulation might not occur because the female may not be stimulated.

Also, a monitoring program will be significant because it will ensure that water diversions during drought years do not erode *X. eiseni's* population. It will also provide insight into how habitat alterations are affecting extant populations of *X. eiseni*. For instance, if land the land use has change near a population then the population can be monitored along with the water quality to see if this is having an affect on the population size or the species characteristics.

Future studies and future monitoring programs are critical to the conservation of *X. eisnei*, a species that is not considered endangered by federal and international organizations but should be, due to their rapid decline in population numbers (Kenway-Lynch 2010, Jelks 2008, Lyons 2011). Research within *X. eiseni's* native habitats will be significant because it ensures that the same findings that were found in the laboratory are found in the wild. Females need to be interested or stimulated by the courting male, otherwise the male and female will not reproduce, as the male does not possess a true gonopodium. Therefore, the male is not capable of circumventing the female without female cooperation. A better understanding of the species breeding biology within a variety of native habitats, with different populations will help ensure that the *X. eiseni* does not continue to decline and it remains a Mexican treasure.

Bibliography

- Arellano-Aguilar, O., & Marciás García, C. (2008) Exposure to pesticides impairs the expression of fish ornaments reducing the availability of attractive males. *Proc. R. Soc. B*, 275, 1343-1351. doi: 10.1098/rspb.2008.0163.
- Bisazza, A. (1997). Sexual selection constrained by internal fertilization in the livebearing fish *Xenotoca eiseni*. *Animal Behaviour*, 54, 1347-1355.
- Burkhead, N. M. (2012). Extinction rate in North American freshwater fishes, 1900-2010. *BioScience*. 62(9), 798-808 doi: 10.1525/bio.2012.62.9.5
- Cain, M. L., Bowman, W. D., & Hacker, S. D. (2011). Ecology (2nd ed.). Sunderland, MA: Sinauer Associates.
- Carmen Uribe, M., Grier, H. J. and Parenti, L. R. (2012). Ovarian structure and oogenesis of the oviparous goodeids *Crenichthys baileyi* (Gilbert, 1893) and *Empetrichthys latos* Miller, 1948 (teleostei, Cyprinodontiformes). *J. Morphol.*, 193, 371–387. doi: 10.1002/jmor.11028
- Crenichthys baileyi*. (2013). *Goodeid Working Group*, Retrieved March 5, 2013 from <http://www.goodeidworkinggroup.com/crenichthys-baileyi>
- Crenichthys nevadae*. (2013). *Goodeid Working Group*, Retrieved March 5, 2013 from <http://www.goodeidworkinggroup.com/crenichthys-nevadae>
- Darwin, C. (1859). On the origin of species. London, United Kingdom. John Murray
- D'Eath R.b. (1998). Can video images imitate real stimuli in animal behaviour experiments. *Biology Review*. 73(3) 267-292 doi: 10.1111/j.1469-185X.1998tb00031.x
- De la Vega-Salazar M. Y., & Macías-García, C. (2005). Principal factors in the decline of the Mexican endemic viviparous fishes (Goodeinae: Goodeidae). Pages 506-513 in M. C. Uribe and H. J. Grier, editors. Viviparous fishes. New Life Publications, Homestead, Florida.
- Doadrio I., Domínguez- Domínguez O., (2004). Phylogenetic relationships within the fish family Goodeidae based on cytochrome b sequence data. *Molecular Phylogenetics and Evolution*, 31(2), 416-430 doi: <http://dx.doi.org/10.1016/j.ympev.2003.08.022>
- Domínguez-Domínguez, O., Boto L., Alda F., Pérez-Ponce De León, G., & Doadrio I. (2006). Human impacts on drainages of the mesa central, Mexico, and its genetic effects on an endangered fish, *Zoogoneticus Quitzeoensis*. *Conservation Biology*, 21(1), 168-80. doi: 10.1111/j.1523-1739.2006.00608.x

- Domínguez-Domínguez, O., Zambrano, Escalera-Vázquez, L. H., Perez-Rodriguez, R., & Pérez-Ponce de León, G. (2008). Changes in the distribution of goodeids (Osteichthyes: Cyprinodontiformes: Goodeidae) in river basins of Central Mexico. *Revista Mexicana de Biodiversidad*. 79, 501-512
- Domínguez-Domínguez, O., Mercado-Silva, N., & Lyons J. (2005). Conservation status of Mexican goodeids: problems, perspectives, and solutions. Pages 515-523 in M. C. Uribe and H. J. Grier, editors. *Viviparous fishes*. New Life Publications, Homestead, Florida.
- Domínguez-Domínguez, O., Mercado-Silva, N., Lyons J. & Grier, H. J. (2005). The viviparous Goodeid species. Pages 526-568 in M. C. Uribe and H. J. Grier, editors. *Viviparous fishes*. New Life Publications, Homestead, Florida.
- Duncan, J.R., & Lockwood J.L. (2001). Extinction in a field of bullets a search for causes in the decline of the world's freshwater fishes. *Biological Conservation*, 102(1), 98-105
doi: [http://dx.doi.org/10.1016/S0006-3207\(01\)00077-5](http://dx.doi.org/10.1016/S0006-3207(01)00077-5)
- Durham, B. (1979, February). The orange-tailed "goody". *Tropical Fish Hobbyist*, 96-102
- Fitzsimons, J. M. (1970). Comparative behavior and systematics of two genera of Goodeid fishes (Atheriniformes, Cyprinodontidae) from the Mexican Plateau (Unpublished doctoral dissertation). University of Michigan, Ann Arbor, Michigan.
- Fitzsimons, J. M. (1972). A revision of two general of Goodeid fish (Cyprinodontiformes, Osteichthyes) from the Mexican Plateau. *American Society of Ichthyologists and Herpetologists*, 1972(4), 728-756.
- Fleishman, L. J., McClintock, W. J., D'Eath, R. B., Brainard, D. H., & Endler, J. A. (1998). Colour perception and the use of video playback experiments in animal behaviour. *Animal Behaviour*. 56, 1035-1040
- Glmenez D.M. (1996). *Crenichthys nevadae*. IUCN Red List of Threatened Species. Version 21013.2 <www.iucnredlist.org> Downloaded on 05 March 2013
- Helfman, G. S., Collette, B. B., Facey, D. E., & Bowen, B. W. (2009) The diversity of fishes: biology, evolution, and ecology. Chichester, UK: Wiley-Blackwell.
- Hollenberg, F., & Wourms, J.P. (1995) Embryonic growth and maternal nutrient sources in goodeid fishes (Telostei: Cyprinodontiformes). *J. Exp. Zool.*, 271, 379-394
- Houde, A. E. (1997). Sex, Color, and Mate Choice in Guppies. Princeton, NJ: Princeton University Press.
- Hubbs, C. L. & Turner, C.L. (1939). Studies of the fishes of the order Cyprinodontes XVI. A revision of the Goodeidae. *Misc Publs University of Michigan Mus. Zool.* 42, 1-80.

IUCN (1994). Categories & Criteria. Version 2.3 <www.iucnredlist.org> Downloaded on 04 March 2013

IUCN (2012). IUCN red list of threatened species. Version 2012.2. <www.iucnredlist.org>. Downloaded on 19 February 2013

IUCN Standards and Petitions Subcommittee (2013). Guidelines for Using the IUCN Red List Categories and Criteria. Version 10. Retrieved from <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>.

Jelks, H. L., Walsh, S.J., Burkhead, N.M., Conteas-Balderas, S., Diaz-Pardo, E., Hendrickson, D. A., Lyons, J., Mandrak, N. E., McCormick, F., Nelson, J. S., Platania, S. P., Porter, B. A., Renaud, C. B., Schmitter-Soto, J. J., Taylor, E. B., Warren, M. L. Jr (2008). Conservation status of imperiled North American freshwater and diadromous fishes. *Fisheries*. 33, 372-407

Kelley, J. L., Magurran, A. E., & Marcías García, C. (2006). Captive breeding promotes aggression in an endangered Mexican fish. *Biological Conservation*, 133, 169-177 doi: 10.1016/j.biocon.2006.06.002

Kenway-Lynch, C. S. (2010). Phylogeography and population structure of the imperiled the redbtail splitfin (*Xenotoca eiseni*): implications for conservation (Doctoral dissertation. Southeastern Louisiana University, 2010). *Dissertation abstract*. Retrieved from http://books.google.com/books/about/Phylogeography_and_Population_Structure.html?id=GcPvZwEACAAJ

Kenway-Lynch, C. S., McMahan, C. D., Geheber, A. D., Hayes, M., & Piller, K. R. (2010). Threatened fishes of the world: “*Xenotoca*” *eiseni* Rutter, 1986 (Goodeidae). *Environ Biol Fish*, 87, 219-220 doi: 10.1007/s10641-010-9584-2

Kingston D. I. (1979). Behavioral and morphological studies of the Goodeid genus *Ilyodon*, and comparative behavior of fishes of the family Goodeidae. (Unpublished doctoral dissertation) University of Michigan, Ann Arbor, Michigan

Kordic-Brown, A. Nicoletto P.F. (1997). Repeatability of female choice in the guppy: response to live and videotaped males, *Animal Behavior*, 54(2), 369-376 Retrieved from: <http://biology.unm.edu/biology/kodric/Pubs/RepeatabiltyOfFemaleChoice.pdf>

Lyons, J., Gonzalez-Hernandez, G., Soto-Galera, E., & Guzman-Arroyo, M. (1998). Decline of freshwater fishes and fisheries in selected drainages of west-central Mexico. *Fisheries*, 23(4), 10-18

Lyons, J. (2011). Conservation status and population trends of Mexican Goodeids, 2011. Retrieved from <http://www.goodeidworkinggroup.com/conservation-status-and-population-trends-of-mexican-goodeids-2011>

- Macías-García, C., & Saborío E. (2004). Sperm competition in a viviparous fish. *Environmental Biology of Fishes*, 70(3), 211-217. doi: 10.2307/3333558. doi: 10.2307/3333558
- Marcías García, C., & Valero, A. (2010). Sexual conflict and sexual selection in the Goodeinae, a class of viviparous fish with effective female mate choice. *Advances in the Study of Behavior*, 42, 1-54
- McLain, D. K., Moulton M.P., & Redfearn T.P. (1995). Sexual Selection and the Risk of Extinction of Introduced Birds on Oceanic Islands, *Wiley-Blackwell*, 74(1), 27-34. Retrieved from <http://www.jstor.org/stable/3545671>
- Mendoza G. (1965). The ovary and anal processes of "Characodon" Eisei, a viviparous Cyprinodont Teleost from Mexico. *Biol Bull.*, 129(2), 303-315
- Miller, R. R., Minckley, W. L., Norris, M. S., Gach, M. H. (2005). *Freshwater Fishes of México*. Chicago, The University of Chicago Press.
- Mohsen, T. (1965). A new key character in males of the family Goodeidae (Cyprinodontiformes). *Nature*, 4976(205) 1127. doi:10.1038/2051127b0
- Moyaho, A., Macías García, C., & Ávila-Luna, E. (2004) Mate choice and visibility in the expression of a sexually dimorphic trait in a Goodeid fish. *Canadian Journal of Zoology*, 82(12), 1917-1922.
- Parenti, L. R. (1981). A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). *Bulletin of the American Museum of Natural History*, 168 (4), 335-557.
- Ptacek, M. B. (2005). Mating singal divergence, sexual seletion, and species recognition in Mollies (Poeciliidae: *Poecilia: Mollienesia*). Pages 72-85 in M. C. Uribe and H. J. Grier, editors. *Viviparous fishes*. New Life Publications, Homestead, Florida.
- Ransier, J. (2005). Tagging livebearing fish: potential role in enhancing and restoring livebearing fish populations. Pages 476-480 in M. C. Uribe and H. J. Grier, editors. *Viviparous fishes*. New Life Publications, Homestead, Florida.
- Ritchie, M. G., Hamill, R. M., Graves, J. A., Magurran, A. E., Webb S. A., Macias Garcia, C. (2007). Sex and differentiation: population genetic divergence and sexual dimorphism in Mexican goodeid fish. *Evolutionary Biology*, 20(5), 2048-2055 doi: 10.1111/j.1420-9101.2007.01357.x
- Rutter C. (1896). Notes on fresh water fishes of the Pacific slope of North America. li. The fishes of Rio Yaqui, Sonora, with the description of new genus of Siluridae. *Proceedings of the California Academy of Sciences*, 6(2), 255-267

- Sorvano, V.A., & Bissazza, A. (2003). Modularity as a fish (*Xenotoca eiseni*) views it: conjoining geometric and nongeometric information for spatial reorientation, *Experimental Psychology: Animal Behavior Process*, 29(3), 199-210. doi: 10.1037/0097-7403.29.3.199
- Sutherland, W.J. (1998). The importance of behavioural studies in conservation biology. *Animal Behaviour*, 56(4), 801-809 doi: <http://dx.doi.org/10.1006/anbe.1998.0896>
- Turner, C.L. (1937). The trophotaeniae of the Goodeidae, a family of viviparous cyprinodont fishes, *Journal of Morphology*, 61(3), 495-523 doi: 10.1002/jmor.1050610306
- Turner, C. L., Mendoza, G., & Reiter, R. (1962). Development and comparative morphology of the gonopodium of Goodeid fishes. *Proceedings of the Iowa Academy of Science* 69, 571-586
- Webb, S. A. (1998). A phylogenetic analysis of the Goodeidae (Teleostei: Cyprinodontiformes). Ph.D. Dissertation, University of Michigan, Ann Arbor.
- Webb, A. S., Jefferson A. G., Macias-Garcia, C., Magurran, E. A., Ó Foighil, D., Ritchie, G. M. (2004). Molecular phylogeny of the livebearing Goodeidae (Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, 30(3), 527-544. doi: 10.1016/S1055-7903(03)00257-4
- Wourms, J. P. (1988). The maternal-embryonic relationship in viviparous fishes. *Fish physiology*. 11, San Diego, CA: Academic Press
- Xenotoca eiseni. (2013). *Goodeid Working Group*, Retrieved March 5, 2013 from <http://www.goodeidworkinggroup.com/xenotoca-eiseni>
- Zahavi, A. (1975). Mate Selection – A Selection for a Handicap. *J. theor. Biol.* 53, 205-214. Retrieved from: <http://www.eebweb.arizona.edu/Faculty/Dornhaus/courses/materials/papers/other/Zahavi%20sexual%20selection%20handicap%20model%20signal.pdf>
- Zuarth, C. G., & Marcías Garcia, C. (2006). Phenotypic differentiation and pre-mating isolation between allopatric populations of *Girardinichthys multiradiatus*. *Proc. R. Sco. B* 273, 301-307 doi: 10.1098/rspb.2005.3323

Appendix

The following shows the results for the dichotomous choice experiment, video playback experiment and open aquarium experiment.

A. Open Aquarium Data:

Table 1 (Appendix A) female size compared to male color

	Estimate	Std. Error	z-value	Pr(> z)
(Intercept)	1.164 e+00	1.827 e-01	6.371	1.87 e-10
fsML	-3.471e-02	2.798e-01	-0.124	0.90126
fsMS	-4.349e-01	3.646e-01	-1.193	0.23295
fsS	-1.758e+01	1.246e+03	-0.014	0.98874
fsL:mcLC	-3.331e+00	1.052e+00	-3.168	0.00154 **
fsML:mcLC	-1.703e+00	5.526e-01	-3.001	0.00206 **
fsMS:mcLC	-8.989e-01	5.117e-01	-1.757	0.07900
fsS:mcLC	1.610e+01	1.246e+03	0.013	0.98969
fsL:mcMC	-6.486e-01	3.882e-01	-1.671	0.09478
fsML:mcMC	-6.139e-01	4.024e-01	-1.526	0.12713
fsMS:mcMC	-2.587e-01	4.441e-01	-0.583	0.56013
fsS:mcMC	1.596e+01	1.246e+03	0.013	0.98978
fsL:mcMCC	5.505e-02	2.481e-01	0.222	0.82441
fsML:mcMCC	-2.344e-01	2.990e-01	-0.784	0.43308
fsMS:mcMCC	-2.137e-01	4.624e-01	-0.462	0.64400
fsS:mcMCC	-7.635e-06	1.762e+03	0.000	1.00000

fs= female size, mc= male color, L= size of female large, ML= size of female medium large

MS = size of female medium small, S= size of female small, LC=Least amount of ornamentation (male), MC= second greatest magnitude of ornamentation, MCC = third greatest magnitude of ornamentation, GC= greatest amount of ornamentation

Table 2 (Appendix A) female size compared to male size

	Estimate	Std. Error	z-value	Pr(> z)
(Intercept)	1.3371	0.1441	9.277	< 2e-16
fsML	-0.5391	0.3139	-1.717	0.085889
fsMS	-0.9479	0.4256	-2.227	0.025924
fsS	-17.8847	1290.8195	-0.014	0.988945
fsL:msML	-0.3399	0.2581	-1.317	0.187726
fsML:msML	0.1576	0.3391	0.465	0.642090
fsMS:msML	0.3292	0.4880	0.675	0.499983
fsS:msML	14.3227	1290.8199	0.011	0.991147
fsL:msMS	-1.7250	0.5047	-3.418	0.000631 ***
fsML:msMS	-0.3824	0.4064	-0.941	0.346766
fsMS:msMS	-0.1602	0.5179	-0.309	0.757103
fsS:msMS	15.8299	1290.8196	0.012	0.990215
fsL:msS	-2.8059	0.7759	-3.616	0.000299 ***
fsML:msS	-1.1859	0.5531	-2.144	0.032028 *
fsMC:msS	-0.2958	0.5427	-0.545	0.585786
fsS:msSS	16.2802	1290.8196	0.013	0.989937

fs= female size, ms= male size, L= size of female/male large, ML= size of female/male medium large

MS = size of female/male medium small, S= size of female/male small

B. Dichotomous Choice Experiment Raw data:

Table 1 (Appendix B), total amount of time a female spent near a particular male during part 1 of each trial

Trial #	Higher Ornamentation (sec)	Less Ornamentation (sec)
1	192.7	179.8
2	317.8	152
3	172.6	358.1
4	445.5	81.5
5	305.3	362.5
6	170.3	244.3
7	60.5	174.1
8	371.2	62.8
9	296.6	116.2
10	411.9	134.7
11	319.5	72.1
12	231.2	110.2
13	450.1	260.1
14	340.2	270.2
15	569.2	327.1
mean	310.31	193.71

Table 2 (Appendix B), total amount of time a female spent near a particular male during part 2 of each trial

Trial #	Higher Ornamentation (sec)	Less Ornamentation (sec)
1	253.5	193.8
2	220.1	142.6
3	509.9	393.7
4	250.4	164.5
5	218	181.1
6	599.7	66.1
7	286.4	76.8
8	75.8	329.9
9	154.7	94.2
10	218.1	172.7
11	60.3	293.3
12	290.2	117.2
13	320.4	350.1
14	278.1	321.1
15	450.2	74.2
mean	279.05	198.09

C. Video Playback Raw Data:

Table 1 (Appendix C), total amount of time a female spent in the zone of preference during part 1 of each trial

Trial Number	Saturated Side (sec)	Unsaturated Side (sec)
1	72.4	59.6
2	57.6	49.6
3	27.3	72.3
4	51	143
5	66.7	47.3
mean	55	74.36

Table 1 (Appendix C), total amount of time a female spent in the zone of preference during part 2 of each trial

Trial Number	Unsaturated Side (sec)	Saturated Side (sec)
1	69.6	43.4
2	80.5	65.3
3	38.7	94.1
4	78.2	170.8
5	73.4	36.4
mean	68.08	82