Comparing the Fertility of Florida's Eastern Mosquitofish, *Gambusia holbrooki*, Based on Differences in Female Traits, Water Quality, and Habitat Type

by

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Abstract

The eastern mosquitofish, *Gambusia holbrooki*, is a surface-dwelling guppy native to the southeastern United States. While resilient to poor water quality, the fish is a voracious predator of the epilimnion, with established populations in most subtropical/tropical regions with surficial freshwater. Eastern mosquitofish are farmed and collected for use as biocontrol agents against the spread of many infectious, often life-threatening, mosquito-borne illnesses. I compared the reproductive success (fertility) of 370 gravid mosquitofish among 32 sites across central Florida to test the hypothesis that fertility is influenced by maternal, ecological, and environmental factors. I found that fish fertility (clutch size) differed significantly by region (Fig. 13a; $R^2 = 0.02$, $p < 0.0001$), habitat classification (Fig. 13b; $R^2 = 0.007$, $p < 0.0001$), preserved female weight (Fig. 13c; $R^2 = 0.58$, $p < 0.0001$), total dissolved solids (Fig. 13d; $R^2 = 0.006$, $p = 0.0004$), and conductivity (Fig. 13e; $R^2 = 0.007$, $p = 0.003$). The strongest predictor of *G. holbrooki* clutch sizes was female weight, which accounted for 87.8% of the observed variation in fertility. Regional distribution was the strongest predictor of eastern mosquitofish weight. Water quality was a relatively weak predictor of mosquitofish characteristics and fertility. I found that females make significant (189%) post-fertilization nutrient contributions to developing embryos. To my knowledge, my study is the first to quantify maternal contributions to developing embryos in *G. holbrooki*. Gravid females displaying superfetation developed two clutches at different developmental stages simultaneously and were nearly three times as fertile as those not.

Chapter One: Introduction

Reproductive Strategies of Fishes

Reproduction is an energy-intensive event. Adaptations of fishes for placing the maximum number of reproducing offspring into the next generation fall within the spectrum of extreme semelparity to iteroparous life histories (Iguchi & Tsukamoto, 2001). No simple generalization can define how fish, marine or aquatic, reproduce. While some species mass-broadcast eggs into the water column for external fertilization, others may guard and rear fertilized eggs attached to substrate, or even practice internal fertilization and development. The most uncommon life history strategy is that of semelparity; to spawn once and die (McBride et al., 2015). Chinook salmon (*O. tshawytscha*) spend years in the open ocean building mass to support a single key spawning event, in which the production of up to 17,000 eggs exhausts the female to the point of death (Fleming, 1996). A staggeringly low percentage of the spawned eggs survive from fertilization to adulthood; even fewer return to spawn after sexual maturation. A return of three reproducing fish per mother would sustain and grow salmon populations; one to replace the mother, one to replace the father, and another individual to increase the population size (Groot & Margolis, 1991). Far on the other end of the spectrum are iteroparous species, which practice more than one reproductive cycle within their life histories. The great white shark (*C. carcharias*) gestates between two and 14 young at a time for over 11 months and ultimately gives birth to well-developed, free-swimming young that are immediately capable of foraging. Having a lifespan of 70 years, females may rear several to many highly successful broods (Bruce, 2008). Iteroparous species may exhibit smaller-scale broadcast spawning, oviparity, and viviparity.

Viviparity is a taxonomically-widespread phenomenon that has evolved independently many times within distantly related groups such as plants (Elmqvist & Cox, 1996), insects (Meier et al., 1999), gastropods (Kohler et al., 2004), echinoderms (Hart et al., 1997), and virtually all classes of vertebrates except birds. The phenomenon first arose among vertebrates within Teleostei (ray-finned fishes) over 500 million years ago, when evolutionary pressures began to favor changes in the physiology of fish egg production, from displaying external to internal fertilization (Wourms, 1981). The perfection of internal fertilization and a decrease in egg abundance encouraged the absorption of nutrients by the yolk sac, allowing intrauterine care of developing embryos to reach advanced developmental stages. All 54 families of fishes host members that bear living young; within Teleosts, an estimated 510 of 18,000 species are viviparous (Wourms, 1981).

Introducing *Poeciliidae*: The "Livebearers". The *Poeciliidae* is a family of small-bodied, Neotropical, surface-dwelling fishes found on every continent, hold Antarctica. As it appeals to evolutionary biologists, it is one of the most extensively studied fish families. Representatives of *Poeciliidae* include species of guppy (*Poecilia reticulata*), mollies (*Mollienesia spp.*), swordtails (*Xiphophorus spp.*), and the revered mosquitofish. Short generation times, ease of mass-culture, and the remarkable diversity of reproductive adaptations within the family, add to their appeal from an evolutionary biology perspective. Reproductive adaptations of *Poeciliidae* include internal fertilization, viviparity, oviparity, lecithotrophy, matrotrophy, and superfetation (Pollux et al., 2009). Oviparity and viviparity are relatively rare in all the fishes, most species display external fertilization. Oviparity and viviparity fall within the classification of iteroparous reproduction, because gravid females exercise maternal care to rear fewer, but more well-developed viable young over several breeding cycles. *Poeciliidae* displays a remarkable array of variation within reproductive adaptations, and therefore fertility (Pollux et al., 2009). All but one species of poeciliids are viviparous, with brood sizes ranging from several to 315 developing young at a time (Wourms, 1981). The production of fewer young born in an advanced stage of development shows that poeciliids exercise quality over quantity, a result of evolutionary processes driven by environment, ecological interactions, and reproductive fitness. Within the family, modifications of reproductive strategies have encouraged variations in fertility to lengthen. Ova size and number, the advancement of placental development and the extent of nutritional supplement from mother to embryo, degrees of superfetation, and variations in gestation lengths and brood intervals all contribute to the variation of reproductive capability and success within *Poeciliidae* (Thibault & Schultz, 1977). Characteristic of oviparous poikilothermic vertebrates (reptiles & amphibians), larger females typically produce more eggs and offspring, thus adding to the variation of fertility within the many species of the family (Thibault & Schultz, 1977).

Maternal Provisioning Within *Poeciliidae*. Within viviparous species of *Poeciliidae*, patterns of maternal provisioning and embryonic development range from strict lecithotrophy, to profound levels of matrotrophy. Variations in such developmental tactics arose from the independent evolution of varying placental forms and functions within the family. The placenta, being a complex vascularized organ, mediates physiological interactions between the mother and the developing embryos. Within *Poeciliidae*, placentas range from being non-existent to highly developed and capable of allowing advanced maternal contributions to developing embryos (Pollux et al., 2009). While lineages within *Poeciliidae* have evolved placentas that are structurally different, they all fulfill a similar function of facilitating mother-to-embryo contributions and are derived from the same tissues (Schindler & Hamlett, 1993).

Lecithotrophy is the more primitive mode of viviparity, in which all nutritional resources are loaded into eggs prior to fertilization. No additional resources are partitioned from mother to embryo following fertilization. The embryos of lecithotrophic species are associated with modestly-sized yolks (> ~2mm), containing sufficient reserves of nutrients to achieve complete embryonic development without additional maternal care (Thibault & Schultz, 1977). Throughout development, embryonic weight decreases as some metabolic energy is lost as heat and, lacking a placenta, maternal contributions are not made after fertilization. *Poecilia reticulata* (the “guppy”), a close relative of the eastern mosquitofish, appears to be a lecithotroph, undergoing a net loss of 25% of embryonic weight during development (Depêche, 1976). Starkly contrasting this strategy of development, very few members of the family that are considered “specialized matrotrophs” contribute significant maternal care during embryonic development. Yolk reserves are initially small and inadequate, requiring extra contributions of maternal nutrients to achieve proper growth. *Poeciliopsis turneri* (blackspotted livebearer) embryos display a weight gain of up to 1,840% during development by utilizing advanced follicular pseudoplacentas (Thibault & Shultz, 1978). Within specialized matrotrophs, placentas are highly vascularized and host more follicles (small secretory glands) through which maternal contributions are transferred to the developing embryo's amnion that provides a direct linkage to the pericardial sac (fibrous tissues surrounding the developing embryo's heart) (Pollux et al., 2009). The phenomenon of superfetation is maximized in species displaying specialized matrotrophy. By reducing the surge of nutrients required by a single egg loading period, more frequent and numerous oogenesis periods are metabolically allowed; so much so that broods of different developmental stages are able to gestate at the same time. Species practicing specialized matrotrophy must rely upon steady nutritional resources to supply the enduring requirements of mother-embryo nutrition transfers (Reznik et al., 1996). Species practicing strict lecithotrophy are typically found within more dynamic, less nutritionally-reliable habitats.

Most species of *Poeciliidae* display unspecialized matrotrophy, a healthy medium to the two previously-stated tactics of reproduction (Wourms, 1981). As opposed to a loss or gain of mass, the weight of embryos remains relatively constant from fertilization to birth by utilizing nutritious maternal contributions in later stages of embryonic development, when yolk contents begin to deplete. *Gambusia* falls within this classification. The utilization of modestly-sized eggs in unspecialized matrotrophic species discourages superfetation, limiting the phenomenon to individuals with the largest body sizes, presumably because they are capable of meeting the metabolic demands associated with skipping brood intervals. Matrotrophy has been identified in many poeciliids, including *Gambusia*, by tracing the addition of identifiable amino acids from mother to embryo (Marsh-Metthews et al., 2001). However, for most species, including *Gambusia holbrooki*, the extent of matrotrophy has not been quantified.

Therefore, a complete understanding or definition of most poeciliid reproduction is still lacking.

Describing the Eastern Mosquitofish

Gambusia holbrooki (eastern mosquitofish) is a viviparous and voracious surface-dwelling guppy, native to the southeastern United States, with a significant global presence. By many counts, *G. holbrooki* is the most abundant freshwater fish on Earth (MacDonald et al., 2012). Its prominence as a popular tank species for hobbyists has resulted in innumerous introduction events over space and time. To some, the species is considered invasive and ecologically disastrous (Pyke, 2008). To others, it is vital agent for preserving public health (Chandra et al., 2008). While being highly competitive, *G. holbrooki*'s niche is provided by most freshwater habitats and its presence is therefore global. The natural range of *G. holbrooki* is limited to the eastern coast of the United States, east of the Appalachian Mountains. Today, the species distribution is widespread across the United States and there are established populations on all continents, hold Antarctica (MacDonald et al., 2012). The present distribution of *G. holbrooki* comprises low-elevation, warm areas with permanent bodies of freshwater. Globally, established mosquitofish populations are located generally between 40° N and 38° S, in areas where temperatures maintain daily averages of at least 20° C (Pyke, 2005; Otto, 1973). The fish is small and silver in color and is highly resistant to poor water qualities that would quickly exclude most other freshwater fish. With its upwards-facing mouth and the ability to exercise rapid vertical dashes, *G. holbrooki* is perfectly adapted for feeding on smaller surface-dwelling prey (Bisazza & Marin, 1995). By advantageously utilizing counter shading, the species exercises predation through ambush-style tactics at the surface. They are aggressive in nature, and by utilizing counter shading, the species is often able to effectively avoid capture by larger fish displaying active predation tactics (Miller, 2015). Mosquitofish are voracious predators of the epilimnion, the most surficial layer of water. Being so highly tolerant to poor water qualities, the *G. holbrooki* is able to out-compete most other small-bodied epilimnetic species (Pyke, 2008).

When numerous mosquito larvae are present, the mosquitofish feeds almost exclusively upon them. The application of *G. holbrooki* presents an inexpensive, safe option for biological control of mosquitoes in Florida and much of the eastern United States (Bence, 1988; Hoy et al., 1971). However, their usage in the States is restricted by the United States Fish & Wildlife Service to areas where they are native. For example, *G. holbrooki* cannot be applied as a biological control in Colorado, where delicate populations of endangered fish species could be negatively impacted by the introduction (Tyus & Saunders, 2000).

Habitat Use. *G. holbrooki* establish populations within slow-moving and stagnant bodies of water with dense aquatic vegetation (Bisazza & Marin, 1995). The fish prefer shallow waters with vegetation to avoid predation from larger fish. In deeper bodies of water, *G. holbrooki* are found along the shallower areas near the banks, where the warmer habitat is safer and provides favorable surface-dwelling food sources. *Gambusia* are extremely resilient to varying water quality, which has facilitated their widespread distribution. The species has been documented in waters with temperatures ranging from 0 – 40°C, salinities of 0 – 41 ppt, pH’s of 4.5 – 9.0, dissolved oxygen of 1 – 11 mg/L, and turbidities ranging 3 – 275 JTU (Alcaraz et al., 2008). The species has even been observed thriving under thin layers of ice for brief periods of time (Otto, 1973).

Importantly, *G. holbrooki* populations can persist in stagnant bodies of water with anoxic conditions, which are ideal oviposition sites for gravid mosquitoes. *G. holbrooki* is able to utilize an upwards-facing mouth to gulp air from the surface-water interface, allowing it to thrive in predator-free waters where all other fish are excluded. From undisturbed swamps, to highly urbanized areas, mosquitofish can thrive in standing bodies of water, even those with low dissolved oxygen and high amounts of pollution (like urban ditches). Mosquitofish have also shown resistance to toxic ingredients found within herbicides, insecticides, and industrial pollutants found in highly urbanized regions (Chambers & Yarbrough, 1979). All of these factors make mosquitofish ideal for curbing adult mosquito numbers by consuming larval populations; they thrive in water bodies that mosquitoes choose to oviposit on.

Reproduction. Mosquitofish are viviparous; fertilization occurs internally, and the mother gives birth to free-swimming young. This evolutionary adaptation is relatively rare in fish and has helped to facilitate broad range expansion of the species, as live young are more resilient to poor water quality than developing eggs. Mosquitofish display an obvious sexual dimorphism, with females being considerably larger than males. Males display pronounced gonopodium, which are utilized to help deposit sperm within the vent of females. Females display rounded anal fins. From birth, both male and female *G. holbrooki* develop at a rate of ~1-2 mm/week until sexual maturity is reached (Bisazza & Marin, 1995). Maturation in females takes slightly longer, as they must grow to be larger than males. Warmer habitats facilitate more rapid reproduction and developmental cycles within the species (Vondracek, 1988, Bisazza & Marin, 1995).

G. holbrooki reproduction occurs within warmer months, when temperatures facilitate the metabolic activity required of females to load eggs with nutrient-rich/costly yolk products. The breeding season is therefore abbreviated in higher latitudes with more variable climates. Where temperatures allow it, *G. holbrooki* reproduction occurs yearlong. Spermatogenesis in males is correlated with warmer seasonal water temperatures, but not necessarily changing photoperiods. Oogenesis in females mirrors this time period (Vondracek, 1988).

Females invest heavily in reproduction, with gonads comprising up to 25% of the total body weight. Males do not invest as heavily, with gonadal weight rarely reaching 4% of total weight within mature individuals (Geiser, 1924). Superfetation is not common among *G. holbrooki*, though females are able to store, maintain, and utilize active sperm within specialized oviducts throughout the breeding season (Pires et al., 2010). Unfertilized eggs are modestly-sized (~2 mm diameter) and costly to produce, discouraging superfetation except in the largest individuals. Females may have several broods over the course of a breeding season, with older/larger females typically completing more cycles annually (Snelson et al., 1986). The gestation period typically occurs within 15–22 days, though higher/lower water temperatures can cause slight variations in this cycle (Bisazza & Marin, 1995). After birth, females typically take less than a week to re-fertilize, as unfertilized eggs are produced and made available during pregnancy (Pollux et al., 2009). Over the course of a six to seven-month breeding cycle, a large female may be able to produce upwards of nine broods. In areas with more warm months (like Florida), more broods may occur per season.

Water Quality and Mosquitofish Health.

All aquatic and marine species share an intimate relationship with their environment, especially fish. Relative to all other sea life, fish are very active and mobile; their metabolism is high. Water is moved through a fish's body at all times, and strongly influences its ability to maintain healthy internal processes. Water quality parameter deviations from normal (healthy) levels may influence a fish's behavior and ability to function.

Temperature. Fish are poikilothermic animals; their body temperature is regulated by the temperature of the water they occupy. Poikilothermic organisms are able to withstand greater temperature fluctuations than homeothermic organisms, but varying temperatures strongly influences metabolic activity. Fish are able to withstand seasonal changes in temperature, while abrupt changes may result in instant shock and/or death. It is a pervasive concept that warmer environmental conditions result in an increase in metabolic rate; mosquitofish adhere to this rule.

Acidity (pH). The optimal pH range for fish is 6.5 to 8.5, a fairly neutral acidity range. Deviations too far from this range in either direction may have detrimental effects on fish health. Sudden fluctuations in pH are often lethal to fish but do not happen naturally. At the seasonal level, water acidity typically increases in the spring with the introduction of snow melt into lakes and streams. At this temporal scale of fluctuation, fish can adapt to changes in water acidity, but only to a certain extent. Strong acids and bases act to impede the progress of enzyme-catalyzed reactions within the fish's body systems that are vital for maintaining a healthy homeostasis. Enzyme-catalyzed reactions depend upon specific proteins being able to function with associated substrate molecules.

Any environmental change that acts to denature or change the shape of proteins will inhibit the reactions. *G. holbrooki* is resilient against water acidity and alkalinity. It is likely that the species is able to utilize a thick layer of mucus within the operculum and on the gills to buffer against acidic/alkaline water, but a definitive explanation is thus far non-existent (Gupta & Jawale, 2013). Water acidity has a significant effect on the toxic action of other substances within the water such as heavy metals, ammonia, and naturally-occurring cyanides; meaning, deviations from normal pH may increase the potency of other pollutants within the water that often have detrimental effects on any organism present.

Dissolved Oxygen. Atmospheric oxygen diffuses into lakes, streams, and rivers in areas where water is turbulent. Additionally, oxygen is produced via the photosynthetic activity of aquatic vegetation. Oxygen is used in the degradation of organic matter by bacteria and by the respiration of all organisms present within the water. This creates a delicate supply and demand dynamic at play within any body of water. Eutrophication is detrimental to most fish species because the end result of the process creates anoxic conditions; there is too much biological demand for the supply of oxygen. Without ample levels of oxygen, most fish species are not able to properly respire and fuel the body processes that require oxygenation of blood. Fish deficient of oxygen become torpid. At first, they do not take food or respond properly to stimuli. Ultimately, oxygen-deficient water causes asphyxiation and fish lose their ability to avoid capture; they die. However, mosquitofish are resilient to low levels of oxygen within waterbodies due to their surface-dwelling nature. As masters of the topwater, mosquitofish are able to gulp atmospheric oxygen by breaching the surface. This has been observed in Florida springs with oxygen levels between 0.1 – 0.3 mg/L and in laboratory environments. When surface breaches are prevented, *G. holbrooki* in low-oxygen environments perish within 20 minutes (Cech et al., 1985).

Total Dissolved Solids. Total Dissolved Solids (TDS) refers to minerals, salts, and metals dissolved within the water column. In Florida waters, tannins released from decaying plant matter strongly influence water clarity and contribute to TDS. Turbidity is a measure of water clarity and can strongly influence the ecological function and behavior of fishes within a waterbody. For example, in waters of low-clarity (high turbidity), juvenile largemouth bass (*Micropterus salmoides*) will more frequently display ambush predation tactics upon adult *G. holbrooki*. In turn, *adult G. holbrooki* demonstrate varying evasion tactics to changing predation techniques (Miller, 2015). In darker colored waters *G. holbrooki* behave more erratically and likely expend more energy by darting laterally about the surface of the water to avoid predation. Turbid water, therefore, may increase the metabolic demand of *G. holbrooki* as compared to waters of improved clarity.

Man’s Worst Enemy is the Mosquito

Globally, an estimated 700 million people are affected by mosquito-borne diseases each year. Of these, one million die from a handful of lethal arboviruses (Caraballo & King, 2014). Additional millions are asymptomatic and unharmed. Unfortunately, many mosquito species participate in a harmful, intimate, relationship with human populations. Mosquitoes thrive in areas where humans are present for several reasons. Humans alter environments in ways that favor mosquito populations by inhibiting the natural drainage processes of landscapes and by providing favorable harborage habitats such as cool, damp areas around buildings. Secondly, humans and livestock are an ideal source of blood meals. Gravid mosquitoes require blood meals to meet the protein requirements for producing eggs. It is during these interactions between humans and mosquitos that arboviruses are transmitted.

Mosquitoes are an aquatic insect. By hindering a landscape's natural drainage capabilities, human habitats encourage the growth of mosquito populations. Ditches, agricultural operations, home gutters, neglected swimming pools, or any object that can hold a teaspoon of water for over 10 days, poses a possible breeding habitat for mosquitoes. As a result of human development, the number of areas in which mosquitoes can reproduce have increased dramatically. Developing nations are at exceptional risk to mosquito-borne illness, as governments have neither the means, nor the motivation, to protect and educate citizens from mosquito-borne illnesses.

Mosquito Biology: A Chink in the Armor. Mosquitoes spend a significant portion of their life cycle in lakes, ponds, and puddles. Two distinct classifications of mosquito exist; flood-borne and raft-laying species. The two groups differ in their ovipositional behavior. Flood-borne mosquitoes oviposit on dry soil, or in areas that will eventually be inundated by water. Upon contact with water, the eggs hatch and initiate the mosquito life cycle. Raft-laying species oviposit their clutch directly onto the water's surface, where the eggs develop into larvae. Once the eggs are in an aquatic environment, the life cycle of the two classifications is identical (Bentley & Day, 1989).

Mosquitoes spend between nine to 13 days developing from egg to adult in an aquatic environment. Within two days of oviposition, the eggs hatch, releasing instar-I larvae. Over the course of a week to 11 days, the larvae complete four instars, growing with each molt. Mosquito larvae reside at the water-surface interface, utilizing trumpet-shaped spiracles and surface tension to supply themselves with atmospheric oxygen. When stimulated by a predator or a disturbance in the surface tension, the larvae sink themselves into the water column in an attempt to evade potential predation.

Mosquito larvae actively feed upon decaying plant and organic matter, and therefore thrive in stagnant, dirty bodies of water. The instar-IV larvae pupates after about a week, entering a stage of rapid development during which the larvae do not consume food. After two days of pupation, an adult will emerge from the water and disperse. Emerging female mosquitoes are ready to mate immediately and survive for about two weeks. During their lifespan, mosquitoes can produce up to six individual clutches of ~100 eggs per clutch. The entire life cycle of the mosquito typically takes place within 30 days. Life cycles are typically more rapid in warmer temperatures (Bentley & Day, 1989).

Individual clutches hatch within the same body of water at the same time. If areas where mosquito breeding is occurring are identified, there are a number of techniques that can be applied to kill the developing larvae, or pupae. The identification of, and regular, repeated monitoring of breeding habitats is imperative for practicing effective mosquito control. The logic is that it is easier to curb populations when all of the individuals are in one spot (ex: a stagnant lake or a neglected pool), rather than when they become airborne and disperse. Greater effort is required to exterminate adult mosquito populations than is needed to kill developing larvae. This concept is central to Integrated Pest Management: use an organism’s biology against it to reduce control efforts.

Biological Control: A Cheap and Effective Alternative?

There are four modes of biological control, each uses a different classification of organism to reduce population sizes of pests: (1) parasitoids act by killing, or permanently immobilizing, pests in the larval stage, using pests as hosts to rear offspring and killing the pest in the process. Adult forms of the parasitoid are innocuous to non-target species, and act to continue the cycle; (2) pathogens are introduced to pest populations to intentionally plague them with disease, causing populations to dwindle and die; (3) competitors are used to lower pest populations by reducing the amount of available space and resources to the target species; and (4) this research focuses on one species that participates in the fourth method of biological control, the introduction of predators into the ecological habitats of pest species. Predators are highly-mobile species (relative to the pest) that consume a large number of prey in their lifespan. It is important that significant ecological consideration be exercised before the implementation of predatory-biological control. When the wrong predators are introduced, they may exceed carrying capacity as controls take place, thus causing their populations to decline after implementation (Christophers, 1960). Imported predators can be safely incorporated into the ecology of an area, but their introduction requires a significant amount of thought and consideration. Pest control can be achieved through the seasons without additional introductions, making this approach cost effective. The mosquitofish is of particular biocontrol interest globally because they are voracious predators of mosquito larvae, resilient to poor water quality, and can be applied most anywhere.

Mosquitofish as Biological Control. As agents of public health, mosquitofish (*G. holbrooki* & *G. affinis*) have shown repeatedly to be effective biocontrollers against mosquitoes, the vectors of deadly arboviruses worldwide. Trials of mosquitofish applications outside of their native ranges for larval control of mosquitoes showed promising results in Europe (Hackett, 1937), India (Menon & Rajagopalan, 1978; Das & Prasad; 1991; Chatterjee & Chandra, 1997), Afghanistan (Rafatjah & Arata, 1975), and the western United States (Tabibzadeh et al., 1971). In the presence of introduced mosquitofish, larval populations display significant reductions in artificial habitats (wells, culverts, ditches, and flooded agricultural fields), as well as in natural ones by up to 96% (Chandra et al., 2008; Rao et al., 1982). When applied to flooded rice fields in densities of 46 fish per square meter, mosquitofish sharply declined larval populations and vector bite counts among nearby local farming communities (Tabibzadeh et al., 1971). Studies that find no significant reductions in larval mosquito populations in the presence of introduced mosquitofish involve low-density stocking (~2 lb/acre) and are often cited by those opposed to the introduction of exotic species as agents of biocontrol (Pyke, 2008; Hoy et al., 1972). As a mode of inexpensive, effective, and long-term mosquito control, the introduction of *Gambusia spp.* to at-risk waterbodies is a pest control/public health tactic that is relied upon globally. This research intends to inform the public of trends in fertility within eastern mosquitofish, with the hopes of increasing the efficacy of pest control operations worldwide that are cost-limited, by identifying locations and water quality characteristics related to more fecund mosquitofish.

Hypotheses

Fertility and Water Quality. I predict that *G. holbrooki* are more fecund in waters of warmer temperatures, such as ditches or other shallow bodies of water. I predict that pH levels deviating from the norm (6.5 – 8.5) result in lower maternal investment and fertility within adult *G. holbrooki*. I predict that low dissolved oxygen content within waterbodies do not affect *G. holbrooki* fertility, or that maternal investment will not be correlated with dissolved oxygen levels; I predict this because mosquitofish utilize atmospheric oxygen from the air-water interface when dissolved oxygen levels are low (Cech et al., 1985). I predict that regional and habitat differences in mosquitofish fertility are minimal. Overall, I predict that water quality parameters are relatively weak predictors of mosquitofish fertility because of the fish's resilient nature towards poor water quality.

Fertility and Fish Characteristics. I predict that individual fish characteristics (length/weight) are the most significant predictors of mosquitofish fertility. Specifically, I predict there is a positively correlated relationship between fish weight and fertility. I predict there is a positive relationship between fish length and fertility, but that fish weight is the more powerful predictor.

Chapter Two: Methods and Materials

Sampling Sites and Habitat Classification

Gravid female eastern mosquitofish were collected from 32 locations and divided into four habitat classifications within two regions in Florida (Fig. 1).

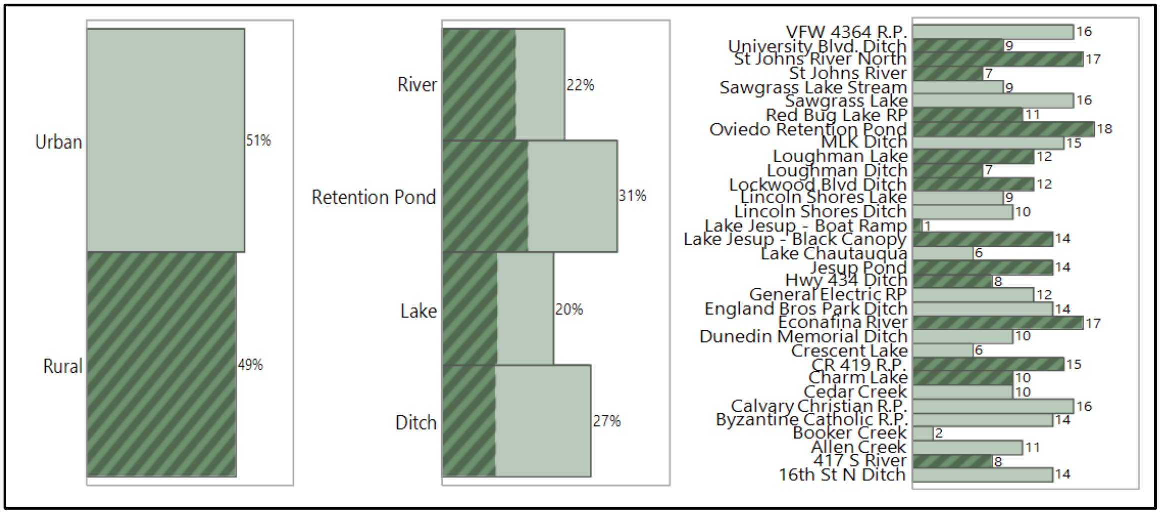

Figure 1: Frequency distribution of data by region, habitat type, and site location. Rural sampling sites are denoted by diagonal lines.

Region and Habitat Designation. Sampling occurred within two regions in the state of Florida: highly urbanized Pinellas County, and a more rural area northeast of Orlando. Within both regions, samples were taken from artificial and natural habitats. Artificial habitats consisted of non-permanent waterbodies, namely ditches and retention ponds. Ditches and retention ponds are more susceptible to fluctuating water level/quality and are therefore classified as non-permanent. Natural habitat, deemed permanent, consisted of lakes and rivers.

Sampling Locations. Sampling occurred within 32 bodies of water within the State of Florida. Eight permanent sites (four lakes/four rivers) and eight non-permanent sites (retention pond/ditch) were sampled from within Pinellas County (highly urbanized). The same occurred east of Orlando (rural), summing a total of 32 sampling sites. Sampling sites all hosted established populations of *G. holbrooki* that may have occurred naturally or have been stocked by local mosquito control districts. Sampling occurred on public and private land at the permission of the landowner or manager, and not in any state parks or wildlife reserves. Water body habitat was classified as permanent/non-permanent and delineated as either natural or artificial. The four classifications included natural lakes and rivers, artificial ditches and retention ponds.

Water Quality and Environmental Measurements

Using a handheld meter (ExTech EC 500 ExStick II), water quality measurements were taken in three separate locations at each sampling site and averaged to give an overall description of water quality characteristics. Water quality parameters included temperature (°C), pH, salinity (ppm), total dissolved solids (ppm), conductivity (microsiemens [μS]), and dissolved oxygen content (mg/L). Dissolved oxygen was measured using a Hach® dissolved oxygen test kit (Model OX-2P).

Mosquitofish Sampling and Collection

Gravid females were collected from each site and immediately sacrificed in 95% ethanol (AMVA 2013, S6.2.1(3)). Females were collected from three randomized locations at each site using a dip-net technique that involved baiting. The net was constructed of a fine-mesh, as to avoid sampling bias towards large individuals. All baited fish were retained within the net, unless the fish successfully evaded the net completely. Bait was introduced to the water surface to attract female eastern mosquitofish. On top of their intrinsically voracious nature, requiring protein to supply their maternal investment drive, female mosquitofish are irresistibly attracted to bait in the water. Mosquitofish were dip-netted out of the water, and gravid females were collected from the net. Gravid females are easily identifiable by a gravid black spot along the sides of their bodies, which shows an enlarging uterine wall. Live mosquitofish measurements of length (snout to tail in cm) and weight (grams) were taken on site before sacrifice. The collected fish were stored and labelled individually in ethanol for observations on maternal investment at a later point.

Observing Fertility and Maternal Investment

The uterine contents of each gravid female were observed by counting, weighing, and classifying the clutches by developmental stage in a laboratory environment. The heads of preserved individuals were removed and the fish's abdomen was opened on the ventral side from the anal pore to just below the operculum. Uterine contents were then easily removed from the fish's abdominal cavity. The entire uterine contents were weighed and compared to the female's total weight to indicate maternal investment. Clutch size was recorded as the total number of developing embryos and was used as the primary indicator of fertility.

The developing embryos were classified into four developmental stages (Fig. 2). I arbitrarily defined the four developmental stages, as there is no previous research that has done so.

Figure 2: Photographic descriptions of embryonic development stages one through four.

1. No Development: No eye pigment formed within embryo. Recent/no fertilization.
2. Slight Development: Eye pigment formed, yolk still substantial and not depleted.
3: Moderate Development: Fish body formed, some depletion of yolk material.
4. Advanced Development: Fish body nearly complete, yolk material all/mostly depleted.

Data Analysis and Dissemination

Multifactored fit models were utilized to determine relationships between fish characteristics, fertility, and environmental conditions. Because individuals were preserved before maternal characteristics were observed, I used the preserved weight of females rather than live weight when conducting multifactored fit models. Live female weight was significantly correlated with preserved female weight (Fig. 3; Regression: $R^2 = 0.95$, $p < 0.0001$). Wilcoxon signed-rank non-parametric testing was conducted to determine statistically significant differences between fish characteristics, fertility, and the not-normally distributed environmental variables (Fig. 4).

Stepwise regression fitness models of all measured variables were conducted to identify the most powerful predictors of the observed variation within fish characteristics and fertility. At each step, non-statistically significant predictors of fish characteristics and fertility within the model were removed to allow the quantification of observed variation from the significant predictors.

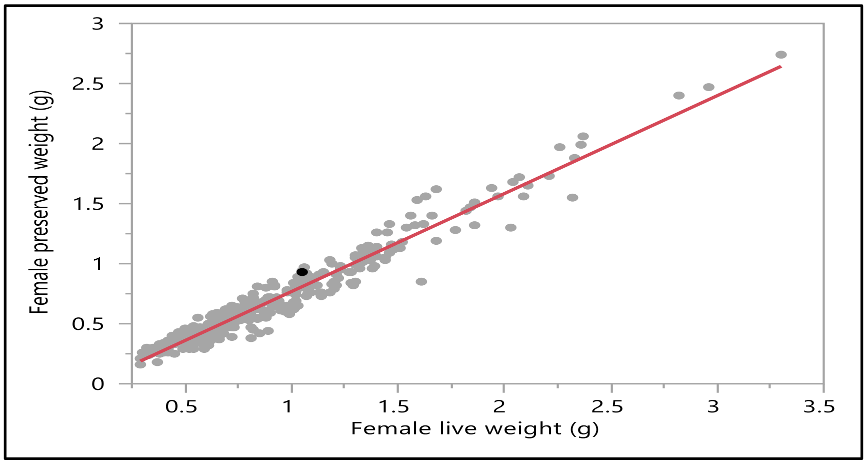

Figure 3: Female preserved weight by female live weight.

Chapter Three: Results

Descriptive Statistics

While live female length was evenly distributed, preserved female weight, clutch size, and preserved female clutch weight was significantly skewed. Of the variation in preserved female weight, 38% was explained by variation in live female length (Linear regression: $R^2 = 0.376$, $p < 0.0001$). The average gravid female mosquitofish weighed 0.903 grams and was 4.08 centimeters long, with 9.46 developing embryos. The largest mosquitofish weighed 3.3 grams and was 5.7 centimeters long, with 25 developing embryos. The most fecund mosquitofish's clutch size was 41; it displayed superfetation (n = 9) and was 4.7 centimeters long and weighed 2.96 grams.

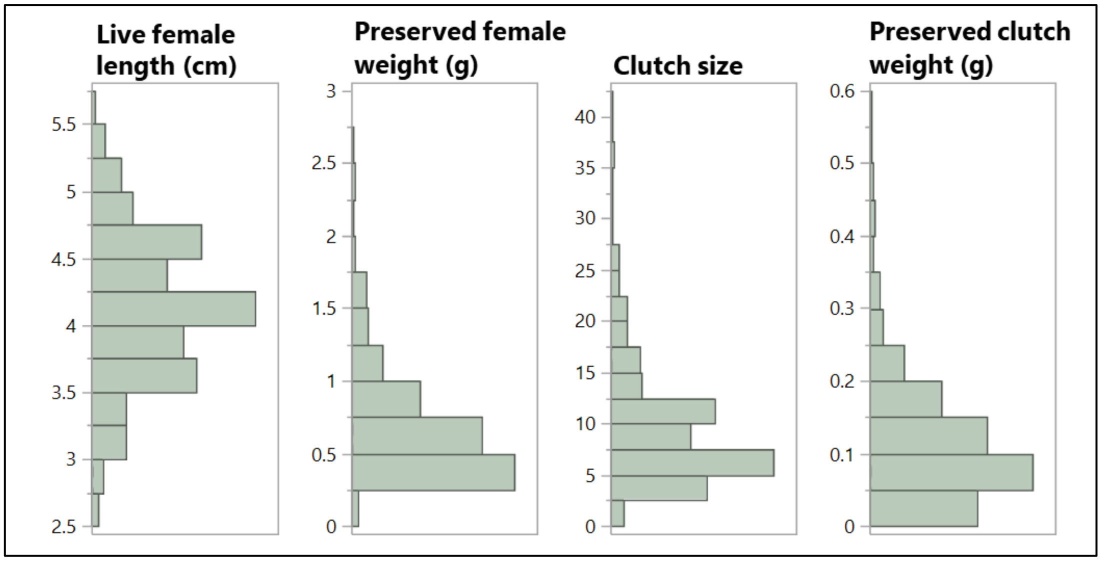

Figure 4: Distributions of live female length, preserved female weight, clutch size, and preserved clutch weight.

Fish Fertility Variables and Environmental Classification

Female Length. Live female length differed significantly by habitat (Fig. 5a; Multifactor model: $R^2 = 0.06$, $p = 0.0036$), region (Fig. 5b; $R^2 = 0.02$, $p < 0.0001$), and date (Fig. 5c; $R^2 = 0.13$, p = < 0.0001). Mosquitofish were significantly longer in riverine habitats and retention ponds than in ditches and lakes. Female mosquitofish were significantly longer in the rural region (East Orlando) than in the urban region (Pinellas County). The fish were also shorter in length at earlier sampling dates. Longer female mosquitofish were found in rural lakes and retention ponds at later sampling dates.

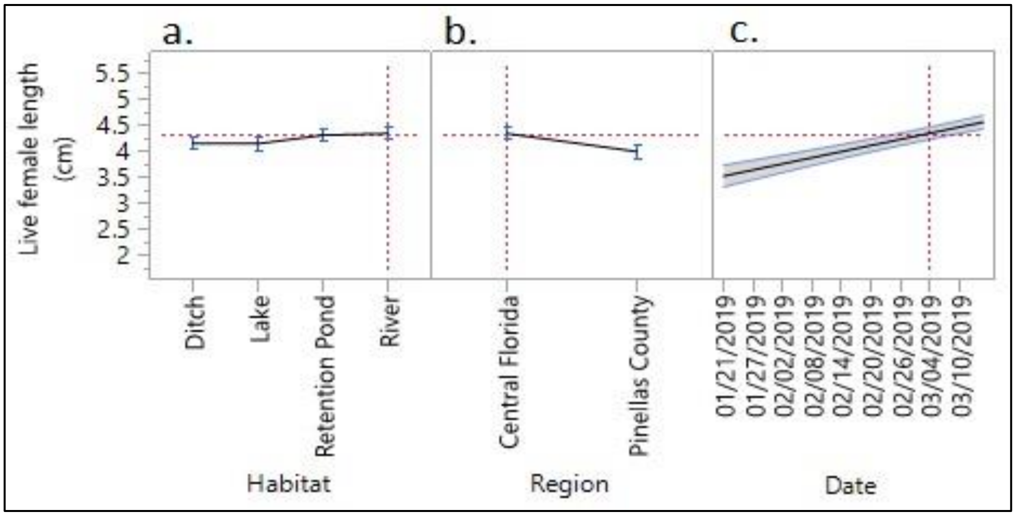

Figure 5: Live female length by habitat, region, and date of collection.

Preserved Female Weight. Preserved female weight differed significantly by habitat (Fig. 6a; Multifactor model: $R^2 = 0.02$, $p = 0.038$) and region (Figure 6b; $R^2 = 0.06$, $p < 0.0001$), but not by sample date (Figure 6c; $R^2 = 0.01$, $p = 0.515$). Female mosquitofish weighed significantly more in ditches, retention ponds, and rivers than in lakes. Female mosquitofish weighed significantly more in the rural region (East Orlando) than in the urban region (Pinellas County). Date did not significantly influence fish weight. The heaviest mosquitofish were found in rural ditches, retention ponds, and rivers.

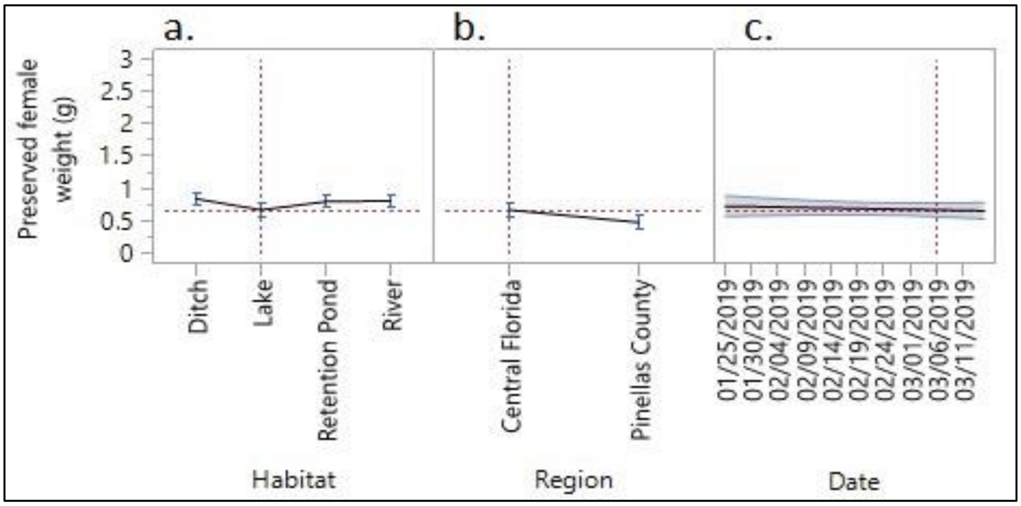

Figure 6: Preserved female weight by habitat, region, and date of collection.

Clutch Size. Clutch size differed significantly by habitat (Fig. 7a; Multifactor model: $R^2 = 0.03$, $p = 0.006$) and date (Fig. 7c; $R^2 = 0.05$, $p = 0.0001$), but not by region (Fig. 7b; $R^2 = 0.02$, $p = 0.771$). Female mosquitofish clutch sizes were the largest in rivers and smallest in lakes. Clutch size decreased significantly as the study progressed from January to March. Region did not significantly influence clutch sizes within female mosquitofish. Riverine habitats at earlier dates hosted the most fertile mosquitofish.

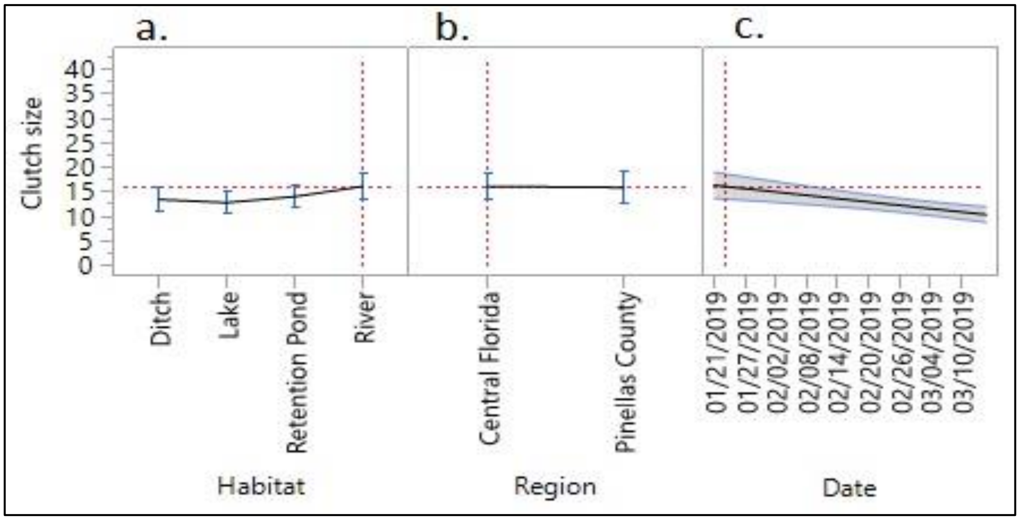

Figure 7: Clutch size by habitat, region, and date of collection.

Preserved Clutch Weight. Preserved clutch weight differed significantly by habitat (Fig. 8a; Multifactor model: $R^2 = 0.04$, $p = 0.002$) and date (Fig. 8c; $R^2 = 0.02$, $p = 0.019$), but not by region (Fig. 8b; $R^2 = 0.02$, $p = 0.436$). Preserved clutches from riverine habitats weighed significantly more than clutches from ditches, retention ponds, and lakes. Preserved clutch weight significantly decreased as the study progressed from January to March. Region did not significantly influence preserved clutch weight. With region not influencing preserved clutch weight, riverine habitats at later dates hosted female mosquitofish with the heaviest clutches.

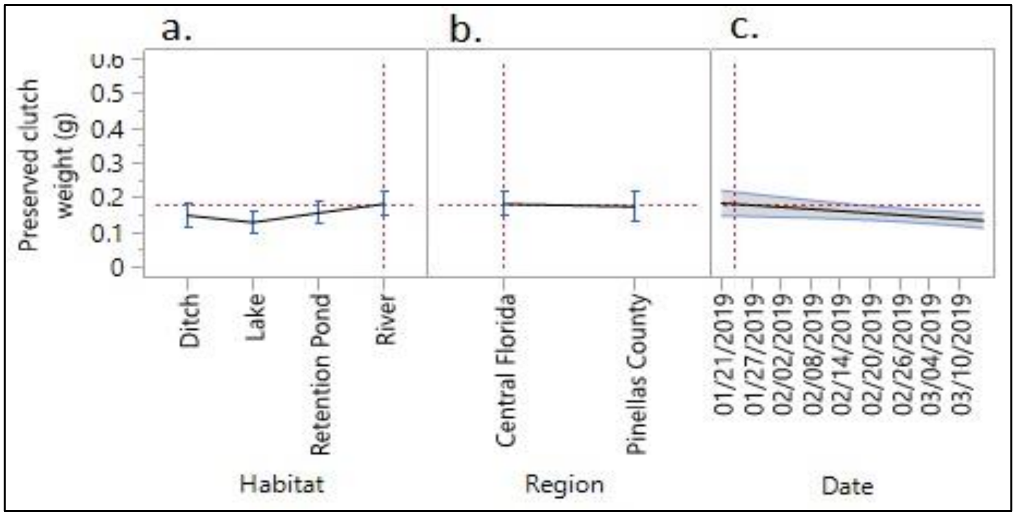

Figure 8: Preserved clutch weight by habitat, region, and date of collection.

Fish Fertility Variables and Water Quality Parameters

Female Length. Live length of female mosquitofish differed significantly by temperature (Fig. 9a; Multifactor model: $R^2 = 0.01$, $p = 0.006$), pH (Fig. 9b; $R^2 = 0.004$, $p = 0.003$), salinity (Fig. 9c; $R^2 = 0.01$, $p = 0.006$), conductivity (Fig. 9e; $R^2 = 0.01$, $p = 0.003$), and dissolved oxygen (Fig. 9f; $R^2 = 0.02$, $p = 0.003$), but not by total dissolved solids (Fig. 9d; $R^2 = 0.01$, $p = 0.279$). Female mosquitofish length increased significantly with rising temperatures, salinity, and dissolved oxygen levels. However, fish length decreased significantly with increasing alkalinity and conductivity levels. The amount of total dissolved solids present did not significantly influence female mosquitofish length.

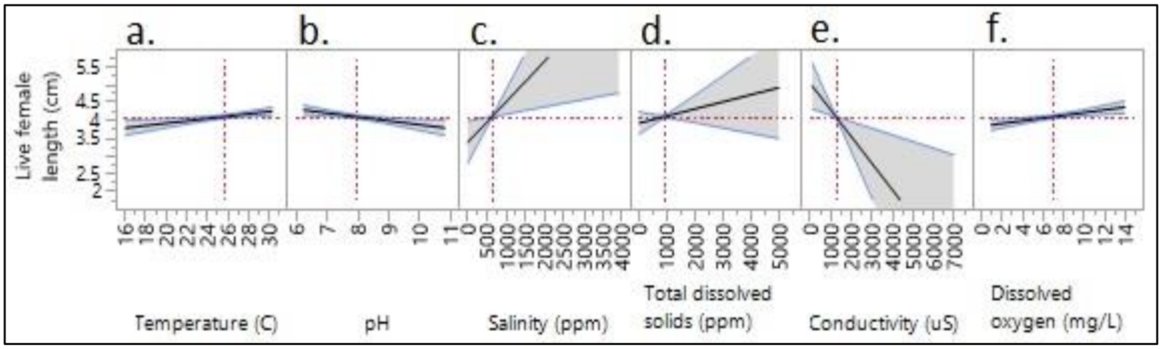

Figure 9: Live female length by temperature, pH, salinity, total dissolved solids, conductivity, and dissolved oxygen.

Preserved Female Weight. The weight of preserved female mosquitofish differed significantly by pH (Fig. 10a; Multifactor model: $R^2 = 0.03$, $p < 0.0001$), salinity (Fig. 10c; $R^2 = 0.009$, $p = 0.002$), conductivity (Fig. 10e; $R^2 = 0.01$, $p = 0.009$), and dissolved oxygen (Fig. 10f; $R^2 = 0.001$, $p = 0.009$), but not by temperature (Fig. 10a; $R^2 = 0.01$, $p = 0.289$) or total dissolved solids (Fig. 10d; $R^2 = 0.01$, $p = 0.376$). Preserved female mosquitofish weight increased significantly with rising salinity and dissolved oxygen concentration, while a significant negative correlation was displayed for alkalinity and conductivity. Temperature and the concentration of total dissolved solids did not significantly influence the weight of preserved female mosquitofish

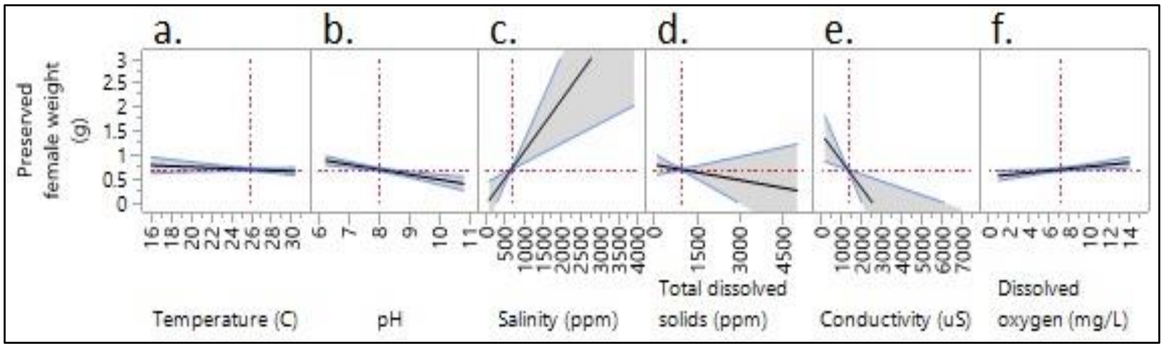

Figure 10: Preserved female weight by temperature, pH, salinity, total dissolved solids, conductivity, and dissolved oxygen.

Clutch Size. The clutch size of female mosquitofish differed significantly with temperature (Fig. 11a; Multifactor model: $R^2 = 0.05$, $p = 0.001$), pH (Fig. 11b; $R^2 = 0.04$, $p = 0.002$), salinity (Fig. 11c; $R^2 = 0.006$. $p = 0.001$), and conductivity (Fig. 11e; $R^2 = 0.007$, $p = 0.0007$), but not total dissolved solids (Fig. 11d; $R^2 = 0.006$, $p = 0.498$) or dissolved oxygen (Fig. 11f; $R^2 = 0.005$, $p = 0.249$). Clutch sizes increased significantly with rising salinity, while a significant negative correlation was displayed for temperature, pH, and conductivity. The concentration of total dissolved solids and dissolved oxygen did not significantly influence the clutch sizes of gravid female mosquitofish.

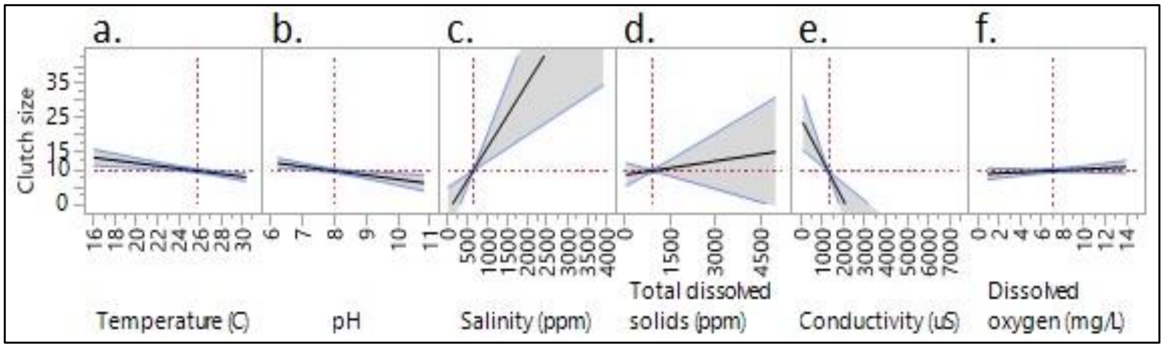

Figure 11: Clutch size by temperature, pH, salinity, total dissolved solids, conductivity, and dissolved oxygen.

Preserved Clutch Weight. The weight of preserved female mosquitofish differed significantly by temperature (Fig. 12a; Multifactor model: $R^2 = 0.04$, $p = 0.009$), pH (Fig. 12b; $R^2 = 0.03$, $p = 0.003$), salinity (Fig. 12c; $R^2 = 0.004$, $p = 0.002$), and conductivity (Fig. 12e; $R^2 = 0.005$, $p = 0.009$), but not by total dissolved solids (Fig. 12d; $R^2 = 0.005$, $p = 0.50$) or dissolved oxygen (Fig. 12f; $R^2 < 0.001$, $p = 0.06$). The weight of preserved female mosquitofish increased significantly with rising salinity. An inverse relationship was evident between the weight of preserved mosquitofish and temperature, pH, and conductivity. The concentration of total dissolved solids and dissolved oxygen did not significantly influence the clutch sizes of gravid female mosquitofish.

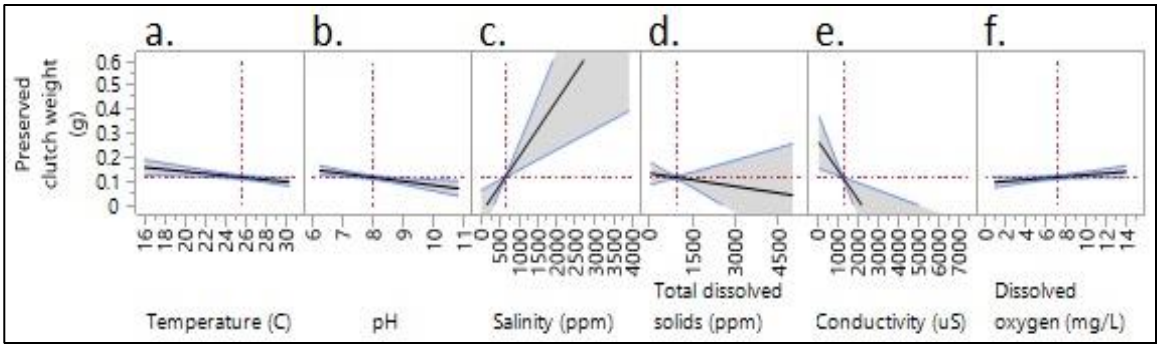

Figure 12: Preserved clutch weight by temperature, pH, salinity, total dissolved solids, conductivity, and dissolved oxygen.

Stepwise Regression of Significant Variables

Predicting Clutch Size. Clutch size differed significantly by region (Fig. 13a; Multifactor model: $R^2 = 0.02$, $p < 0.0001$), habitat classification (Fig. 13b; $R^2 = 0.007$, $p < 0.0001$), preserved female weight (Fig. 13c; $R^2 = 0.58$, $p < 0.0001$), total dissolved solids (Fig. 13d; $R^2 = 0.006$, $p = 0.0004$), and conductivity (Fig. 13e; $R^2 = 0.007$, $p = 0.003$). Of the observed variation in clutch size from these variables (Sum of Squares), preserved female weight was the strongest predictor (87.7%) of clutch size. Region predicted 2.8% of the observed variation, habitat classification 5.3%, total dissolved solids 1.9%, and conductivity 2%.

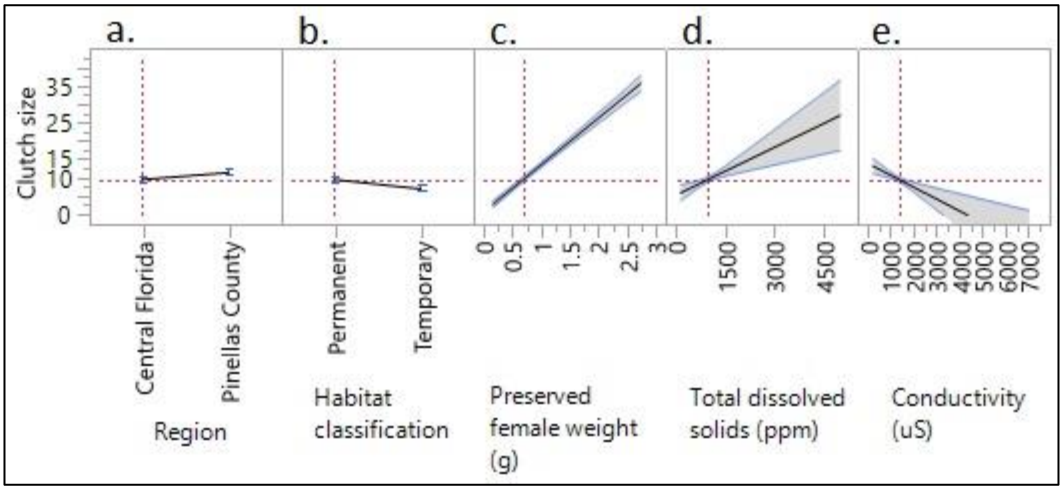

Figure 13: Clutch size by region, habitat classification, preserved female weight, and conductivity.

Predicting Preserved Fish Weight. Fish weight was the strongest predictor of fertility (sizable clutches). The weight of preserved females differed significantly by region (Fig. 14a; Multifactor model: $R^2 = 0.06$, $p < 0.0001$), pH (Fig. 14b; $R^2 = 0.03$, $p = 0.013$), salinity (Fig. 14c; $R^2 = 0.01$, $p = 0.01$), conductivity (Fig. 14d; $R^2 = 0.01$, $p = 0.0004$), and dissolved oxygen (Fig. 14e; $R^2 = 0.001$, $p = 0.003$). Of the observed variation in preserved fish weight from these variables (Sum of Squares), region was the strongest predictor (31.6%) of preserved fish. Conductivity predicted 22.3% of the observed variation, salinity 20.1%, dissolved oxygen 15.1%, and pH 10.8%.

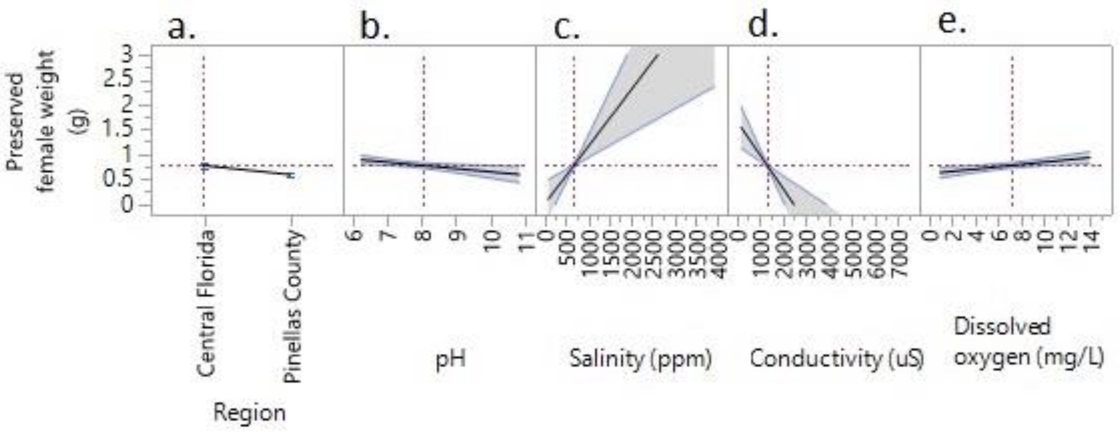

Figure 14: Preserved fish weight by region, pH, salinity, conductivity, and dissolved oxygen.

Observations on Reproductive Strategies

Predicting Superfetation. Gravid female mosquitofish that displayed superfetation were significantly heavier (n = 9, $\bar{x}$ = 1.89 g) than individuals that did not display superfetation (n = 361, $\bar{x}$ = 0.88 g) (Fig. 15; One-way Wilcoxon rank-sum test: $p < 0.0001$). Gravid female mosquitofish that displayed superfetation were also significantly longer than individuals that did not display superfetation (One-way Wilcoxon rank-sum test: $p = 0.006$).

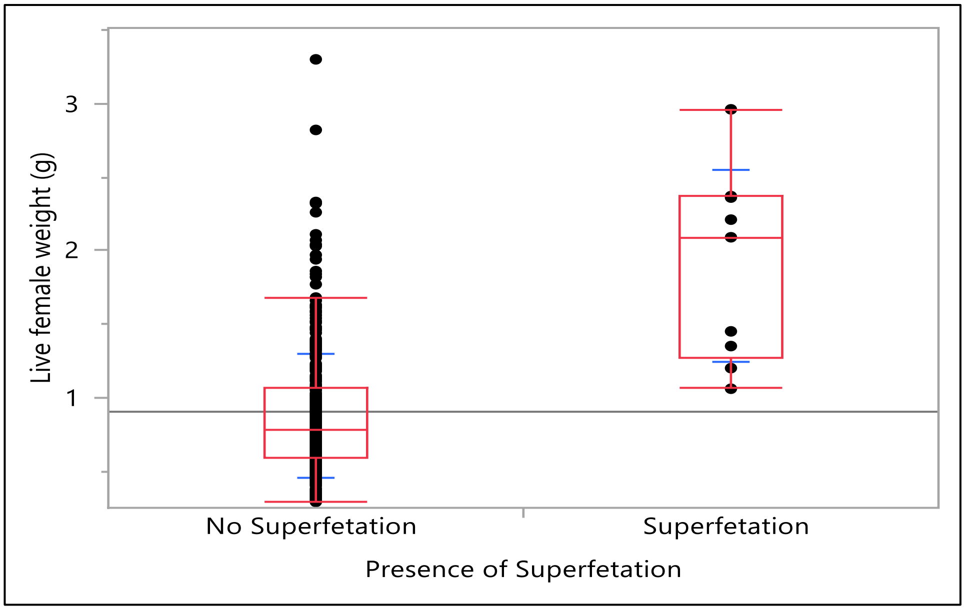

Figure 15: Live female weight by the presence of superfetation.

Classifying Matrotrophy. Embryo weight increased linearly throughout the developmental stages, representing significant post-fertilization maternal contributions (Fig. 16; Multifactor model: $R^2 = 0.29$, $p < 0.0001$). Stage four embryos ($n = 22$, $\bar{x} = 0.018$ g) displayed weight gains of 189% from the first developmental stage ($n = 146$, $\bar{x} = 0.0095$). On the spectrum of strict lecithotrophy to specialized matrotrophy, mosquitofish fall within the classification of unspecialized matrotrophs.

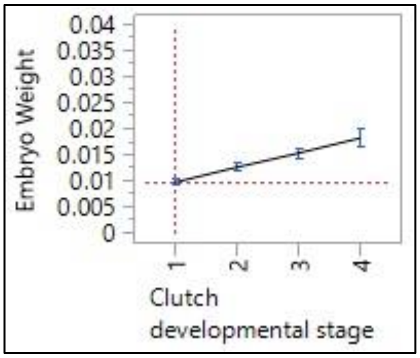

Figure 16: Embryo weight by clutch developmental stage.

Chapter Four: Discussion

Conclusion

In the present study, water quality parameters and habitat classification were minor predictors of mosquitofish fertility. Rather, individual maternal characteristics (length and weight) were the strongest predictors of mosquitofish fertility. Preserved female weight was the strongest predictor of clutch size. Preserved female weight was the largest in rural habitats. I found that fish displaying superfetation were by far the most fecund individuals; they consistently weighed more and were longer than individuals not displaying superfetation. Mosquitofish maternal contributions resulted in embryo weight gain of 189%. To facilitate the most effective biocontrol for mosquito control, pest control managers should collect or culture larger and heavier breeders. These individuals will be the most fecund.

Predictors of Mosquitofish Fertility

Fish Characteristics (Weight). Fertility is defined as the ability of an organism to produce an abundance of new offspring. For fish, the traditional metric of fertility is clutch size. Through stepwise analysis, fish weight was the strongest predictor of fertility; heavier fish had significantly larger clutches. Of the total range in female clutch sizes, 87.8% of the observed variation was explained by the preserved weight of the breeding female. This supports the findings of previous research that documents larger *Poeciliids* displaying higher fertility (Meffe & Snelson, 1993; Wourms, 1981; Riesch et al., 2012).

My research differs from previous work in several ways. First, I have quantified the relationship between maternal traits, environmental variables, and mosquitofish fertility within natural landscapes. Previous research is centered around other *Poeciliid* species and is focused on lab-raised individuals; no environmental information was considered (Wourms, 1981; Riesch et al., 2012) I assert that, from a pest control operator's perspective, any treatment that increases fish weight will in turn raise the fertility of female mosquitofish.

Environmental Classification. Female mosquitofish weighed more in ditches, retention ponds, and rivers than in lakes. Female mosquitofish also weighed more in the rural region (East Orlando) than in the urban region (Pinellas County). Region was the most powerful environmental predictor of mosquitofish characteristics, but not fertility. Within my stepwise analysis, 31.6% of the observed variation in preserved female weight was from regional differences. Heavier mosquitofish were found in the rural region. As for clutch size (fertility), habitat classification was a stronger predictor (5.3%) than region (2.8%). I assert that regional differences indirectly influenced mosquitofish fertility, to an extent, by causing variations in female weight.

In conclusion, with sampling date not a significant predictor of preserved weight, the heaviest mosquitofish were found in rural ditches, retention ponds, and rivers. As water quality characteristics were weak predictors of fish weight, I conclude that trophic interaction influences the variation in fish weight by region and especially within naturally occurring lakes. I conclude that rural habitats hosted heavier, more fertile mosquitofish because the populations of predatory species are more diffuse, or that the mosquitofish displays a higher ability of predator evasion (Stearns, 1976).

Water Quality. Female mosquitofish length increased with rising temperatures, salinity, and dissolved oxygen levels, while fish length decreased with increasing alkalinity and conductivity levels. Preserved female mosquitofish weight increased with rising salinity and dissolved oxygen concentration, while a significant negative correlation was displayed for alkalinity and conductivity. Dissolved oxygen content was the sole water quality indicator that correlated positively with both fish length and weight. These initial findings appear not to support my original hypothesis that dissolved oxygen would not influence mosquitofish fertility. However, despite being correlated to fish characteristics, dissolved oxygen content was not a strong predictor of clutch size, which does support my hypothesis. Within my stepwise analysis, water quality indicators, when significant, displayed overall weak predictive power over the size and fertility of mosquitofish.

Trophic Controls. The weak correlation between fertility, environmental classification, and water quality provides evidence that there are other, stronger, factors acting upon mosquitofish populations within Florida's varied waterbodies. I hypothesize that with individual fish characteristics, trophic interactions with other species, between habitat types and regions are the true determinants of varied regional fertility within the eastern mosquitofish.

Specifically, I hypothesize that gape-limited piscivorous predators (larger fish) exercising top-down control more-strongly influence the composition of mosquitofish populations, and that they do so differently between habitat type and region. Being gape-limited, predatory freshwater fishes' body size must be sufficiently large to completely engulf prey species. In habitats with varying prey and predatory species compositions (species type, age/size of individuals), top-down controls are exerted differently. When available, adult largemouth bass feed almost exclusively upon mosquitofish (Nowlin, 2006). Gape limitations reserve the largest of mosquitofish to be preyed upon by larger predatory largemouth bass, and populations of juvenile largemouth bass will consume small-bodied mosquitofish at higher frequencies (Hoyle & Keast, 1987).

Photoperiod and Metabolism. Both the length and weight of gravid female mosquitofish were positively correlated to clutch size. Through my stepwise analysis of predicting sizeable clutches, fish length was excluded because its prediction power in relation to all other measured variables was weak. Still, I find it important to note that my data showed longer female mosquitofish were found in rural lakes and retention ponds at later sampling dates. Of the significant environmental variables, sampling date was the strongest predictor of fish length. These findings align with my prediction that metabolic activity increases with rising temperatures during the sampling period (Cech et al., 1985). However, sampling date was a stronger predictor of female length than temperature. This suggests that rather than water temperature, photoperiod is the true determinant of metabolic activity within gravid female mosquitofish. Although longer mosquitofish were sampled at later dates, preserved female weight was not significantly influenced by sampling date, despite the powerful correlation between live female length and preserved female weight. I predict seasonal variation in metabolism and nutritional provisioning within *Gambusia* encourages individuals to grow in length throughout the spring without gaining additional weight.

Reproductive Strategies of Mosquitofish

Superfetation and Fertility. Superfetation is characterized by the occurrence of more than one stage of developing embryo found in an organism at the same time. First reported by W. P. Seal in 1911, the phenomenon is now well-recognized within *Poeciliidae* (Seal, 1911; Scrimshaw; 1944). I have shown that within *Gambusia*, the occurrence of superfetation is a phenomenon reserved for larger individuals. “Super-fish” displaying superfetation were twice the weight ($\bar{x}$ = 1.89 g versus 0.88 g), and nearly three times as fecund (clutch size), as regular fish ($\bar{x}$ = 26.56 versus 9.03). Within unspecialized matrotrophic species like *G. holbrooki*, superfetation is limited to individuals with larger body sizes capable of meeting the metabolic demands of skipping brood intervals. There were no significant environmental predictors for superfetation; the occurrence of the phenomenon was strictly dictated by fish characteristics. I also found that breeding females with clutches in a later stage of development weighed more.

Observing Unspecialized Matrotrophy. While eastern mosquitofish have been classified as unspecialized matrotrophs based on yolk loading patterns within *Poeciliidae* (Wourms, 1981, Marsh-Matthews et al., 2001), the extent of post-fertilization maternal contributions to developing embryos has not yet been quantified. I observed such contributions by plotting embryo weight against developmental stage in a multifactored model. Throughout all developmental stages, embryos gained a significant amount of weight in a linear pattern. Within *Poeciliidae*, maternal contributions are facilitated by highly vascularized placentas and consist of lipids and amino acids. On the spectrum of strict lecithotrophy to profound matrotrophy, eastern mosquitofish appear to lie somewhere in the middle. Close relatives of the eastern mosquitofish display both net losses and gains in embryonic weight throughout development. Guppies, which are closely related enough to hybridize with mosquitofish, utilize modest-sized eggs with sufficient yolk stores and undergo a net loss of 25% of embryonic weight (Depêche, 1976). The blackspotted livebearer displays weight gains of up to 1,840% during development (Thibault & Shultz, 1978). From my findings, I have shown that *G. holbrooki* embryos display embryonic weight gains of 189% by use of follicular transfer of post-fertilization maternal contributions. This is the first quantification of maternal contributions to embryos for the species.

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