THE EFFECTS OF GILL PARASITIZATION BY AN UNDESCRIBED SPECIES OF GENUS AETHYCTERON ON THE REPRODUCTIVE CAPABILITIES OF ETHEOSTOMA KENNICOTTI, THE STRIPETAIL DARTER

by

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THESIS

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences in The Graduate School of the University of Alabama in Huntsville

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2020
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The aim of this experiment was to determine what effect gill parasites have on reproduction in stripetail darters. Nearly 450 stripetail darters were collected from Estill Fork stream in Jackson County, Alabama. Their gonads were excised and the oocytes were photographed, counted, and then classified into one of four developmental stages. Their gill parasites, flatworms belonging to genus *Aethycteran*, were also removed and counted. Sexual dimorphism in size was discovered in the stripetails, as well as a high gonadosomatic index and large (2.0 mm) size of clutch oocytes. Reproductive maturity was observed in females as small as 19.3 mm in length. The number of males found at the 25> mm length range far outnumbered females. Prevalence and intensity of parasitic infection were both high, with prevalence nearing or reaching 100% in breeding males and females. Two hypotheses were advanced to explain these phenomena: fecundity compensation in females is likely causing the early reproductive maturity in stripetail females, and the lack of older females may be the result of a high cost of reproduction being paid at the expense of long-term survivability. The combination of both of these factors may ultimately be forcing a shift in life history in stripetail females that favors semelparity.
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SIGNED CONSENT FORM
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I. INTRODUCTION

The planet is a diverse and complex place comprised of several different terrestrial biomes and marine and fresh waters. These areas contain multitudes of habitats and niches that foster all kingdoms of life. Just as many relationships exist between the residents of these habitats and those around them. Producers rely on light and/or chemical sources to produce energy, and they, in turn, provide energy to the herbivorous consumers that ingest them. Other consumers, carnivores and omnivores, prey upon these herbivores, taking a portion of that energy for themselves. Many organisms develop symbiotic relationships with other organisms that allow them to work together for the benefit of one or the both of them. One form of symbiosis, parasitism, enables one organism, the parasite, to prey upon another without causing immediate death. It does this by attaching itself to a host organism and siphoning off nutrients needed by its host. A successful parasite may spend its entire life slowly draining life from its host. Depending on the size of the host and parasite, and the intensity of the infection, this relationship may prove catastrophic to the host, or it may prove a minor hindrance to the host causing little in the way of adverse health effects.

One example of a parasite having an adverse effect on one species, but no observable effect on another, is the freshwater pearl mussel. The life cycle of this mussel, *Margaritifera margaritifera*, includes an obligate parasitic (glochidial) phase that requires fishes as a host (Marwaha et al., 2017). Many fish species, game and non-game alike, contribute to the population of freshwater mussels by serving as a host for these parasitic larvae. Two preferred hosts for freshwater pearl mussels are Atlantic salmon and brown trout (Ieshko et al., 2016). The mussel larvae initiate their host-parasite
relationships by attaching themselves securely to the gill filaments of either species of fish (Ieshko et al, 2016). The immune system of the fish host responds by encapsulating the glochidia within a cyst, unwittingly providing protection for their parasite (Ieshko et al, 2016). From the safety of this cyst the parasitic larvae are able to siphon off the nutrients that they require to fuel their metamorphosis into a non-parasitic stage, a process that takes several months (Ieshko et al, 2016). Excessive gill parasitism by these glochidia have been linked to a decrease in swimming capacity coupled with an increase in mortality in brown trout (Ieshko et al, 2016). However, in the study conducted by Ieshko (2016) on the effects of parasitism by these glochidia on Atlantic salmon populations in northwest Russia reported no significant connection between parasite intensity and the mass of their captured salmon.

This study was conducted in order to answer the question of whether or not parasitism by a different gill parasite, a monogenoidean ectoparasite belonging to genus *Aethycterons*, has an adverse effect on its host, the stripetail darter. This research focused primarily on the reproductive system of the darter, in stripetail females particularly, and the extent to which parasitism affects gonadosomatic index (GSI) values, oocyte size, and clutch number. There were two hypotheses being tested by this study, the first being that the large nutrient investment being made into reproduction on the part of female stripetails is likely proving detrimental to their long-term survivability. This is known as the cost of reproduction hypothesis. The second hypothesis being tested is that stripetail females may have undergone a form of fecundity compensation in which they responded to exposure to gill parasites by sexually maturing at an earlier age than they would have had they not been exposed to parasites.
I-A. Stripetail Phylogeny

Like all members of genus *Etheostoma*, *E. kennicotti* belongs to the Percidae family, which encompasses fishes (chiefly perches, darters, and walleyes) that occupy the freshwater and brackish environments of North America and Europe (Stepien & Haponski, 2015). *Etheostoma* is a large genus consisting of nearly 250 species of darter that have been further sorted into 18 subgenera (Near et al., 2011). These darters are distinguished from other members of the Percidae family by their smaller body size, the appearance of nuptial colorations on males in many darter species, and the general lack of a functional swim bladder (Near et al., 2011). *Etheostoma kennicotti* in particular belongs to subgenus *Catonotus* which is characterized by nest guarding behavior in males and by a distinctively flat nonbifurcated shaped genital papilla in females (Page et al, 1992). On a species level there are a number of physical characteristics by which a researcher may distinguish *E. kennicotti* from other members of *Catonotus*. These include a scaled belly but unscaled nape and operculum, lack of red and blue pigmentation, and also the 6 to 11 black vertical stripes on its caudal fin from which the stripetail darter derives its name (Kuehne and Small, 1971). One other characteristic found only in stripetails and other egg-clustering nest guarding species of *Catonotus* are egg mimics, pronounced fleshy bulbous knobs located on the tips of the spines on their dorsal fins (Page & Bart, 1989).

![Male stripetail darter. Egg mimics are clearly visible at tips of dorsal spine.](image)

Figure 1: Male stripetail darter. Egg mimics are clearly visible at tips of dorsal spine.
To date, no true fossil record exists for the darters of genus *Etheostoma* (Carlson et al., 2009). Much of what is known about the phylogeny of stripetails and other darters has been compiled using morphological traits, like those described in the previous paragraph, and also behavioral data. Efforts to accurately classify darters, particularly among Etheostomatinae, has been complicated by the large number of species it contains (Carlson et al., 2009). Altogether the Percidae family of North America boasts a species-richness that is second only to the Leucisidae family which boasts 320 species of minnow (Near et al., 2011). Even more impressive than its numbers are the staggering extent of morphological diversity that darters display. Darters are able to occupy a wide variety of different freshwater habitats from slower moving lakes with deeper waters to shallow streams with faster water currents. Species isolation and divergent evolution have contributed to an explosion of diversification among members of the *Etheostoma* genus, with the end result being the development of multitudes of different morphological variations (Near, 2002).

With the advent of molecular approaches, efforts have been made to revisit the taxonomy of *Etheostoma* using other methods than simply relying on morphological data alone. One such effort involved using a combination of nuclear DNA and mitochondrial DNA (mtDNA) sampled from 245 of the 248 recognized darter species together with Bayesian phylogenetic analysis to create a rank-free clade-based classification that clarified the phylogenies of not only *Etheostoma*, but also three other darter genera, *Percina, Crystallaria, and Ammocrypta* (Near et al., 2011). *Etheostoma*, the authors of that study claim, is the oldest of this grouping with an age estimate of between 26.1 and 33.5 million years (Near et al., 2011). Based on the results of their DNA analysis, Near et
al. also proposed that *Etheostoma* subgenus *Nothonotus* be elevated to its own genus (2011).

I-B. The Stream at Estill Fork: An Ideal Stripetail Darter Habitat

*Etheostoma kennicotti* takes its name from Robert Kennicott the naturalist and herpetologist who first discovered stripetail darters in 1856 while studying the fauna living in the rocky brooks of Union County, Illinois (Stripetail, 1977). Since their initial discovery in Illinois, stripetails have also been found living in freshwater steams and river systems in Ohio, Kentucky, Tennessee, Mississippi and Alabama (Page & Smith, 1976). The stripetail specimens collected for this study were taken from Estill Fork in Jackson County, Alabama (34 54 38’N 86 10 04’W). This stream proved a prime stripetail darter collection point for a number of reasons, including water depth and oxygen retention levels, cooler temperatures, the availability of nesting substrates, and a rich variety of accessible food sources.

The depth of the stream, as well as the pace of the water current and temperature at collection sites, facilitate habitation by multiple genera of fishes, not just darters. It wasn’t uncommon to observe chubs, shiners, sunfish, daces, and sculpins captured in the same seine net along with multiple species of darter. The site where the stripetail specimens were collected possesses both shallow riffles and slightly deeper slab pools that ranged in depth from approximately 50 cm (January) to approximately 20 cm (June). As a benthopelagic fish, darters are able to make their nests within the substrate, and also hunt and feed on prey throughout the entire water column from substrate to surface,
provided that this column isn’t too deep (Stepien & Haponksi, 2015). Neutral buoyancy, the ability of a fish to remain static in a water column, neither rising nor sinking, requires a swim bladder (Liem, 1989), a gas-filled organ which darters from genus *Etheostoma* do not have (Near et al., 2011). This lack of a swim bladder translates to a lack of buoyancy, which, in turn, makes it easier for a benthic fish to hug the substrate (Liem, 1989). Swim bladders also function as respiratory organs in some teleosts, but darters rely on gill respiration to supply their oxygen requirements. Through the use of countercurrent oxygen exchange, fish uptake oxygen from their surrounding waters into their gills and then cycle it throughout their circulatory systems (Hills, 1970). This method of respiration relies on the surrounding water having a high concentration of oxygen because countercurrent oxygen exchange relies on the diffusion of oxygen from an oxygen-rich environment to an oxygen-poor environment (Hills, 1970). This limitation means that oxygen-poor and mostly anoxic environments are devoid of all but the most hypoxia tolerant of darter species (Ultsche et al., 1978).

A study hasn’t been conducted on stripetails specifically to determine whether they are limited to their particular habitat as a result of respiration, but this research has been carried out on other members of *Etheostoma*. Ultsche set out to discover whether any interrelation existed between the oxygen content of a body and water and habitat selection in darters (Ultsche et al., 1978). By testing six darter species (*E. ruﬁlineatum*, *E. ﬂabellare*, *E. squamiceps*, *E. duryi*, *E. boschungi*, and *E. fusiforme*) Ultsche et al. were able to determine each species’ level of tolerance to hypoxia and how this affected their habitat selection (1978). The six darters varied in habitat from a fast-moving current with a high oxygen content (*E. ruﬁlineatum*), to a mid-range current (*E. ﬂabellare*, *E. duryi*, *E.
squamiceps, and *E. boschungi*), to one of the rare darter species capable of living in swamplike environments (*E. fusiforme*) (Ultsche et al., 1978). Ultsche et al. captured specimens from each of these species and kept them contained within streamwater aquaria (Ultsche et al., 1978). Oxygen content was manipulated by altering the temperature of these aquaria between 10°C and 20°C, roughly the winter and summer temperatures of their Alabama stream habitats, and then allowing the fish to deplete the aquaria water of their oxygen supplies (Ultsche et al., 1978). What they discovered was that *E. rufilineatum* was highly limited to its fast-moving environment as it was unable to survive in waters with reduced oxygen levels, but none of the other species shared that limitation (Ultsche et al., 1978). Each of the five species accustomed to living in somewhat slower waters were able to tolerate lower levels of oxygen (Ultsche et al., 1978). *Etheostoma kennicotti*, which tends to avoid faster currents in favor of the slower moving waters of slab-pools, appears to be in very little danger of suffering oxygen deprivation at its preferred depths of 20 to 50 cm.

In addition to respiration, shallower waters and cooler temperatures are also more conducive to spawning. Lawrence Page explains that of nine stripetail spawnings that he observed in nature, all occurred in shallow waters ranging from 19 °C to 22 °C (Page, 1975). Males, he reported, began establishing and guarding nests beginning in March and primarily in waters less than 30 cm in depth (Page, 1975). He did also note that when forced to, perhaps by competition for prime nesting locations, some stripetails chose to nest at the margins of deeper slab-pools, but that ultimately any depth of 40 cm and greater was avoided (Page, 1975). Page’s observations at Big Creek in Illinois indicate that the spawning season of *E. kennicotti* spans the months of March, April, and May.
During that time, the temperature of the stream at Estill Fork measured 16.7, 21.4, and 23.5 °C. In another study, stripetails were captive bred and their eggs were successfully incubated at temperatures that ranged from 18-22 °C and 22-26 °C (Simon, 1987).

The second characteristic of the stream at Estill Fork that supports darter activity is the range of materials resting on the bottom of the stream bed, the substrates. These substrates are of crucial importance for benthic and hyperbenthic darter species because these are the materials in which they build their nests, hunt for their food, and hide from predators. It was observed during collection trips that Estill Fork possesses a wide variety of nesting substrates from fine grain sand and mud to slab-rock. During breeding months, the majority of stripetail darters were collected in the vicinity of wide flat slab-rocks and smaller cobble-type stones, which is understandable given that stripetails males make their nests on the underside of flat rocks (Tiemann & Sherwood, 2011). During the rest of the year, adult stripetails of both genders prefer that same habitat as it provides them protection from predators (Page, 1975). These observations were further borne out by Page (1992) when he reported that all members of *Catonotus* save two, *E. flabellare* and *E. percnurum* (the fantail darter and duskytail darter, respectively), had been found residing in the headwaters of their habitat streams, particularly those headwaters with a rockier substrate. The young the year, however, were not nearly so selective in their habitat as their parents and were often found mingling among coarse gravel, presumably because they are small enough to be able to utilize it as cover (Page, 1975).

Nesting and shelter aside, the substrate can also be a good place for a fish to find a meal. In fact, if the fish is purely a benthic species, it is likely the only place to find one. This brings us to the final piece of the puzzle, the third characteristic of the stream at
Estill Fork that makes it a prime darter ecosystem: its proximity to easily accessible food sources. The prey items of stripetails may differ depending upon which river system one finds them in, but the published findings all point to a diet high in small aquatic insects, the larvae of these small aquatic insects, and even some small crustaceans, particularly snails (Ross, 2001.) During his study at Big Creek, Illinois, Page studied the stomach contents of 333 stripetails and found that the remains were predominantly immature insects and whatever crustaceans were small enough for the stripetail to fit into their mouths (1975). As far as insects went his subjects seemed to prefer the larvae of midges and mayflies while the crustacean menu consisted of copepods, seed shrimp (ostracods), and water fleas (cladocerans) (Page, 1975). Though the stomach contents of the specimens collected at Estill Fork were not examined, it is worth noting that the stream is bordered on both sides by a thick stand of mature deciduous trees. This ecosystem serves as the home for a diverse community of plants, fungi, insects, and other animals. The stream itself sustains a wide variety of small crustaceans, including many snails, and aquatic insects such as water beetles (Hydrophilidae), as well as numerous species of mosses, grasses, and other aquatic plants.

Even though some male darters are known to be territorial when it comes to guarding their nests, they aren’t above eating the very offspring that they’re guarding. Filial cannibalism is quite common among nest-guarding species and other species in which the male parent provides parental care (Lindstrom, 2000). This form of cannibalism has been documented so far in two species of Catonotus, the fantail darter and the spottail darter. While the spottail darter seems to engage in the practice primarily to remove eggs infected with the aquatic fungi, Saprolegnia (Bandoli, 2016), males of
other egg tending species, such as fantails cannibalize their own eggs to gain nutrients (Lindstrom & Sargent, 1997). As of yet no works have been published tying stripetails to filial cannibalism, but given that it is a nest-guarding species it seems highly likely that a stripetail father might feel the need to satisfy his hunger by consuming one or more of his own offspring.

I-C. Stripetail Reproduction

Males:

Stripetails reproduce by external fertilization (oviparous) meaning that female stripetails lay clutches of oocytes that are then fertilized by the males. Beginning in March male stripetails will attempt to find a suitable nest, preferably within a cavity on the underside of a wide, flat rock (Tiemann & Sherwood, 2011). The competition for prime nesting grounds can be fierce. Male stripetails face off against rivals who may be older and larger than they are, and in the game of nests size matters. Page was fortunate enough to observe this ritual in stripetails while studying them in Illinois (1975). He found that while a male stripetail may be sexually competent at one year of age, it was the older, larger males who held most of the nests and therefore engaged in most of the spawning (Page, 1975). Stripetail males may also be forced to contend with the males of co-occurring darter species. The spottail darter, for example, also builds its nests beneath flat slab-rock putting them in direct competition with stripetails nesting real estate (Page, 1974). The situation only becomes trickier for younger stripetails when you take into consideration that spottails do not reach sexual maturity until their second year making them larger and more difficult to compete with (Page, 1974). The male stripetails
collected and analyzed during Page’s study may have been reproductively capable at one year old, but size-wise they ranged from 30 to 39 mm in length with the heaviest being 0.89 g (1975). The majority of his two-year old spottail specimens measured between 41 and 51 mm, and the heaviest spottail tipped the scales at 2.16 grams (Page, 1974).

Females:

Female fish begin the production of oocytes, or oogenesis, as early as September (Page, 1975). Oogenesis begins with the production of the primordial germ cells (PGCs) that will soon transform into oogonia and then undergo meiosis to become oocytes (Lubzens et al., 2009). Newly developed, or primary, stripetail oocytes start out small (<1.00 mm in diameter), are nearly translucent or gray in color, and have a roughly spherical shape. Structurally primary oocytes are at their least developed. They consist of a pair of membranes: an outer layer called the chorion and a clear inner plasma membrane that is known by a variety of names, including the pellucid membrane and the zona pellucida (Laale, 1980). Contained within these membranes are the yolk and the oocyte’s cytoplasm, or ooplasm (Laale, 1980). In primary oocytes, this ooplasm is clear. As they progress in their development, oocytes gain size and take on a pronounced yellow coloration. This stage of development is called vitellogenesis and is characterized by synthesis of maternal RNA within the oocyte, the accumulation of the vitellogenin phospholipoglycoproteins required to fuel the growth of the yolk, and the incorporation into the yolk of those vitamins, carbohydrates, proteins, and lipids that are vital to embryonic development (Lubzens et al., 2009). At their most mature, just prior to deposition, they average approximately 2.1 mm in diameter and are roughly spherical in shape (Simon, 1987) with a slight invagination along one side. Their ooplasm is clear in
appearance but possesses a single fully developed yolk that is large and yellow-orange in color (Simon, 1987).

I-D. Courtship and Spawning

Any stripetail male of any age fortunate enough to claim and hold a nesting territory leaves it for only one purpose: to woo potential mates (Page, 1975). It is during this courtship that a characteristic unique to egg-clusterers like stripetails is used. It was previously mentioned that egg-clustering nest-guarding *Catonotus* species possess bulbous knobs on the tips of their dorsal spines. These fleshy knobs were believed to have evolved in nest guarders in order to allow them to move among their eggs without damaging them (Page & Swofford, 1984). Having these soft coverings on their dorsal spines would potentially benefit fish species, like stripetails, who lay their eggs on the underside of rock surfaces. In certain members of *Catonotus* these dorsal knobs have further evolved in structure and function, increasing in size and taking on the color of mature darter eggs (Page & Bart, 1989). Because of this resemblance to eggs, this type of dorsal knob has been called an egg mimic (Page & Bart, 1989). Egg mimics are believed to be an improvement on the dorsal knob design because they still permit nest guarders to tend to their offspring without causing damage, but they also serve to attract potential mates to a male’s nest (Page & Bart, 1989). In three separate aquarium studies, the first performed on spottails, the second on stripetails, and the third on fantails, it was observed that females of all three species only spawned in nests where fertilized oocytes were already present (Page, 1974 & 1975, Knapp & Sargent, 1989). It has been hypothesized
that egg mimics evolved as a way of stimulating females with this preference to spawn by “tricking” them into thinking that oocytes were present when in reality they were not.

Interestingly enough, egg mimics are not always physical structures like the enlarged knobs one sees in stripetails. Egg mimicry based entirely on pigmentation has been observed in an isolated population of the striped darter, *E. virgatum* (Porter et al., 2002). These unique mimics appear as conspicuous white spots that strongly resemble the fertilized oocytes of striped darters (Porter et al., 2002). Rather than adorning the dorsal spines, the striped darter’s egg mimics are located on their pectoral fins which they fan out during their mating display to entice potential reproductive partners (Porter et al., 2002).

When a female, lured in by the sight of the egg mimics, approaches the male and his nest the male commences the courtship ritual with a mating dance. During this dance the male displays his breeding colors and spreads his fins (Page, 1975). The male stripetail may also attempt to engage in direct contact with the female by nudging her suggestively with his snout or slapping his tail against her body (Page, 1975). If sufficiently impressed with the male and his dance, the female stripetail will enter the nest, find a spot on the underside of the flat rock, invert her body so that she can lay eggs while upside-down, and then press her genital papilla against the rock (Page, 1975). The male will follow suit, inverting himself and lying next to his mate so that the pair are arranged in a head-to-tail position (Page, 1975). The two will physically tremble, her as she releases one oocyte at a time, and he as he releases sperm (Page, 1975). Once her oocytes have been fertilized, the female ceases all involvement with them. The
developing embryos will be guarded and cared for solely by their male parent until they hatch 6 days later (Tiemann & Sherwood, 2011).

I-E. The Cost of Reproduction Hypothesis

The first hypothesis being explored during this thesis is that of cost of reproduction. As the name suggests, there is a fitness cost associated with reproduction that often comes in the form of a tradeoff between reproductivity and long-term survivability. Trade-offs between procreation and longevity are common in the natural world and the cost of reproduction to an organism’s fitness has been studied extensively by researchers in a variety of different biological fields.

Physiologically, costs involved with reproduction include the energy that must be expended in order to court a mate and produce gametes, like oocytes in stripetails for instance (Rose & Bradley, 1998). This energy output has been quantified in previous studies by joules expended per production of each gamete and caloric costs associated with mating (Rose & Bradley, 1998). Along with the energy cost comes material cost, chiefly the nutrients required to maintain the organism’s survival and participate in reproduction. A deficiency in crucial nutrients would translate directly into a deficiency of calories available to perform energy-intensive activities like reproduction. Because females tend to burn far more energy during reproduction, nutrient limitations affect reproduction in females far more than it does males (Rose & Bradley, 1998).

For that same reason trade-offs between reproduction and fitness can be far more pronounced in females. An experiment conducted on Drosophila melanogaster illustrated
this point. By culturing a sample of *D. melanogaster* at 25°C the researchers were able to postpone first reproduction in these *Drosophila* from as early as a few days or weeks to as much as eight weeks (Rose & Bradley, 1998). The result was a “significantly increased lifespan” in these females (Rose & Bradley, 1998). Their conclusion was that decreased early reproduction in *Drosophila* is positively correlated with an increase in adult survival (Rose & Bradley, 1998). These experiments were repeated in conditions of nutrient abundance and low crowding, and then again in conditions of limited nutrients and crowding. What they discovered was that the significant correlation between early reproduction and increased longevity disappears when *D. melanogaster* has access to an abundance of nutrients and enough room for their population to grow (Rose & Bradley, 1998). In these test groups, they observed no cost to reproduction in their females. Conversely, in those *Drosophila* that were cultured with a limited food supply and far less room, they observed a six-fold greater cost to reproduction (Rose & Bradley, 1998). Adult longevity was being sacrificed in order to facilitate short-term reproduction when nutrients were limited, as is usually the case in the wild.

A second experiment explored the costs of reproduction by examining the metabolic rates and lipid storage amounts in two different test groups of *Drosophila*, one that had been selected for early reproduction, and the other selected for reproduction later in life (Rose & Bradley, 1998). In the group selected for early reproduction it was observed that lipid stores were lower and the metabolic rate was higher than in those *Drosophila* selected for later reproduction (Rose & Bradley, 1998). In a study similar to that one, *Drosophila* that had been selected for later reproduction were cultured in an environment of highly limited nutrients, including water and yeast, they discovered that
the metabolic rates of this group did not differ from flies selected for earlier reproduction (Rose & Bradley, 1998). In both test groups, stress levels were high and nutrients stores were low. The authors of that study concluded that accumulation of energy stores is critical for reproduction and health of the organism. Of particular importance is the yeast which contains both the B vitamin complex necessary for reproduction. In addition, cholesterol provides lipids needed for membrane and hormone synthesis, and also for egg production in female Drosophila (Rose & Bradley, 1998). Energy stores in male Drosophila was also tested and it was observed that lipid and carbohydrate levels in males with a reproductive partner were substantially lower than non-mated males, suggesting that males also contribute essential nutrients to the reproductive process (Rose & Bradley, 1998).

Limited access to yeast could easily limit health and curtail reproduction in both males and females of D. melanogaster simply because reproduction could prove too costly to these organisms when they have only enough nutrients to survive. Stripetail darters, like the Drosophila studied in these experiments, also alter their reproductive output in response to nutrient deprivation. Unlike D. melanogaster, however, this deprivation hasn’t been caused by the withholding of yeast and other nutrients during an experiment. Rather, in our sample of stripetail darters, nutrient loss occurs as a result of another factor: parasitism.
I-F. The Fecundity Compensation Hypothesis

The fecundity compensation hypothesis holds that in host organisms involved in a host-parasite relationship a trade-off may occur that prioritizes current reproduction over future reproduction. In this sense, fecundity compensation and the cost of reproduction hypotheses can be interrelated. Immune responses to a parasite or pathogen can prove costly to an organism, particularly to reproduction. Resources necessary to initiate the sustained immune response necessary to combat an invading organism often have to be reallocated from reproduction, energy stores, and other critical aspects of a host organism’s life. Ultimately a considerable amount of resources is invested in a fight that there is little to no guarantee that the afflicted organism will win. Rather than divert these nutrients away from reproduction, many host organisms choose not to wage a potentially costly battle against a parasite or pathogen, but instead invest these resources into reproduction. As with the Drosophila, they are compensating for the loss of nutrients and fitness by increasing their fecundity. Fecundity compensation can be termed an “adaptive non-immunological host defense”, an adaptive response that operates outside of the host’s immune system (Heins, 2012).

One advocate of the hypothesis observed one such trade-off while studying three-spined sticklebacks (Gasterosteus aculeatus) infected by a tapeworm Schistocephalus solidus (Heins, 2012). Sticklebacks become hosts to S. solidus by ingesting copepods that are themselves host to the larval form of the tapeworm (Benesh, 2012). Sticklebacks are the intermediate host for S. solidus, meaning that this parasite grows to adulthood while inside the digestive tract of the stickleback, but can’t reproduce until it reaches the guts of its final host, seabirds (Benesh, 2012). Sticklebacks infected with these tapeworms
undergo behavioral modification that induces them to swim to the surface of the water where they stand a greater chance of being eaten by a seabird ensuring that the tapeworms are able to reach their final host (Benesh, 2012). Obviously, this form of infection poses such a demonstrable threat to the health of the stickleback that long-term reproductive success is greatly diminished. Because the chances of future reproduction are reduced in fish infected with *S. solidus*, sticklebacks compensate for this risk to future reproduction by making a trade-off between current and future progeny. According to a study in which the exact effects of fecundity compensation in sticklebacks are explored, this trade-off came in the form of oocyte mass and number. Infected stickleback mothers were able to maintain the same reproductive capacity as uninfected mothers by producing larger numbers of oocytes that were smaller in size than those of the uninfected sticklebacks, essentially trading quality for quantity until the intensity of infection ultimately rendered them unable to reproduce at all (Heins, 2012).

A second study confirmed that fecundity compensation can be triggered by life-threatening events such as exposure to pathogens or even bodily injury, but also that host organisms will resort to fecundity compensation more often when the parasite or pathogen involved poses a significant risk to their survivability. The experiment in question required testing three different clonal lines of aphids against four different challenges. Three of these challenges involved exposure to a pathogen while the fourth was accomplished by simply piercing the aphid’s body with a sterile needle in order to cause it bodily harm but without introducing a pathogen (Leventhal, 2014). The first challenge was exposure to a gram-negative bacteria, *Enterobacter cloacae* (Leventhal, 2014). The second challenge involved exposure to an unnamed gram-positive bacteria,
and the third pathogenic challenge came from the fungus *Eryna neoaphidis* (Leventhal, 2014). The goals of the study were to observe the effects of each challenge on the test groups by comparing differences in both short-term and long-term reproductive rates against controls, to determine what extent fecundity compensation played a role in these differences, and lastly to determine how these challenges effect survivability of the test groups (Leventhal, 2014).

The authors discovered that of the three clonal lines tested, two of them responded to the challenges by lowering reproductive output (Leventhal, 2014). The third responded by increasing reproductivity in the short-term (Leventhal, 2014). This initial burst in productivity came at the cost of reduced output in later reproductive episodes (Leventhal, 2014). As far as the sterile piercing tests went, there did not appear to be a homogenous reaction to this challenge across the three lines tested; they all responded differently. The authors of the study also noted that all four challenges ultimately reduced the survival rate of all of the test aphids when compared to controls (Leventhal, 2014).

What the authors concluded from this study was that the degree of fecundity compensation initiated in response to an impending threat posed to the aphid depended on the severity of the threat (Leventhal, 2014). When the threat is perceived as weaker, an organism may engage in an immunological response as opposed to a non-immunological response like fecundity compensation. In instances like these resources are pulled away from reproduction and allocated instead toward to the immune response (Leventhal, 2014). The authors believe that this was what had occurred in the two aphid lines that exhibited a reduced reproductive output. Conversely, when the threat is greater, as is the case with a highly virulent parasite, like *S. solidus* in sticklebacks, or life-threatening
injury or infection, fecundity compensation is the result (Leventhal, 2014). Rather than reallocate crucial resources away from reproduction in order to engage in a potentially costly immune response with no guarantee of success, the affected organisms instead increase their reproductive output in the short-term, producing a larger number of offspring compared with controls (Leventhal, 2014).

Fecundity compensation doesn’t always entail altering the number of offspring, it can also involve a shift in reproductive timing. This form of fecundity compensation has been observed in the water-flea, *Daphnia magna* (Chadwick & Little, 2005). When exposed to their parasite, the microsporidian *Glugoides intestinalis*, water-fleas were able to shift the timing of their life-history to reproduce earlier than in fleas who were not affected by the microsporidians (Chadwick & Little, 2005). In a laboratory setting multiple lines of parasite-free *Daphnia* were bred and tested against fleas that had been exposed to the microsporidians (Chadwick & Little, 2005). Fleas that had been exposed to the parasite reproduced earlier in life than the uninfected water-fleas. Long-term exposure to the *G. intestinalis* resulted in a loss in reproductive capability (Chadwick & Little, 2005). Just as the stickleback and pea aphid had, the water flea resorted to a form of fecundity compensation when faced with a serious threat to its survival and/or fitness.

Another study set out to identify the mechanism behind fecundity compensation by studying stress responses in nematodes infected with *Staphylococcus aureus*. The experiment relied on testing three different model host strains of *Caenorhabditis elegans* against a control group (Pike et al., 2019). The three strains of *C. elegans* were: one wild type (N2), a strain bred to exhibit heightened stress responses (*daf-2*), and a strain possessing suppressed immune and stress responses (*sek-1*) (Pike et al., 2019). Each
strain was exposed to *S. aureus* and then tested for mortality and number of offspring produced (Pike et al., 2019). Results varied depending on the strain of *C. elegans*. Both the N2 and *daf-2* strains exhibited fecundity compensation, but the *sek-1* strain, lacking both immune and stress responses, did not (Pike et al., 2019). Of the wild type and *daf-2* strains, the survival rate of *daf-2* exceeded that of wild type, but N2 generated greater numbers of F1 progeny across both the control and test groups (Pike et al., 2019). The authors linked stress response to fecundity by explaining that the p38 MAPK pathway is involved in both stress responses and the positive regulation of egg-deposition behaviors in *C. elegans* (Pike et al., 2019). The authors concluded the study by stating that hosts with an elevated stress response, like *daf-2* host strains of *C. elegans*, demonstrated a higher degree of fecundity compensation than did hosts with a suppressed stress response, as was the case with *sek-1*. It stands to reason that an invading parasite or pathogen able to cause death or eventual sterility in its host could greatly elevate a stress response no matter which organisms were involved, be it nematodes, water fleas, pea aphids, sticklebacks, or stripetails.

I-G. **Genus Aethycteron: A Look at the Parasite**

The parasites plaguing stripetail darters belong to a Class of ectoparasitic flatworms known as Monogenea. The members of this group, referred to as monogenoideans, rate among the most strongly host-specific parasite groups (Whittington et al., 2000). For this reason, many are undescribed if the host species hasn’t been well-studied. While comparatively little has been published about the stripetail darter, at least in relation to other darter species, the particular species of gill parasite
written about in this report is undescribed. What is known is that it belongs to genus *Aethycterorn*, the genus that includes monogeneans of darters (Norena & Brusa, 2015).

Monogenoideans are simple morphologically. They are miniscule in size, ranging from 100 to 300 µm in length with only marine species typically exceeding 2 cm (Norena & Brusa, 2015). Their most obvious feature is their sheath which is elongated in shape with a smooth white/translucent exterior. At the anterior end of the sheath are two pairs of eyespots and the sensory nerve bundles that form the parasite’s central nervous system (Hanson & Stallsmith, 2013). At the posterior end are two pairs of 4A-hooks, called hamuli, situated within a disc-like surface called the haptor (Reed et al., 2012). Ringing the perimeter of the haptor are between 14-16 smaller marginal hooks (Hanson & Stallsmith, 2013). Both sets of hooks serve as anchors, attaching the posterior end of the parasite’s sheath firmly to its host’s tissues (Hanson & Stallsmith, 2013; Suriano & Beverley-Burton, 1982). Monogenoideans seem to lack circulatory and respiratory systems, but they do possess reproductive systems. Adult monogenoideans are self-fertilizing hermaphrodites (Reed et al., 2012). Genus *Aethycterorn*, like their darter hosts, are oviparous. Eggs are deposited in the substrate, ideally in the same substrate in which its host resides (Reed et al., 2012). When the larvae emerge from their eggs, they use their cilia and the surrounding water currents to carry them to their preferred host fish (Reed et al., 2012). Once the larvae have located a suitable host, they situate themselves within the host fish’s gill arches and then secure themselves using their haptor apparatus (Reed et al., 2012).

Because this species of *Aethycterorn* has never been studied, the exact nature by which it parasitizes its host is not yet known. We have, however, been able to observe
how parasite load synchronizes with their hosts, increasing in number in tandem with the fish’s breeding cycle, and rapidly decreasing when the reproductive cycle is complete. This timing suggests that parasitism is closely associated with the reproductive process. Their choice of host, predominantly males, suggests that an increase in testosterone may be at least partially responsible for the increase in parasite load. Testosterone increases have been linked to suppression of the immune system. As stripetail males prepare to mate, their testosterone levels increase, rendering them more susceptible to parasitism by *Aethycteran*. This phenomenon is known as the immunocompetence handicap hypothesis (Habig, 2018).

We have also been able to observe the effect that parasitism by *Aethycteran* has on stripetail darters. The females that we’ve collected from Estill Fork have become reproductively mature at an age far earlier than has been previously observed in stripetail populations. In addition, the stripetail oocytes that we’ve excised from our specimens have been smaller in diameter than oocyte sizes recorded in previous studies. These observations had led us to conclude that, like *Daphnia magna*, stripetail females are responding to parasitism by producing their progeny at an earlier age in order to maximize short-term reproductive output. Similarly, as is the case with the sticklebacks who produce smaller oocytes but in greater number when exposed to parasitism, stripetails females have been depositing smaller oocytes than what has been observed in stripetail populations unaffected by gill parasitism. Both methods are examples of fecundity compensation in action. We’ve also witnessed evidence of the cost of reproduction hypothesis at work in our sample population of stripetail females. We’ve discovered that the number of male stripetails collected outnumbered females by nearly
2:1. This discrepancy was far more pronounced in fish at two years of age, suggesting that far fewer females are surviving past their first year of life than males. Because female stripetails are reaching maturity at an earlier age, it is highly likely that this has an adverse on their longevity. A previous paper has been published regarding the negative correlation between early reproduction and long-term survival in *Drosophila* and we believe that we’ve witnessed a similar phenomenon in stripetails.

II. MATERIALS AND METHODS

II-A. Specimen Collection and Measurement

The stripetail darters examined during this research were taken from Estill Fork in Jackson County, Alabama (34 54 38’N 86 10 04’W) over the course of 11 months (August 2016 through June 2017). Collections were done by way of seine netting and “darter dancing”, shuffling forward and disturbing nesting substrate in order to drive fish into the net. This involved a seine net and minnow bucket, and a crew of three or more people. The goal was to drive any fish inhabiting that 2 to 3-meter area downstream into the seine net. Stripetail darters were taken from the net and placed in the minnow bucket. A total of 449 Stripetail darters was collected from the stream using this method. Each specimen was then euthanized by immersing them in a solution consisting of 10 mL of clove oil and 95% ethanol per every 200-300 mL of water. Each fish was measured, weighed, catalogued by month collected, and then finally preserved in a 10% phosphate buffered formalin solution. Standard length (SL) of the fish was taken by measuring the fish from the tip of their mouth to the narrowest point of their caudle peduncle. This measurement was recorded in mm. Somatic mass, recorded in grams, was obtained by
using an Ohaus Explorer balance. The SL and somatic mass values collected were used to
calculate Fulton’s Condition Index (FCI), also called Fulton’s Condition Factor. FCI is a
method of determining the condition or health of a fish by using weight and length data
(Froese, 2006). The FCI equation was named for T.W Fulton who first proposed, in
1904, that as a fish grows in length it also increases in weight, or “fatness”, but the ratio
between length and weight varies depending on the seasons and reproductive episodes
(Froese, 2006). The expected range for a healthy fish is between 2.5 and 3.5 (Froese,
2006).

\[ K = \frac{\text{Somatic Weight} \times 100,000}{\text{Standard Length}^3} \]  

(1)

II-B. Gonad Retrieval and Gonadal Mass Measurement

We dissected with a lengthwise incision down the ventral surface starting just
below the jawline and ending at the fish’s anal fin. A pair of perpendicular incisions was
also made, the first of these being immediately posterior to the gill arches and the second
just anterior to the anal fin. Once the body could be sufficiently pinned open, each
specimen’s gonads were located and then excised. Tools used during dissections were: a
scalpel, forceps, dissecting scissors, pins, straight and angled needles, a wax dissecting
tray, and a Motic K Series dissecting scope. Dissections were limited to those fish that
measured 18 mm SL and greater. As gonads were excised, they were weighed on an
Ohaus Explorer balance that was used to measure the somatic mass of the fish. Each
specimen’s gonadosomatic index (GSI) was calculated using the somatic and gonadal
mass data, and then by calculating the percentage:
GSI = (Gonadal Mass/(Total Fish Mass – Gonadal Mass)) × 100 \hspace{1cm} (2)

GSI is calculated for the purposes of determining when a particular species’ breeding cycle begins and ends. As a fish starts its cycle its gonads increase in size yielding a higher GSI value than during periods of non-breeding. As the cycle ends, these GSI values drop off until they reach non-breeding levels.

II-C. Photographing and Counting of Gonads

Gonads were photographed using an Olympus SZX7 stereo microscope equipped with cellSens software. The Egg Helper program (Tarver & Tarver, 2014) was used to count and categorize each oocyte. Oocytes were measured to the nearest 0.001 mm, and then classified into developmental stages based on their size, shape, and color. At their most underdeveloped state, Stage 1, these oocytes are small (between 0.3 and 0.8 mm in diameter) and translucent in color. As they mature, they gain in size and color. A Stage 2 oocyte is larger (typically between 0.6 and 1.2 mm in diameter) and has taken on an opaque appearance and dull yellow coloration. By Stage 3 they have increased to between 0.8 and 2.0 mm in size and the accumulation of vitellogenin phospholipoglycoproteins have turned the ooplasm a vibrant golden yellow color. They are also at their most spherical in shape at this point. At Stage 4 they are at their most mature and are ready to be deposited. They continue to gain in size between stages 3 and 4, and by this point their vibrant golden yellow hue has coalesced into a large golden yolk. After counting and classification, all gonads, male and female, were fixed in 10% phosphate-buffered formalin within individually labelled plastic microtubes. Stage 3 and
4 oocytes may be referred to collectively as clutch oocytes which are mature, or nearly mature enough to be deposited and fertilized in a single reproductive event.

II-D. Gill and Gill Parasite Removal

The gill arches of each stripetail darter measuring 25 mm SL and longer were excised. This was accomplished by holding open the outermost gill filament and then making incisions around the entire gill structure. This was done to separate the gill tissue from the other connective tissues located in the cranial area of the fish. The entire gill structure was then excised and observed under a Motic K series dissecting microscope. In order to locate the parasites, the eight gill arches of the stripetail darter were carefully separated with a dissecting probe. The gill parasite is distinct in appearance, with a round sausage or potato-like shape. Identifying them among the gill tissue was straightforward. Each parasite was removed from the tissue using forceps, photographed using cellSens software and then stored in 10% phosphate-buffered formalin within plastic microtubes. The number of parasites per fish, or parasite load, was used to determine the mean intensity of infection and the prevalence of infection. The mean intensity of the parasitic infection between specimens was found by counting the total number of parasites found and dividing that by the total number of fish found to be hosting parasites. and the percentage of individuals infected within the stripetail population. Prevalence is the proportion of stripetails infected out of the total number of stripetails that had their gill arches excised and examined (Bush et al., 1997)
II-E. Lipid Extraction and Analysis

Lipid extractions were performed per collection month in order to examine changes in the somatic storage lipid fraction of both sexes before and during the mating period (Stallsmith, unpublished data). The method used was that of Huelett et al. (1995). While Huelett et al. measured the somatic lipid content in an intact sexually immature fish, the stripetails in this study had sexually matured and had their gonads removed beforehand. 12 fish were selected (six males and six females) from the months of January, March, and April. Six fish were selected (three male and three female) from the months of December, February, May, and June. These 60 total stripetails were sliced into small pieces which were dried in a drying oven at 55 °C for two days. Each sample was weighed at this point to obtain their pre-extraction mass. Lipid extraction occurred via the addition of 2 mL of petroleum ether to each stripetail specimen. After the addition of the ether each specimen was covered and allowed to sit for 60 minutes before the ether was drained. This type of ether wash was repeated five more times before the samples were further incubated at 55 °C and then weighed to determine post-extraction mass. The difference in mass between the pre-extraction sample and the post-extraction sample is attributed to storage lipids. The amount of lipids extracted is expressed as a percentage of the initial somatic mass.
II-F. Statistical Analysis

The alpha level used for all statistical analysis was 0.05. A Shapiro-Wilk test for normality of distribution was used to determine whether a sample of organisms collected from a wild environment can be considered representative of the population as a whole, and whether or not the variables found in the test sample are evenly distributed throughout the population as a whole. This test was performed first on standard length and somatic mass in order to determine whether these two sets of data were equally distributed. The Shapiro-Wilk test was repeated using gender and standard length as variables being tested.

A paired samples t-test and a linear regression model was used to examine the relationship between standard length and somatic mass in all 449 collected stripetail specimens. The paired samples t-test was chosen for this analysis because the statistical difference between standard length and somatic mass of each individual fish were being compared to each other. GSI values were obtained using MS Excel’s statistical functions, and a Levene’s test for equality of variances was applied to the GSI values to assess the relationship between gender and GSI percentage. The Levene’s test quantifies the differences between variances in two or more groups. In this case, the two groups being compared were the male and female genders, and the variances being tested were GSI percentage. Following that, SPSS’s Welch ANOVA tests with Games-Howell post-hoc tests were used to explore possible statistical correlations between GSI values and fish standard lengths, GSI values and lipid extraction percentages, gonadal mass and lipid percentages, GSI values and somatic mass, GSI and gender, GSI and total oocyte count, and GSI values and parasite load. The Welch’s ANOVA test was used to compare the
means of the different groups being tested. The Games-Howell post-hoc test was used because it doesn’t require that the groups being tested have equal variances. Possible links between gender and parasite count, and gender and lipid percentage, were explored using independent samples t-tests per gender and month of collection. Linear regression analysis was used to examine potential relationships between the number of oocytes in a clutch and the size of the oocytes. A follow-up 2-tailed t-test was performed on total oocyte counts per stage and total mean diameters for that stage.

The next round of tests focused on the gill parasites and their possible effect on stripedetail reproduction and health. An independent t-test was conducted for a possible relationship between gender and parasite count. The independent t-test is designed to compare the means from two seemingly unrelated test groups in order to determine whether there exists a statistically significant difference between the two. It is known that Aethycterorn parasites infect both males and females. Because only the females produce oocytes, subsequent tests involving parasite counts were limited solely to the 38 dissected females which met the following two criteria: (1) they were hosting at least one gill parasite, and (2) they were carrying a clutch of Stage 3 and Stage 4 oocytes. Paired samples tests were used to test for any correlation between parasite load and the sizes of the oocytes produced within the clutches of their host fishes. A second round of paired samples t-tests examined the relationship between parasite load and the number of clutch oocytes. These were also carried out per month of collection.

The somatic storage lipids percentage was examined. Because eight of the 60 randomly sampled fish had been too small to dissect and could not be separated by gender, they have been excluded from this study. The remaining 52 fish were comprised of 29 males
and 23 females. Mean percentages of lipid extractions for both males and females were calculated, and linear regression analysis was used to determine whether a correlation exists between somatic lipid percentages and gonadal mass in males and females. This was followed up with t-tests and then further linear regression analyses into possible correlations between lipid extraction and GSI values. These t-tests were broken down by gender and month of collection. Lipid percentages and somatic mass was tested in females using both linear regression and t-testing. The final lipid analysis involved linear regression testing for a potential relationship between lipids and parasite count.

All statistics for this study were completed using SPSS and Microsoft Office 2016 Tools.

III. RESULTS

The Shapiro-Wilk test for a relationship between SL and somatic mass was performed separately on males and females and returned a value of $p < 0.01$, leading to the conclusion that these data sets are not equally distributed. The Shapiro-Wilk test used to examine whether gender was equally distributed in the sample likewise returned a value of $p < 0.01$, indicating that our sample collected from Estill Fork consisted of more of one gender than the other. Table 1 illustrates the numbers of both genders collected from August 2016 to June of 2017.
Figure 2: Differences in length between male and female stripetails of reproductive age. Male average SL is 33.2 mm, with a range of 25.5 to 47.7 mm. Female average SL is 28.9 mm, with a range of 19.31 to 44.8 mm.

Table 1: Breakdown of dissected samples by gender and month collected

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III-A. Sexual Dimorphism in Somatic Length

The gender of each fish was determined through dissection and the excision of their gonads. Dissections were carried out initially on fish measuring 35 mm and greater SL. Of the 60 stripetails that met this criterion, 50 were male and 10 were female, a 5:1 ratio. This male/female imbalance was consistent for every month of collection except for the month of April in which none of the male fish captured exceeded 35 mm, but four females did. Reducing the length to 25 mm revealed less of a gender disparity, though males were still the predominant gender. 180 total stripetails were measured between 25
mm to 35 mm SL. Of these, 119 were identified as male, and 61 were identified as female, a 1.95:1 ratio. In an effort to determine precisely at which length *E. kennicotti* reached reproductive viability, the length at which a fish was dissected was reduced to 18 mm. This length was chosen after one researcher discovered viable oocytes inside a 19 mm female. The genders of only 13 stripetails could be positively identified at 19 mm SL, and all 13 were female. Reproductive viability was determined to be 19 mm as no gonads could be positively identified in the dissected fish within the 18 mm range. Of the 220 stripetails dissected, 142 were male and 78 were female, a roughly 2:1 ratio (Figure 2, Table 1). The mean length and standard deviation (SD) of the males was 33.33 ± 1.99 mm, and the mean length and SD of the females was 28.86 ± 3.80 mm. (Fig. 3).

![Length Comparisons Males vs. Females](image)

Figure 3: The mean differences in length (mm) between male and female stripetails depending upon the month of collection. Total mean length in males is 33.57 ± 1.99 mm, and total mean length in females is 29.72 ± 3.80 mm. An independent samples t-test performed indicated a strong relationship (p<0.01) between gender and SL. A similar p-value was observed (p<0.01) for the relationship between gender and somatic mass.
A Levene’s test for a relationship between the mean SL values between males and females confirmed a strong positive relationship between gender and length ($P < 0.01$). A similar test returned a similar p-value for the relationship between gender and somatic mass ($P < 0.01$). This is unsurprising considering there is also a strong relationship between SL and somatic mass in both males and females (Fig. 4a, 4b).

(a)

Relationship between SL and Somatic Mass in Males

\[
y = 0.0372e^{0.0824x} \\
R^2 = 0.7384
\]
Figure 4: Scatterplot demonstrating a clear relationship between SL and somatic mass in (a) male stripetail darters, and (b) females. A paired samples t-test of these two variables returned a p-value of $P < 0.01$ with a t-value of 93.55 with 449 df.

### III-B. GSI Values

GSI values were calculated for those fish from which gonads were extracted.

These values were plotted against month of collection for both males and females (Fig. 5).
Figure 5: The mean GSI by sex and month. Error bars indicate standard error of the mean. A Levene’s test for equality returned a P-value of <0.01 indicating a very strong relationship between gender and GSI for both males and females.

For males, whose GSIs were, predictably, lower than that of females, a small increase was observed in January. This was the only collection month in which the GSI value for males exceeded 1%. The male GSI value steadily declined after this. The female GSI values, on the other hand, spiked dramatically in the month of March, increasing from 3.3% in February to 20.24%. It is worth noting that the sample size for February (n=19 total, 9 males and 10 females) was lower than that of both January and March (n=36 and n=29, respectively). Because the highest GSI values were observed during the months of March, April, and May, this is apparently the breeding period for stripetail darters.
For every month of collection GSI was tested for correlations between SL and somatic mass. The results indicated that a strong relationship existed between GSI and SL in both males and females (p<0.001) with one exception. The p-value returned for females in March was p=0.32. The t-testing for GSI against somatic mass returned a very strong relationship between GSI and total body mass in females (p<0.001), but the relationship in males was mixed. For the months of August, February, March, May, and June, the p-value returned was p<0.001. October, December, January, and April ranged from p=0.08 in October to p=0.67 in December.

A strong statistical relationship was observed between GSI and total oocyte count for each female (p< 0.01). The relationship between GSI and lipid percentages differed by gender. In males, the weakest relationship was found in June (p=0.03), but was still statistically significant for a strong relationship. For females, this relationship was also strong, save for January and May, where the values were p=0.39 for January, and 0.14 in May. Every other month was p<0.003.

When a possible link between GSI values and parasite counts was tested for, a significant relationship was found (p=0.047), meaning that higher GSI values were accompanied by greater parasite loads. Breaking it down month-by-month, it was discovered that this strong positive relationship between GSI and parasite load held true for males for every month with the exception of June (p=0.69). For females, the link between GSI and parasite load was nonexistent initially (January: p=0.93, and February: p=0.81), but became very strong as females entered peak breeding season. The range of p-values for March through June, for females, was p=0.02 in June, and p<0.001 in May. This would seem to suggest that gill parasites are demonstrating a strong affinity for fish
with a higher GSI in general, but especially breeding females. This correlation between higher GSI values and higher parasite counts does suggest that parasitism and reproduction in males and females is closely linked. These results, in addition to the small SL and somatic weights in reproductively mature females, seems to points to an early reproduction maturation as a fecundity compensation mechanism.

III-C. Oocyte Development

All oocytes were examined and then classified into one of four developmental stages.

Figure 6: Stripetail oocytes in stages 1 through 4. Stage 1 (a) oocytes are translucent and small in size, typically between 0.3 and 0.8 mm in diameter. During Stage 2 (b) vitellogenesis begins and the oocytes darken as the vitellogenin proteins accumulate. By
Stage 3 (c) the oocytes have increased in size to between 0.8 and 2.0 mm in diameter. Vitellogenesis is complete, or nearly complete, at this point. At Stage 4 (d) the vitellogenin has condensed into a yolk leaving the ooplasm clear. An obvious invagination has formed along one side of the oocyte.

**Table 2:** Total oocytes and mean diameter per stage of oocyte development

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total Oocytes</th>
<th>Mean Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1708</td>
<td>0.73</td>
</tr>
<tr>
<td>2</td>
<td>1716</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>778</td>
<td>1.47</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>1.97</td>
</tr>
</tbody>
</table>

The total number of oocytes from each stage was tallied and the total mean diameter for each stage was taken. These values are listed in Table 2. The follow-up t-test between oocyte number and oocyte diameter returned a p-value of 0.04. Four linear regression analysis tests performed for a possible significant relationship between SL of the fish and total oocyte count per each of the four stages proved inconsistent for all months save for May when all four stages returned a value of p<0.001. In April, all oocytes but stage 2 shared the same strong relationship with SL. January, February, March, and June showed no discernible pattern between oocyte size and relationship to SL at all. January and March returned only one strong p-value (p=0.03 and p=0.02, respectively), and those were for stage 1 in January and Stage 3 in March. February had no strong relationships out of any of the four developmental stages. June was so devoid of clutch oocytes that it was impossible to get an accurate t-test value at all. Stage 1 oocytes in June returned a p=0.51, and stage 2 oocytes came back with a p-value of 0.07. For these reasons I am unwilling to say that a relationship between the SL of a female stripetail and the number of her oocytes exists.
III-D. The Effects of Parasite Load on Oocyte Size and Count

Parasite examination was limited only to the months of January through June of 2017 and only to fish that exceeded 25 mm in length. The extremely diminutive size of the gill arches significantly impaired our efforts to excise them from smaller fish using the tools we had on hand (Fig. 7). Attempts at gill arch removal from fish smaller than 25 mm resulted in destruction of the gill arches and much of the anterior end of the fish as well.

Figure 7: Undescribed species of monogenoidean gill ectoparasite belonging to genus *Aethycterion*. This parasite specimen was found within the gills of a stripetail darter collected from Estill Fork, Alabama (Stallsmith, 2016).

The first measure taken was the prevalence of infection by genus *Aethycterion* in stripetail darters (Fig. 8). These calculations, as well as the calculations for intensity of infection, were performed on both genders.
Figure 8: Percentage of fish infected with gill parasites broken down by month and gender. The sample size for females was $n=3$ in the month of June, and none of them were found to be hosting gill parasites.

Parasite intensity was determined by taking the mean number of parasites extracted per month and dividing the resulting value by the number of fish found to be infected for that month. (Fig. 9).
The overall independent t-test conducted on parasite and gender returned a p-value that was statistically insignificant (p=0.2), so while there may be a difference in the parasite load experienced by males and females, this difference doesn’t appear to be statistically significant for most months. It is worth noting that, when these tests were broken down month-by-month, April returned a p-value of 0.06. This still does not meet the alpha level established for rejecting the null hypothesis, but it is also unlikely that this is a coincidence given that April is also the month in which female GSIs are at their highest.
That having been said, the intensity of infection does vary between males and females depending on season, their life cycle, and likely the life cycle of the gill parasite. Parasites were found to be more numerous on those fishes possessing a higher GSI, females during their breeding cycles for instance. Females during the peak stripetail breeding month, April, were parasitized to a far greater degree than males were (Fig. 9). Otherwise, male stripetails bore the brunt of the infection as their larger sizes made them more appealing to parasites, and their immunocompromised state made them more vulnerable to parasitic attack. By April, the GSIs of males are on the decline, and so is parasite load.

Paired samples t-tests were performed for clutch oocytes of both stages, comparing each of them with the parasite count. This was done to ascertain whether or not parasite load has an effect on the size of clutch oocytes. The p-value for clutch oocytes was p<0.001, so, the null hypothesis, which would state that parasite load has no effect on the size of clutch oocytes that a stripetail female carries, is able to be rejected. Along with the significant p-value, linear regression analysis was used to try to confirm whether parasite load impacts the size of clutch oocytes (Figure 10). Though the scatter plot of Figure 10 doesn’t appear near as well-clustered as Figure 11, it would appear that gill parasitism does appear to have some kind of impact on clutch oocyte sizes.

If stripetails respond to gill parasitism in the same manner that sticklebacks do, then parasite load will directly impact the size of the clutch oocytes. The more parasites that a host organism carries, the more nutrients it is being robbed of. This ultimately means that this host organism is able to devote fewer of these crucial resources towards reproduction. There is some evidence to the notion that stripetails experiencing gill
parasitism produce smaller oocytes. Previously published works have recorded a mean diameter of 2.1 mm for clutch oocytes (Page, 1975). The mean diameter of clutch oocytes for our samples collected from Estill Fork stream was 1.98 mm.

Figure 10: Linear regression analysis between parasite load and the diameter of clutch oocytes (mm) found in the 38 dissected females. The p-value performed between parasite load and clutch oocyte size was $p<0.001$. 

$$y = -0.0165x + 1.5741$$

$R^2 = 0.0376$
Table 3: Total Clutch Oocyte Counts by Stage per Month along with Total Parasites Collected that Month

<table>
<thead>
<tr>
<th>Month</th>
<th>Average Clutch</th>
<th>Average Parasites Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan-17</td>
<td>18.5</td>
<td>9</td>
</tr>
<tr>
<td>Feb-17</td>
<td>17.8</td>
<td>6.2</td>
</tr>
<tr>
<td>Mar-17</td>
<td>19.3</td>
<td>8</td>
</tr>
<tr>
<td>Apr-17</td>
<td>13</td>
<td>3.5</td>
</tr>
<tr>
<td>May-17</td>
<td>11.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Jun-17</td>
<td>14</td>
<td>1.7</td>
</tr>
</tbody>
</table>

The tests performed to determine whether gill parasites affect clutch oocyte size were the tests conducted to determine what effect they might have on clutch oocyte count. Clutch oocyte counts were tested via a paired samples t-test, and each returned a p-value that did not exceed our alpha level of 0.05. Another set of t-tests, these broken down by gender and month collected, returned a more in-depth set of results. The p-values returned for the number of clutch oocytes a possible link with parasite load were overwhelmingly significant statistically, p<0.001. This suggests that parasitism by monogenoideans increases during those times when the number of oocytes carried by females is at its greatest. This further aligns with the results between parasite counts and fish, particularly females, with high GSIs. Two notable exceptions to the t-tests between parasite load and oocyte count are the stage 4 oocytes in the months of April and May. Both p-values for the stage 4 oocytes for those months exceeded 0.05. This, I believe, is due to the lack of stage 4 oocytes compared to the other stages because stage 4 oocytes are deposited by the female soon after reaching maturity.
The t-tests were followed up with linear regression analysis, plotting the number of clutch oocytes removed from the dissected stripetail females against the number of parasites recovered (Figs. 11 and 12). Like parasite sizes, both the t-tests and linear regression analysis points to a link between parasite load and clutch oocyte count.

Figure 11: Linear regression analysis between the total number of oocytes and the number of parasites found in 50 female stripetails. A t-test performed on these oocytes and parasites returned a value of $p<0.001$. 

$y = 2.9x + 55.14$

$R^2 = 0.1028$
Figure 12: Linear regression analysis between parasite load and the number of clutch oocytes found in the 38 dissected females. The p-value performed between parasite load and clutch count was \( p < 0.001 \).

Table 4: Mean Number of Oocytes and Mean Number of Parasites Collected per Month.

<table>
<thead>
<tr>
<th>Month</th>
<th>Average Clutch</th>
<th>Average Parasites Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-Jan</td>
<td>18.5</td>
<td>9</td>
</tr>
<tr>
<td>17-Feb</td>
<td>17.8</td>
<td>6.2</td>
</tr>
<tr>
<td>17-Mar</td>
<td>19.3</td>
<td>8</td>
</tr>
<tr>
<td>17-Apr</td>
<td>13</td>
<td>3.5</td>
</tr>
<tr>
<td>17-May</td>
<td>11.4</td>
<td>2.2</td>
</tr>
<tr>
<td>17-Jun</td>
<td>14</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Figure 13: Trendline representation of the relationship between the mean number of oocytes (Stages 1 through 4) collected from female stripetails and the mean number of gill parasites excised from them that same month.
III-E. Lipid Percentages

T-tests were performed in order to determine whether a higher concentration of storage lipids translates to having a GSI value. The resulting p-values were p<0.001 for both tests, indicating that a statistically significant relationship between the two variables does exist. The next step involved testing the percentages of lipid extracted from both genders (Fig. 14), focusing on the same factors of gonadal mass and GSI. For males, both t-tests confirmed a very strong relationship between the percentage of lipids extracted from the same fish and both its gonadal mass and GSI values (p<0.001 for each). T-tests broken down monthly confirmed the same. For all months tested, December through June all results were significant. For females, the results were mixed. The same monthly t-tests were performed for females and two of the seven months did exceed 0.05 (Jan p=0.4; May p=0.15). These results suggest that the relationship between lipids and GSI in males is fairly straightforward, but also that this is not the case for females. It is true that GSI values and lipids both fluctuate far more in females than they do in males, because the metabolism of females is focused on oocyte production. That may be a contributor to the weaker link between female GSI and lipid concentrations, particularly during the breeding months of April and May when oocyte growth and deposition is increased.

While the relationship between lipid percentage and gonadal mass (Figs. 15 and 16) confirmed a very strong relationship (p<0.001) in males, lipid percentage vs. GSI values (Fig. 17) returned the opposite (p=0.97), suggesting that there isn’t a relationship in females. I believe that this result is due to the high lipid percentage of stripetail
oocytes. Females with higher GSIs would have lower storage lipid levels because of the high lipid investment they make in these oocytes.

Because GSI is the percentage of the fish’s gonadal mass divided by its somatic mass minus its gonadal mass, I tested the lipid percentages of only the females against their somatic mass (Fig. 18) in an attempt to determine where the disconnect in the relationship takes place. The results from December through June primarily were significant, indicating a statistically significant relationship between the somatic mass of the female and their lipid content. The resulting linear regression analysis suggests that the greater the somatic mass of the female, the lower her storage lipid percentage was, possibly because she is capable of investing more of these lipids into her oocytes. May was the only month that did not return a statistically significant value (p=0.16). This testing could be better done taking into account seasonal effects on lipid composition with a larger sample size given the small sample used.
Figure 14: Mean percentages of lipids extracted from males and females per month of collection. (Stallsmith, unpublished data).

Figure 15: Linear regression analysis of the relationship between lipid percentage and gonadal mass in grams of the 29 confirmed male stripetail darters randomly sampled for lipid extraction. The p-value performed between lipid percentage and gonadal mass in male stripetails returned a result of $p<0.001$.

$$y = -1.324\ln(x) + 1.1296$$

$R^2 = 0.2231$
Figure 16: Linear regression analysis of the relationship between lipid percentage and gonadal mass in grams of the 23 confirmed female stripetail darters randomly sampled for lipid extraction. A p-value testing the relationship between lipid percentage and gonadal mass in females returned a result of p<0.001.
Figure 17: Linear regression analysis of the relationship between lipid percentage and GSI value (%) in of the 23 confirmed female stripetail darters randomly sampled for lipid extraction.

\[ y = -0.0551x + 9.8148 \]
\[ R^2 = 0.0085 \]

Figure 18: Linear regression analysis of the relationship between lipid percentage and somatic mass in grams of the 23 confirmed female stripetail darters randomly sampled for lipid extraction. The trendline suggests that larger stripetail females invest more of their lipid stores into oocyte production.

\[ y = -5.945\ln(x) + 3.6913 \]
\[ R^2 = 0.2455 \]
The research progressed to identifying what relationship lipids may possibly have with a host fish’s parasite load. An earlier t-test had confirmed that, overall, no significant difference existed between the extent of gill parasitism in males and females, so for testing parasite load against lipid percentages all 52 sample specimens were tested as one group. The results of the linear regression analysis and t-test appear in Fig. 19. Fish with a higher lipid percentage were found to have fewer parasites.

Figure 19: Linear regression analysis of the lipid extraction percentages of all 52 sampled fish vs. the number of parasites collected from them. The test resulted in a value of p<0.001, meaning that there is a statistically significant relationship between higher parasite load and a lower storage lipid composition.
III-F. Fulton’s Condition Index

Fulton’s Condition Index (FCI) is a method for determining the health of an organism, fish in this case, by using weight-length relationships (WLR). The assumption behind FCI is that a fish of a certain length should possess a proportional degree of fatness in order to be considered healthy (Froese, 2006). A fish that is either too fat or too thin for its length would not be considered in good physical condition. The exact interpretation and use of FCI have been debated for decades, chiefly because what is considered a “healthy” FCI value differs widely between species depending on their shape.

The Fulton’s Condition Index was taken for each specimen, but this study focused on those that were mature enough to classify as either male or female. Mean FCI values were taken for both genders for all nine collection months (Fig. 20). It was in the months of February and March that the mean FCI peaked for both sexes. Values for both sexes dropped sharply to 1.7 in April and remained low, especially the FCI values in males.

A subsequent t-test (P<0.001) between FCI and lipid percentage confirmed the relationship between FCI and “fattiness” in stripetails, meaning that a higher lipid composition results in a higher FCI.
Figure 20: Comparison of the mean FCI values for both genders per collection month. The spikes in FCI in February and March may mark the onset of the stripedetail reproductive period which spans from March through late May. FCI measures the relative fitness of a fish via a ratio between the fish’s length and body mass, so it’s not surprising that a t-test examining the relationship between FCI and lipid percentage returned a p-value of p<0.001 indicating a positive relationship between body mass, lipid percentage, and FCI.

A t-test was performed to examine the relationship between parasite count and FCI. This test was performed on a pooled sample of all specimens found to be carrying at least one parasite. The result was p=0.01, indicating that there is a relationship between fish condition and gill parasitism (Figs. 21 and 22). FCI for both males and females peaks in February and March, with high parasite counts in both sexes, but especially males. It is likely that the gill parasitism is the cause of the low FCI scores seen in all of the stripedetail specimens.
Figure 21: Relationship between FCI in stripetail males and the parasite intensity for each month of collection.

Figure 22: Relationship between FCI in stripetail females and the parasite intensity for each month of collection.
IV. DISCUSSION

The aim of this research was to study the link between stripetail darters and their gill parasites and determine what effect, if any, these parasites have on the stripetails’ ability to reproduce. This work proved challenging due to a variety of reasons. First, specimens were comprised solely of what could be captured during monthly collection trips to Estill Fork. In this way we could be certain that we were working with a sample representative of the overall stripetail population at Estill Fork, but it also limited the number and size of specimens examined. Secondly, while the life history and courtship behaviors of darters have been studied extensively, there is comparatively little literature available on reproduction in stripetails. Lastly, though there are some studies describing genus *Aethycterone*, no literature exists for the undescribed species inhabiting stripetail darters.

Through data collection and statistical analysis, relationships were established between gender and physical characteristics (length, mass), as well as gender and reproductive characteristics (GSI and maturation rate) in stripetails. The relationships between somatic storage lipids, somatic mass, and gonadal mass were explored. Finally, links were discovered between parasite count and both the size and number of clutch oocytes confirming that parasitism does influence reproduction in stripetails. Lastly, parasite load was also linked to lipid percentage and to FCI, indicating that intensity of infection may very well have an adverse effect on general health in our test specimens. These results are consistent with the studies cited earlier on this thesis.
IV-A Sexual Dimorphisms and Oocyte Development

Before we could ascertain what the effect of parasitism on reproduction could be, it was necessary to determine how the two genders differed. The results of the Shapiro-Wilk tests for normalcy among males and females were unsurprising. Far more males of larger sizes (>25 mm) were dissected than were females who were typically smaller (169 males compared to 84 females). This is evidence of a clear sexual dimorphism in size and also a large discrepancy in count between males and females. Page (1975) published very similar findings. During his studies of the stripetail population in Big Creek, Page noted that males of the species grew at a much faster rate than did the females, far exceeding females in size by their third year of life (Page, 1975). Another fascinating observation made by Page was that before a year of age, when both sexes still qualified as young-of-the-year, females outnumbered males 1.4 to 1 (Page, 1975). They continued to outnumber males by 1.7 to 1 as adults of 1+ year old, but by year 2 males predominated (Page, 1975). Both genders declined significantly between one and two years of age, but females died out in greater numbers (Page, 1975). He offered no explanation for the swift decline in population from one year to two years of age, except to say that stripetail males seem to possess a greater longevity than females (Page, 1975). Page concluded his study of stripetail aging by noting that none of the stripetails, male or female, that he studied lived to three years of age (Page, 1975). A lifespan of two years is not uncommon among smaller darter species, but the discrepancy we’ve observed between the numbers of males and females past their first year of life suggests that females are not surviving as long as their male counterparts. This was our first indication that there was a possible trade-off being made in females that wasn’t being made in males.
Our study found far fewer females at 1+ year of age than we did males, but it is also true that only 220 of the 449 fish captured were large enough to be dissected (>18 mm). The remaining 229 stripetail specimens were unable to be included in most of the tests. The end result of only being able to count the 229 dissected specimens was a distribution gender-wise that differed from Page’s. Had we been able to collect more fish between the 18 mm and 25 mm length range, we doubtless would have been able to examine more females and this distribution between genders would have been more equal. Having access to more literature on stripetail development from larva to adulthood would really have benefitted this study at this point.

During the month of March, the onset of the three-month breeding period in Estill Fork, GSI in females reached a mean of 20.24%, meaning that a fifth of their total body mass upon capture was due to the oocytes they carried. In males captured in the same month, the mean GSI was 0.38%. GSI values in females plummeted during non-breeding months, but they still far exceeded those of the males (Fig. 5). Such a high GSI is the direct result of the large number of early stage oocytes, and then later also in the size of the late stage clutch oocytes. In late March, when mean GSI values in females were at their highest, 21.5 mean oocytes were collected per female. Out of our sample size of 12 females, all of them carried clutch oocytes. During the month of April, 16 mean oocytes were collected per female, but the mean GSI had decreased to 15.2%. Every female (n=22) was carrying clutch oocytes. During the final breeding month, May, the number of oocytes collected from the eight females had decreased to a mean of 9.4. All were still carrying clutch oocytes. All eight still carried stage 1 and/or stage 2 oocytes. The mean
GSI for May was 8.2% for females. By June, GSI in the three collected females had returned to low pre-breeding period levels.

Page (1975) observed that spawning in the stripetails at Big Creek began on the 5th of April. Our March collection took place on the 25th of March, 11 days before Page observed the first spawning at his location. All 12 of the females in our collection had clutch oocytes. By our April collection, which took place on April 29th, all collected females ($n=23$) females had clutch oocytes. This suggests that stripetails at Estill Fork, just like those in Big Creek, begin spawning either in late March or early in the month of April. These findings align well with those of Page when it comes to oocyte development. However, some discrepancies were noted when it came to rate of ova development as it relates to size in females. Page (1975) observed that older females, those that measured 30 mm in length and longer, developed mature ova earlier in the season than smaller females, those as short as 27 mm. In this project stripetail females as small as 19 mm were observed to be not only producing ova, but they typically produced mature ova at the same rate as larger females. One exception that bolsters Page’s claims is the female designed EKC-22, the lone female found to be without clutch oocytes. She was the smallest female dissected at 19.31 mm. Her GSI was 10.92%, below the mean of 20.24%, but still far greater than GSIs observed in many darter species. By contrast, the highest GSI recorded in April, 74.6%, belonged to a female of 24.41 mm of length with a somatic weight of 0.27 grams. She was found carrying 12 clutch oocytes. The fact that we were finding clutches of mature oocytes in fish measuring below 20 mm, a size that is still considered juvenile in most of the other members of genus *Etheostoma*, is
fascinating, but also concerning in that it lends credence to the idea that an early sexual maturation is taking place among this population.

IV-B Lipids and their Contribution to General Health and Reproductive Success

Fulton’s Condition Index (FCI) is a measurement of the fitness of a fish given its standard length and somatic mass. A portion of this somatic mass can be attributed to storage lipids. Storage lipids are those triglycerides and other fatty acids that have been stored by the body for future energy use which may include growth, maintenance, and reproduction (Heulett et al., 1995). A fish that has eaten well during non-breeding periods will have more fat reserves held over than a fish that has eaten less. Figure 14 illustrates the percentages of storage lipids that were extracted from the 60 randomly sampled stripetails throughout the months of December through June. It is during the month of December that the lowest mean percentage of storage lipids (4-5%) was extracted from the samples. This may be because only six specimens were examined. It is also possible that this is caused by a lack of food items, but this is unlikely given that many of their preferred food sources, such as the larvae of midges and stoneflies, are capable of surviving underwater through the winter (Page, 1975). Stonefly nymphs made up the bulk of the diet of stripetails in Big Creek, Illinois in December and January (Page, 1975). An examination of the stomach contents of the stripetail population in Estill Fork was not conducted, but it stands to reason that the residents of that stream would also have a food source available to them throughout the winter. As with the Drosophila studies referenced earlier in this work, it is very likely that a lack of food during the winter
months may act as a limiting resource. This situation can only be exacerbated by exposure to gill parasitism.

The amount of lipids extracted increased from a mean of 4–5% in December to between 10 and 12% in both genders during the month of February. This is the period of time in which large numbers of oocytes were produced, but the majority of them were still in stages 1 and 2. Vitellogenin, a phospholipoglycoprotein, is being produced by the females for packaging into the developing oocytes. Among males, this is the month just before the scramble to secure nesting sites. Because they cannot hunt while guarding nests, they would spend this time hunting and building up their fat reserves. Lipid levels decrease in March to approximately 8%. In females vitellogenesis is nearly complete and vitellogenin has accumulated within stage 2 and stage 3 oocytes. Males spend March competing with other nest-guarding males for nesting spots, relying upon their energy reserves instead of hunting for food.

During the month of April, the percentages of storage lipids extracted from males and females differed dramatically. Mean lipid extractions in females fell to 5% while the percentage of lipids extracted from males jumped to 13%. Females would be mobile during this time and able to hunt for food between oocyte depositions, but since they are still engaging in oocyte production, their lipid stores are still going toward vitellogenesis. Males who were able to secure a nest and fertilize oocytes now have a readily available food source and are likely engaging in filial cannibalism.

During the final breeding month, May, oocyte production is on the decline. It is possible that instead of allocating their lipids toward vitellogenesis in their remaining oocytes, females may be reabsorbing their less developed oocytes, reclaiming those vital
nutrients for themselves or to increase the chances of more developed oocytes. There exists no direct evidence for this, however. Males would still have access to fertilized eggs within their nests likely through both May and June.

Figure 20 demonstrates the mean FCI percentages by month for each gender. FCI, as a measure of a weight and length, is closely tied to lipid content. Like the lipid extraction chart (Fig. 14), the FCI percentage chart shows a lower percentage before and after breeding periods. Though both also show a sharp spike in the month of February, FCI percentages are still high (>2.00) in the month of March while lipid levels fall. One reason for the discrepancies between lipids and FCI is the presence or absence of oocytes in the calculations. While the oocytes had been removed before the 60 samples were processed for lipids, FCI calculations take total somatic mass into consideration. This includes the gonadal mass. A fish’s length and weight measurements could indicate a high FCI value while still possessing a low lipid content if a large amount of their lipids were sequestered within oocytes. Consequently, the highest FCI percentages for females, indicating a greater degree of fitness, were during February and March when they still contained the greatest amount of vitellogenin and maturing oocytes. As the stripetail breeding period progresses, and oocytes are deposited, the FCI of females decreases. For males, the highest FCI value indicated was in February when they would have been producing large concentrations of testosterone and increasing their fat reserves in preparation for three months of fighting, mating, and nest-guarding. Consequently, February is also the month when the highest mean number of parasites was collected, providing evidence for the immunocompetence handicap hypothesis. FCI in males decreases after that, as does parasite load.
IV-C  Fecundity Compensation: A Solution to the Parasite Problem?

Research analysis carried out during this work centered on finding and examining possible relationships between oocytes, lipids, and parasites. Strong relationships exist between parasites and lipid extraction percentages, FCI values, and the size and count of clutch oocytes. This suggests that parasites play an integral role in the life and reproduction of stripetails, to the detriment of their hosts.

So, what part do monogenoidean gill parasites play in the life cycle of a stripetail darter? The prevalence of infection in stripetails was high. Between 30% and 90% of the population acted as hosts from January to May (Fig. 8). On top of that, infection intensity was also high with infected fish hosting, on average, between 1 and 10 parasites (Fig. 9). The fact remains, the exact effect that these gill parasites have on their hosts is unclear. The low FCI values for the collected fish, combined with the low lipid counts for both males and females suggest that some factor is at work that is proving deleterious to fish health.

The strong link between parasite count and the percentage of extracted lipids provides a likely explanation. It is likely that the gill parasites gain nutrients by siphoning away the nutrients essential for the development of lipids. This, in turn, would directly affect the ability of the fish to store fatty acids for future use, and would impair the production of a phospholipoglycoprotein like vitellogenin. Siphoning away the building blocks for other macromolecules like carbohydrates, proteins, and nucleic acids would ultimately have the same effect on oocyte development and fish health.
The size of the oocytes provides another likely explanation for the low levels of lipids found in females. Testing the lipid content of the oocytes was outside the scope of this study, which focused instead on the percentage of lipids extracted, and the size and count of oocytes in relation to the intensity of parasitic infection. In future experiments of this sort, it may prove beneficial to perform a more comprehensive analysis of the oocytes. In fish species like stripetails in which the oocytes are especially large and yolky, this type of testing could prove especially enlightening.

The highest mean number of oocytes collected (\(n=128.1\)) was in February, but the greatest mean number of parasites was in March (Fig. 13) when there are a greater number of clutch oocytes. Similarly, in months where the mean number of oocyte numbers decreased, we also saw a decrease in mean parasite numbers. This correlation was confirmed by t-tests for clutch oocytes (\(p<0.001\)). The linear regressions (Figs. 11 & 12) provides even more evidence for the relationship between parasite count and the number of clutch oocytes. Figure 10 indicates that parasite count has some kind of effect on the size of clutch oocytes in millimeters (\(p=0.003\) for stage 3 and \(p<0.001\) for stage 4).

Figure 13 indicates a correlation between the mean number of oocytes collected in a month and the mean number of parasites collected in the same month (\(p=0.01\)). A total of 725 oocytes were collected in February, 89 clutch oocytes, 232 stage 2s, and 404 were stage 1. Vitellogenesis was in full effect, but the average GSI for February was only 3.33% so the majority of these developing oocytes were still quite small. The two mean largest number of parasites collected (\(n=9.0\) and \(n=7.6\)) were in January and March. Even so, mean parasite count dropped slightly (\(n=6.2\)), in February, and then continued to drop after March. I will admit to being a little confused on this aspect of the oocyte/parasite
relationship, but what the decline in mean oocytes and parasites beginning in April seems to suggest is that as mean oocyte counts drop (presumably as mature clutch oocytes are being deposited) mean parasite numbers also seem to decline. This graph also seems to depart a bit from the results of earlier statistical analysis which indicated that as GSI values increase, so do parasite loads. It could be that GSI values are highest in April, even as mean oocyte numbers are declining because of the size of the clutch oocytes and not oocyte count.

A relationship between parasites and oocytes being apparent, the question then becomes: does parasitism by monogenoidean gill parasites cause a directly negative impact on oocyte size and clutch count by siphoning off material crucial for the development of those oocytes? Considering how large clutch oocytes can grow (up to 2 mm each), it’s difficult to imagine them growing any larger. Or is there a more indirect process at work here?

Evidence for fecundity compensation among stripetails might be found in their high GSI percentages, large oocyte sizes, and small clutch counts. Among darters, who frequently produce a large number of oocytes at a time and then release them all in the water to be fertilized, carrying 2.0 mm diameter oocytes is a challenge, particularly given how small stripetails are. One member of the darter genus, the saffron darter, *Etheostoma (Ulocentra) flavum*, has been known to produce mature oocytes of 1.5 mm in diameter, but saffron darters don’t reach maturity until they are at least 27 mm in length (Simon & Wallus, 2006). 27 mm is a far cry from 19 mm. One darter species, the least darter, *Etheostoma microperca*, has been observed to have GSI values reaching as high as 35%, but they also don’t reach maturity until approximately 29 mm (Johnson & Hatch, 1991).
The larger GSI value is the result of many smaller clutch oocytes (88 per clutch) rather than few large ones, as we see in stripetails (Johnson & Hatch, 1991).

Sticklebacks, in the face of an impending loss of fertility caused by parasitism, shifted from producing fewer, larger oocytes, to generating a greater number of smaller oocytes. By producing many more oocytes than they normally would, sticklebacks are guaranteeing current reproductive success because there may not be future reproduction. The atypically large oocytes found in stripetails may also be the result of fecundity compensation, albeit in a different form from that seen in sticklebacks. Stripetails are a nest-guarding species and, as such, don’t need to produce a large number of oocytes because the fertilized eggs will have a male parent to care for them and help ensure their survival. For that reason, instead of producing a larger brood, as the sticklebacks did, stripetail mothers might have accomplished the reverse and invested their precious resources in fewer, but larger, oocytes. This mode of reproduction is common in nest-guarding fishes.

The small lengths and masses found in reproductively mature stripetail females points to an early maturation rate in these fish. It is possible that, as with the water flea, *Daphnia magna*, this early sexual maturation in stripetail females may be a form of fecundity compensation in response to parasitism by genus *Aethycteron*. Females experienced the greatest prevalence of infection and intensity of infection in the months of January and April. The females dissected in this study were found to be carrying oocytes in January. December females were also examined, but most of the fish collected in this month were too sexually immature to classify. It is likely that many young stripetail females reach sexual maturity between the months of December and January of their first year. A
fecundity compensation hypothesis in this instance would suggest that the increase in parasite load experienced by the stripetail population in January results in an early maturation of stripetail females at such small sizes as 19.3 mm.

**IV-D The High Cost of Reproduction: A Shift to an Annual Life History**

One final, but extremely important, factor to consider when exploring the relationship between the stripetails and their gill parasites is the survival rates of the stripetails, especially how they relate to cost of reproduction. Page (1975) observed that far fewer of his female stripetail specimens survived to 2 years old than did the males. It is not uncommon for a darter to survive into a second year and never reach a third, parasites aside. What was surprising about this study was how few females we found at the 33+ mm size range compared to smaller sizes (Fig. 2). Because size is an indicator of fish age, this lack of larger females directly translates to a lack of older females. This may be a result of a smaller fish not being able to compete with the larger fish in their environment, but it may also be evidence of another factor, that of the sheer costs involved in reproduction. Stripetail females are reproductively mature as early as 19 mm in length, far smaller than other darter species. This shift to an early sexual maturation is likely a result of fecundity compensation in response to gill parasitism. Ultimately, in this regard, their life history differs from other darters, but it also differs in regards to their atypically large oocyte sizes and extremely high GSI values. Both of these factors indicate a high investment in current reproductive success.

The lack of females in older age categories suggests that such a high investment made by stripetail females during their first reproductive year comes at the expense of not only...
future reproduction, but even at the expense of their own survivability. This may very well be an example of the cost of reproduction hypothesis at work. A potential tradeoff has been made between longer life and reproduction here, just as it had been made in the *Drosophila* studied in previous experiments. Nutrients crucial for maintenance of immune responses, energy reserves, and other bodily functions necessary to ensure survival of the mother have, instead, been allocated toward survival of her young. The end result is that, though stripetails, as a species, are capable of living to their second year of life (and some females do), functionally this heavy investment in reproduction may be relegating female stripetails to an annual life history.

This hypothesis, and the large oily appearance of the clutch oocytes, were the driving forces behind the decision to perform the lipid extraction portion of this research. What was discovered through limited lipid extraction (Fig. 14) was that females experience a drop in storage lipids leading up to their peak breeding period, ultimately bottoming out at approximately 5% in April. Interestingly enough, lipid percentages in females spike in May, reaching approximately 18% before dropping back to 8% in June. Whether this spike and subsequent drop is a result of these fish hunting for food and then storing their lipids away into their remaining oocytes, or accessing existing fatty acid stores and burning them for energy, or simply a fluke by-product of a low sample size, I cannot say. We did not collect stripetails after June 2017 and knowing how lipid levels progressed during the fall and winter months would have been helpful towards this study. However, we do have lipid levels from fishes collected in December of 2016 and those, like April, are exceedingly low (4.5% for females). It is not difficult to imagine that food sources through the winter would be scarce, which makes the accumulation of fat reserves
throughout the rest of the year even more of a necessity. A fish who was unable to gather these reserves would find themselves at a much higher risk for mortality in times of food scarcity, particularly if these fish were straddled with the added burden of gill parasitism.

IV-E Conclusion

One thing that was made clear from this research was that parasitism by flatworms belonging to genus *Aethycterum* has been linked to virtually every facet of a stripetail darter’s reproductive life, from the presence of the parasites impacting the size and count of stripetail oocytes, to the substrate in which oocytes are deposited serving as the same nursery for developing flatworm eggs.

Whether or not early sexual maturation in stripetail darters is the result of a form of fecundity compensation, as is the case with *Daphnia magna*, or whether stripetails compensate for gill parasitism by investing more resources into their developing oocytes than they would had they not been exposed to genus *Aethycterum*, it is clear that exposure to gill parasites has altered the life history of stripetails in a way that is deleterious to their survival. The number of females surviving past their first reproductive year is low. FCI in nearly all of our specimens, was highest as the reproductive period begins, but were otherwise low. Beyond that, determining the exact effects that this species of flatworms has on stripetail health and reproduction is still not known, precisely. Perhaps a longer study, utilizing more samples of both species would have proven helpful.

Had both stripetails and this particular *Aethycterum* species been better studied, that would also have undoubtedly been of assistance in this study. Until further studies
into the nature of parasitism demonstrated by *Aethycterion* are published, understanding the specific mechanisms by which these flatworms function within their host, how they gain their nutrients, and what the long-term prognosis is for infected stripetails will be difficult to determine.
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