Perpetua: The UAH Journal of Undergraduate Research

Volume 2 | Issue 2 Article 7

5-1-2017

# Observations on the Reproductive Biology of the Darter Fish Etheostoma kennicotti in Response to Gill Parasite Infections

Zeina Sleiman
*University of Alabama in Huntsville*

Corinne Peacher
*University of Alabama in Huntsville*

# Observations on the reproductive biology of the darter fish Etheostoma kennicotti in response to gill parasite infections

Zeina Sleiman and Corinne Peacher
Department of Biological Sciences

***Abstract –*** *Etheostoma kennicotti*, the stripetail darter, is found in streams in Tennessee, Ohio, Kentucky, Illinois, Alabama, Georgia, and Mississippi. The specimens for this study were collected in Estillfork in Jackson County, Alabama from August 2016 to June 2017 [12]. After collection and dissection, data were found to illustrate a peak reproduction period during the months of March to May 2017. The purpose of this study is to determine whether or not parasitic infection affected fecundity within the species as well as to contribute information for *E. kennicotti*, an otherwise poorly studied species. Parasitic load was found to be highest in the month of March and steadily decreased as the reproductive season progressed. Similar trends were found in the mean intensity of infection. There was a negative correlation between mean intensity and clutch size in that the clutch sizes increased into the month May, the end of reproductive season.

## I. Introduction

Fecundity compensation is a non-immunological defense mechanism against parasite infections [11]. It is defined as a triggered response to parasitic activity by the use of an increased clutch size with a drawback of decreased size of the oocytes [8]. This may happen under the theory that the increase in fecundity has a lower energy cost than maintenance of an immune system [5]. The fecundity compensation may be a result of the *Aethycteron* species gill parasite infection in the host *Etheostoma kennicotti*, the stripetail darter.

Similar research with *Etheostoma flabellare* [8], a close relative of *E. kennicotti*, suggests that the Monogenean gill parasite, *Aethycteron moorei*, or a parasite of the same genus, may inhabit *E. kennicotti* as suggested in a study that compared two similar species and their microhabitats [7]. The assumption can be drawn due to the concept of the monogenean species' capability of shared microhabitats, with different preferences such as morphological attachment organs and an abundance of various parasites being the only restriction on niches and not interspecific interactions. We assume that an undescribed *Aethycteron* species inhabits the gills of the host, *E. kennicotti*, enabling us to determine if there is possible evidence of the fecundity compensation hypothesis.

The genus *Aethycteron* falls under Monogenea which is a phylum subclass of Trematoda, a Class that consists of primitive flukes with monozoic bodies and an affinity for fish [9]. This Class has no digestive tract or any internal cavity for the parasites' internal organs, instead the internal organs are encompassed in the parenchymatous tissue. The *Aethycteron* parasites attach to the gill tissues through an attachment organ comprised of hooks, typical of this subclass.

*Trematodes* have two different effects on fish through the causation of non-immunological defense: complete castration, or a reduction of the host's reproduction, instead of complete nullification [4]. *Trematodes* in one study apparently caused the reduction of reproductive output and physiological condition of mussels due to the parasites' castrating capabilities [3]. The capabilities of the host to have a compensatory mechanism created would enable the continuation of a species despite parasitic abundance or pressure, through fecundity compensation.

With the possibility of a non-immune defense mechanism in *E. kennicotti*, the host can be studied. Currently there has not been any extensive research done on this particular species. The only prior information available was that of *E. kennicotti's* habitat of hiding under rocks and underbrush in small creeks, which guided our finding and collection of the fish.

## II. Materials and Methods

### Collection and Storage of *Etheostoma kennicotti*

All *E. kennicotti* specimens were collected monthly for eleven months, August 2016 to June 2017, from Estillfork (34 54 38'N 86 10 04'W 437 m) in Jackson County, Alabama. The *E. kennicotti* were distinguished from other members of genus *Etheostoma* by a characteristic striped pattern on each of *E. kennicotti*'s caudal fin. The fish were euthanized

using 10 mL of 10% clove oil and ethanol solution for every 200-300 mL of water in the holding bucket of the collected fish. All samples were given an identification number and fixed individually in 10% phosphate buffered formalin.

**Identifying Parasite and Count**

The gills of each sexually matured *E. kennicotti* were extracted under a Motic K Series dissecting microscope at 50X by opening the outer gill flap with a probe and, with the use of a scalpel blade, making cuts around each of the gill arches to disconnect them from connective tissue. The gill arches were removed and observed under an Olympus SZ7 dissecting microscope. The eight gill flaps were flipped through and each *Aethycteron* parasite was counted and removed from the flaps. The parasites and gill arches were stored in corresponding ID tubes in 10% formalin. The parasite counts were used in calculating parasitic load to determine percentage of infection and mean intensity to determine if either gender was infected more heavily and in which months.

**Body Size and Reproductive Data**

Each *E. kennicotti* was measured in terms of somatic weight and standard length (SL). The somatic weight was taken before any dissection with the use of an Ohaus Explorer balance to 0.0001 gram. The SL was also done before dissection and measured from the snout to the caudal peduncle [7] and measured to the 0.001 mm.

Originally, dissections were limited only to specimens of 30mm length or greater on the basis of previous experiments on the larger species of *E. flabellare*. However, with finished dissections of all the collected *E. kennicotti* samples of 30mm length and longer, we observed a strong male bias. Thus, with an apparent sexual dimorphism, the dissections were adjusted to include specimens of 25mm in length and greater. This small change in procedure resulted in the dissection of a far greater number of female specimens, confirming our suspicions that a dimorphism related to body length between the sexes does exist. Unlike many other species of the genus *Etheostoma* that demonstrate full reproductive maturity at a length of 30mm, females of *E. kennicotti* exhibit sexual maturity at 25mm or less.

The pectoral fins were removed in order to ensure an easier and cleaner dissection. The primary incisions were made below the lower jaw of the fish, continued down the length of the abdomen, and terminated at the pelvic fins. The testes were located underneath the intestine and were attached to the stomach via mesorchium. Typically, the testes were translucent and small in size, which meant that their general location and connectivity within the abdominal cavity was crucial to identifying the reproductive organs.

In contrast to the testes, the ovaries of the females were quite large and were easily discernible from the surrounding organs. The gonads were weighed to 0.0001 gram upon removal and stored in tubes of 10% formalin. The ovary of an *E. kennicotti* may contain upwards of 20-30 mature oocytes. Photographs of all the gonads and the oocytes were made with the use of the Olympus SZX7 stereo microscope and extra illumination from a goose neck lamp. With the oocyte photographs, the Egg Helper program was used to count all oocytes and five oocytes of each developmental stage were measured. Depending on their stage of development, the oocytes progressed in color and size from small and nearly translucent to a bright yellow, indented orb approximately 1 mm in diameter.

## III. Results

Once the mass for both the somatic body and the gonads were obtained, the gonadal weight values were used in conjunction with the somatic weight of the fish to determine the gonadosomatic index, or GSI, for each specimen. The equation used to calculate GSI:

**Equation 1** - GSI

$$\frac{\text{Gonadal Weight}}{(\text{Total Somatic Weight - Gonadal W eight})} \times 100$$

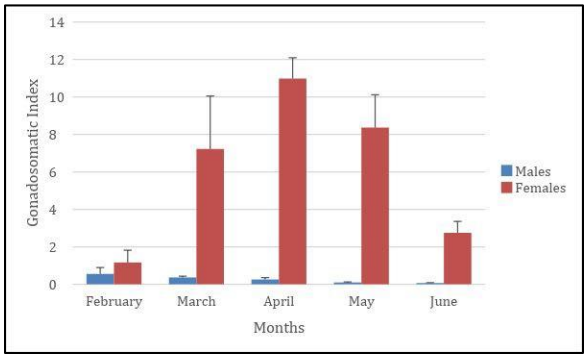


**Figure 1:** GSI
*Through the usage of their GSI, as seen in Figure 1, there was a normal distribution of GSI between the months of February and June with April being the peak month of reproduction. Using this observation, these five months were the focus of this ongoing study. Error bars represent the standard error.*

Through the extraction of the gills, fish found with at least one *Aethycteron* parasite were totaled and divided by the total number of fish collected for each month to determine the percentage of infection.

**Equation 2** - Percentage of Parasite Load

$$\frac{\text{Total Number of Infected Fish}}{\text{Total Number of Fish}} \times 100$$

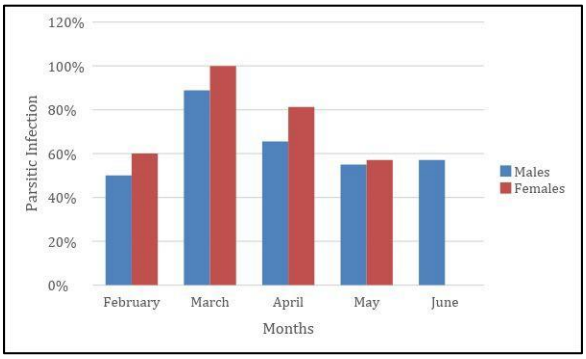


**Figure 2:** *Percentage of infection during reproductive season. The parasite load peaked in March and decreased drastically in April, then had an increase recovery in May.*

Determining mean intensity was done by totaling the number of parasites in the infected specimens and dividing the total by the number of affected fish per month.

**Equation 3 –** Parasite Mean Intesity

$$\frac{\text{Total Number of Parasites}}{\text{Total Number of Infected Fish}} \times 100$$

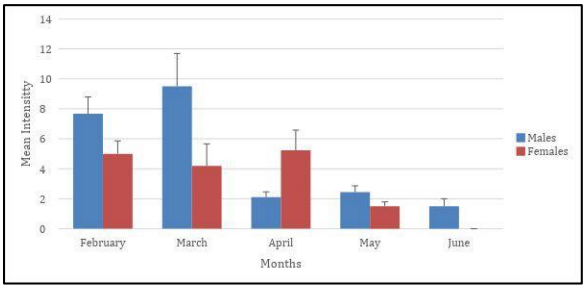


**Figure 3:** *Mean intensity of parasite load of male and female fish during peak reproductive season. The trend illustrates males holding a higher intensity for two out of three months with the exception being in April. Another trend seen in the mean intensity can be pictured to support the parasite load percentage in* ***Figure 2****, which holds a strong positive correlation. The trend of mean intensity is seen decreasing throughout the months portraying the correlation with the decreasing trend of the parasite load.*

The eggs in females were categorized into four different types: latent, maturing, mature, and mature ripening. After type classification, clutch size was determined to be the number of type III and type IV ooctyes. **Figure 4** shows the average in clutch counts in each month of the reproductive season.

**Equation 4** – Clutch Count Average

$$\frac{\text{Total Number of Type III and Type IV Oocytes}}{\text{Total Number of Oocytes}}$$

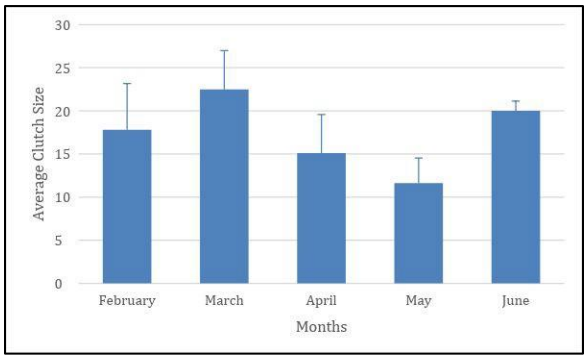


**Figure 4:** *Average clutch size with error bars representing the standard error.*

**IV. Discussion**

With a high diversity of the darter species, little research on the life histories of the host-parasite relationships has been conducted [5]. This is true of *E. kennicotti* and its relationship with the *Aethycteron* gill parasite, creating a line of research that can provide further evidence on whether the fecundity compensation hypothesis is true.

Due to the lack of research done on *E. kennicotti*, reproduction periods were initially only assumed. GSI values were first calculated to determine the effect of parasitic infection on reproduction patterns. GSI was determined and the trend portrayed in **Figure 1**, providing data that the reproduction peak periods are in March through May because of the female ovaries growing heavier with maturing oocytes.

Parasite abundance appeared to have a seasonal pattern in the host, *E. kennicotti*, due to their temperature specificity. Water temperatures in Estillfork ranged from 12° C in February to 25° C in June, but the life cycle of monogenean parasites are known to be temperature dependent with the optimal temperature range of 14° to 20° C [9]. Temperatures ranged from 16° C to 23° C throughout *E. kennicotti's* reproductive season, which largely correlates to the optimal temperature range for the reproduction of the monogenean parasite. As represented in **Figure 2,** the highest percentage of parasitic load occurred in the month of March and slowly decreased into May, which can be explained by the combined variables of optimal water temperature and host reproductive conditions, enabling the monogenean infection to peak in the month of March and then slowly decrease in May as water temperatures increased. The trend in **Figure 3** also confirmed this correlation with March having the higher mean intensity in males and slowly decreasing into the months of April and May.

According to Page [10], males guard the eggs during the reproductive season portraying a different behavioral pattern than the females, consistent with the discovered trends shown in **Figure 3** and **Figure 4.** The parasite *Aethyceteron* would have a higher rate of infection in males during the month of March due to optimal water temperatures and higher host activity.

Fecundity compensation is the post-infection mechanism of an organism's reaction to a parasitic infection. The change in reproduction rather than immunological activity is the key to identifying fecundity compensation, but we are yet unable to make a strong conclusion with *E. kennicotti*. Reproduction time and pattern of parasitic association can be confirmed. With such close association between reproductive timing in the host *E. kennicotti* and its *Aethyceteron* parasite, it can be concluded that the parasites have matched their reproductive patterns similar to their host to enhance higher success in parasitic infection due to the energy costs of reproduction within the host [1]. Further research is needed on *E. kennicotti* to confirm direct correlation between parasitic infection and fecundity.

**References**

[1] Adrian A.B, Holmes B., and Stallsmith B.W. (2012) Impact of a Gill Parasite upon the Minnow *Notropis telescopus. Southeastern Naturalist* 11(1): 35-42

[2] Baker, J. A. et al.(2015) Life-History Plasticity in Female Threespine Stickleback, *Heredity* 115, no. 4: 1–13, doi:10.1038/hdy.2015.65.

[3] Feminella, Jack W. (2008) Parasitic Mite and Trematode Abundance Are Associated with Reduced Reproductive Output and Physiological Condition of Freshwater Mussels, *Hydrobiologia* 610: 25–31, doi:10.1007/s10750-008-9419-8.

[4] Granovitch, A. I., E. B. Yagunova, A. N. Maximovich, and I. M. Sokolova. 2009. Elevated Female Fecundity as a Possible Compensatory Mechanism in Response to Trematode Infestation in Populations of Littorina Saxatilis (Olivi). *International Journal for Parasitology* 39 (9): 1011–19. doi:10.1016/j.ijpara.2009.02.014.

[5] Hanson, Robert H. (2013) Patterns of Infection by Monogenodians in an Assemblage of Darters, *Journal of Freshwater Ecology* 28: 3, 385-396.

[6] Heins, David C. (2012) Fecundity Compensation in the Three-Spined Stickleback Gasterosteus Aculeatus Infected by the Diphyllobothriidean Cestode Schistocephalus Solidus, *Biological Journal of the Linnean Society* 106, no. 4: 807–19.

[7] Kadlec, D., A. Šimková, and M. Gelnar. (2003). The Microhabitat Distribution of Two Dactylogyrus Species Parasitizing the Gills of the Barbel, Barbus Barbus. *Journal of Helminthology* 77: 317–25. DOI:10.1079/JOH2003183.

[8] Million, Kara M., Crissy L. Tarver, Sean Hipe, and Bruce W. Stallsmith. (2017). Does Infection by the Monogenean Gill Parasite *Aethycteron Moorei* Affect Reproductive Ecology of the Darter *Etheostoma Flabellare* in Mill Creek, Tennessee? *Copeia* 105 (1): 75–81. DOI:10.1643/CE-16-403.

[9] Olsen OW. (1968). Types of Monogenic Trematodes, in *Animal Parasites: Their Life Cycles and Ecology.* 199. Mineola (NY): Courier Dover Publications.

[10] Page, L. (1983) Handbook of Darters. T.F.H. Publications, Inc. USA. 271 p. 163

[11] Parker, Benjamin J et al., Non-Immunological Defense in an Evolutionary Framework, *Trends in Ecology & Evolution* 26, no. 5 (2011): 242–48, DOI:10.1016/j.tree.2011.02.005.

[12] Simon, T.P. and D.J. Faber. (1987) Descriptions of Eggs, Larvae, and Early Juveniles of the Iowa Darter, *Etheostoma exile* Girard from Lac Heney, Quebec. *Canadian Journal of Zoology* 65: 1264-1269.