University Honors Program Research Project

APPROVAL PAGE

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Several Studies on Dactylogyrus and Their Hosts: *Lythrurus fasciolaris* and *Notropis telescopus* with respect to parasite seasonality, host reproductive impact, influence of 11-ketotestosterone on parasite load, and the description of two new species: *Dactylogyrus lythruri* and *Octomacrum lamiaruthis*.

Abstract:

Dactylogyrus is a genus of flatworm that infects the gills of cyprinid fishes. They are highly host specific and little is known about their life history. Our purpose of this study was to determine whether Dactylogyrus exhibited seasonality in its life cycle, if there was any effect upon reproductive effort as a result of their infection, and if 11-ketotestosterone (11-KT) levels had any correlation to severity of parasite infection. We examined 323 telescope shiners, Notropis telescopus, and 31 scarlet shiners, Lythrurus fasciolaris, finding 1048 flatworms, Dactylogyrus spatulus and Dactylogyrus crucis, on the gill arches of fish collected from February to September 2007. Parasite counts yielded a peak in the average number of parasites present per fish in May, a significant relationship between host length and infection, and a slight negative correlation with higher parasite load and gonadosomatic index (GSI). There was also a significant relationship with 11-KT and infection in the scarlet shiner. During the study, a previously undescribed species of Dactylogyrus was found on the gills of L. fasciolaris, and a novel species of Octomacrum was found upon the gills of N. telescopus and are described herein.

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Several Studies on Dactylogyrus and Their Hosts:

Lythrurus fasciolaris and Notropis telescopus with respect to parasite seasonality, host reproductive impact, influence of 11-ketotestosterone on parasite load, and the description of two new species: Dactylogyrus lythruri and Octomacrum lamiaruthis.

Senior Honors Thesis

Submitted in partial fulfillment

Of the requirements for the

Honor's Certificate upon Graduation

By

Andrew Blake Adrian
University of Alabama in Huntsville
Huntsville, Alabama 35899
Fall 2009

Abstract:

Dactylogyrus is a genus of flatworm that infects the gills of cyprinid fishes. They are highly host specific and little is known about their life history. Our purpose of this study was to determine whether Dactylogyrus exhibited seasonality in its life cycle, if there was any effect upon reproductive effort as a result of their infection, and if 11-ketotestosterone (11-KT) levels had any correlation to severity of parasite infection. We examined 323 telescope shiners, Notropis telescopus, and 31 scarlet shiners, Lythrurus fasciolaris, finding 1048 flatworms, Dactylogyrus spatulus and Dactylogyrus crucis, on the gill arches of fish collected from February to September 2007. Parasite counts yielded a peak in the average number of parasites present per fish in May, a significant relationship between host length and infection, and a slight negative correlation with higher parasite load and gonadosomatic index (GSI). There was also a significant relationship with 11-KT and infection in the scarlet shiner. During the study, a previously undescribed species of Dactylogyrus was found on the gills of L. fasciolaris, and a novel species of Octomacrum was found upon the gills of N. telescopus and are described herein.



TABLE OF CONTENTS

List of Figures and Tables		4
Chapter		
Ī.	Introduction	6
	a. Dactylogyrus	6
	b. Lythrurus fasciolaris	7
	c. Notropis telescopus	7
II.	Materials and Methods	8
	a. Collection of Fishes	8
	b. Sex determination	8
	c. Gill Inspection	9
	d. ELISA for 11-Ketotestosterone	10
	e. Description of a new species	11
III.	Results and Discussion	11
	a. Dactylogyrus Seasonality	11
	b. Host Reproductive Impact	15
	c Influence of 11-KT on Parasite Load	16

		Adrian 5
	d. Description of two new species,	17
	i. Dactylogyrus lythruri	17
	ii. Octomacrum lamiaruthis	18
IV.	Conclusion	19
V.	Acknowledgements	20
VI.	References	21
VII	. Annotated Bibliography	23

List of Figures and Tables

Figure	1. Dactylogyrus crucis, w.m. 1% acetocarmine stained at 100x mag. Photo by Andrew Adrian. Note apparent posterior haptor and anterior paired eyespots
Figure	2. Lythrurus fasciolaris. Photo by David Eisenhour
Figure	3. Notropis telescopus. Photo by Garold Sneegas
Figure	4. Ovarian developmental stages
Figure	5. Oocyte developmental stages
Figure	6. Dactylogyrus on a partial gill arch of N. telescopus. The parasite is $\sim 300 \mu m$ in length. w.m. 1% acetocarmine. $30x$ magnification. Photo by Andrew Adrian
Figure	7. Parasite Prevalence per Month. Error bars represent 1 SE9
Figure	8. Proportion of infection per month. Low/Med/High classes are defined as possessing a number of parasites in the bottom, mid, or top 33% of total infection counts, respectively
Figure	9. Average monthly GSI % for females (black bars) and males (white bars). Error bars represent 1 SE
Figure	10. Ovarian developmental stages by percentage for months with observable development. Stage 1=vertical shading, stage 2=diagonal shading, stage 3=horizontal shading, stage 4=solid shading

Figure 11. Dactylogyrus infection per fish length
Figure 12. of Average GSI per Below and Above average parasite loads. Data is restricted to female hosts. N=131. Error bars are 1 SE
Figure 13. Percent of Average GSI per month at Below (Blue) and Above (Red) Average Parasite Loads. Average = 4 Error bars represent one standard error
Figure 14. Parasite load versus 11-ketotestosterone levels
Figure 15 Dactylogyrus lythruri sclerites. a. Anchor, b. Ventral bar, c. Dorsal bar, d. Ventral anchor (4A), e. Hook, f. Cirrus, g. Accessory piece
Figure 16. Dactylogyrus lythruri at 400x. w.m. unstained. Haptor and cirrus obvious



I. Introduction

Within this section, I describe the two host species examined for this study and the genus of parasitic Monogenean found upon the host species. In May 2009 I set out to quantify seasonal *Dactylogyrus* infection on N. telescopus and to see if there were any effects from infection upon the reproductive effort of N. telescopus. The fishes used in the study were from a prior study of ours which imparted GSI and reproductive development data which expanded my initial seasonality analysis into the realm of parasite impact on reproductive effort. Later, I investigated whether fluctuations in 11-Ketotestosterone due to reproductive seasonality had any effect upon infection severity. During this study, I found a new species of parasite that Donald Cloutman had also seen on other members of *Lythrurus*, along with *D. Crucis*, but my investigation was first to find these on *L. fasciolaris*.

Dactylogyrus

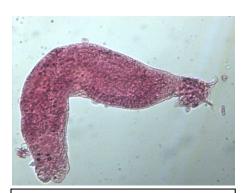


Fig 1. *Dactylogyrus crucis*, W.M. 1% acetocarmine stained at 100x mag. Photo by Andrew Adrian. Note apparent posterior haptor and anterior paired eyespots.

Dactylogyrus (Diesling 1850) (Phylum Platyhelminthes: Class Monogenea) is a flatworm species of gill fluke that infects freshwater fishes, primarily Cypriniforms. Members of Dacytlogyrus possess an elongate body with well-defined head organs, though this characteristic is not always apparent. They possess a haptor with one pair of conspicuous dorsal anchors associated with the dorsal transverse bar; ventral anchors and bar vestigial (negating genus Neodactylogyrus). The haptor also possesses fourteen marginal hooks of similar shape, but varying in size—and an extra pair of hooks is often present, although not readily apparent without phase contrast microscopy (Mizelle and McDougal 1963). The cirrus is tubular,

variable in shape with a visible accessory piece. Vagina present, ventral, usually on the right side of the body. Vitellaria coextensive with intestine and extending laterally to body margin (Hoffman 1999). It is thought that every species of cyprinid fish hosts a unique specie of *Dactylogyrus*.

We chose to examine fishes for *Dactylogyrus* gill flukes based upon their host specificity, and the lack of knowledge about their life history. *Dactylogyrus* are thought to have a direct life cycle, with no intermediate host. New hosts are infected by free swimming larvae called oncomiracidium. At 20C, related species hatch in 5 days and worms mature in 6 to 10 with the life cycle completing in 18 to 20 days (Olsen 1986). The three Dactylogyrus species found herein are *D. spatulus* (Cloutman 1988), *D. crucis* (Rogers 1967), and *D. lythruri* (Cloutman and Adrian 2009).

Lythrurus fasciolaris



Fig 2. *Lythrurus fasciolaris*. Photo by David Eisenhour.

Lythrurus fasciolaris (Scarlet Shiner) inhabits small to medium sized upland streams with moderate current, usually over gravel and rubble substrates (Mettee and others 1996). The scarlet shiner persists in the Ohio basin from Ohio south to Alabama and was just recently elevated to species status from Lythrurus ardens fasciolaris in 1991. In Alabama, spawning occurs from April through July, often

over sunfish nests. Their diet consists of algae, midges, mayflies, odonates, and terrestrial insects taken at the surface (EPA 2008). They are host to at least two species of Dactylogyrus gill flukes, *D. crucis* and *D. lythruri*. The scarlet shiner is a sexually dimorphic species, in which males are distinctly larger, aggressive, and more colorful than females during breeding months. This dimorphism is a result of many combined effects, one of which is the expression of high levels of 11-KT. During breeding season, alpha males have been shown to possess 100 fold increases of the sex hormone 11-ketotestosterone over reproductive females (Schade 2009). 11-KT is a potent form of testosterone present in fish. It is suspected that 11-KT possesses similar immunosuppressive qualities to testosterone (Duffy and others 2000). We chose to examine Lythrurus based on their local availability, their obvious sexual dimorphism, and widely varying 11-KT levels.

Notropis telescopus



Fig 3. *Notropis telescopus*. Photo by Garold Sneegas.

Notropis telescopus (Cope) (Telescope Shiner) occur in two populations, one throughout the river drainages of the Tennessee and another inhabiting the white and black river systems in Arkansas and Missouri. Where found, it is a common species that prefers small to medium sized upland streams in flowing water near riffles over gravel or rock substrates. Its reproductive

biology in Alabama was recently described (Holmes and others 2009) and its diet consists of small terrestrial insects and aquatic insect larvae (Mettee and others 1996). Telescope shiners exhibit peak breeding condition from April to July, descending as water temperatures begin to peak. They are broadcast spawners and likely spawn multiple times throughout their breeding season. Until 2009, little was known of the reproductive biology

of the telescope shiner aside from observations of spawning behavior from mid-April to mid-June in Tennessee (Etnier and Starnes 1993). The telescope shiner is a host of *D. spatulus*, possessing on average four individuals per fish year round. This relative frequent occurrence of *Dactylogyrus* allowed easy collection of data for a seasonality profile. Furthermore, we were able to use the same fishes from a previous study (Holmes and others 2009) in order to determine reproductive impact of *Dactylogyrus*.

II. Materials and Methods

The following section contains a summary of the major methods used throughout this study.

Collection of Fishes

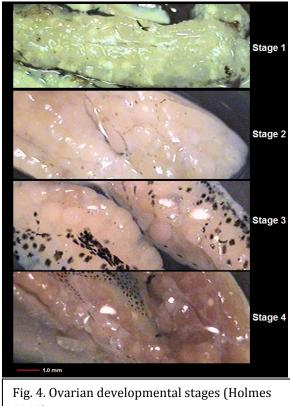
Fish used in this study were collected from three sites: Estill Fork, Jackson County, AL, Limestone Creek at Mooresville Road, Belle Mina, AL (N 34°, 40.535′ W-86°, 52.725′) and Hurricane Creek ($34^{\circ}59'12''N$, $86^{\circ}05'41''W$). Fish collections were performed using seine nets (3.0-m L x 1.3-m D; 3.0-mm mesh). All collections were made in late morning in the first week of a given month. Fish were not selected by size or sex, except for in cases for L. fasciolaris when alpha males were desired. Along with the fish, data for pH, temperature (°C), and TDS (ppm) were recorded. Fish collections from Estill Fork (performed January 2008—September 2009) and Limestone Creek (performed August 2008—August 2009) consisted mainly of L. fasciolaris and N. telescopus and the fish collected were executed with either clove oil or MS-222 (Tricane Methanesulfonate) and then placed on ice for an hour until being fixed in 10% phosphate buffered formalin. Collections made at Hurricane Creek consisted of ~ 30 N. telescopus (as per our permit†) which were executed using MS-222 and immediately transferred to 10% phosphate buffered formalin. Following collection, the fish were blotted dry and weighed (.001g) using a digital scale, and then measured for standard length (.01mm).

[†]This work was made possible with the cooperation and permission of the State Lands Division of the state of Alabama through Forever Wild Tract Research Permission Number 0701.

Sex Determination

In order to determine the GSI (Gonadosomatic index) and the sex of the fish, a gonadal dissection was performed by making an incision lengthwise down the ventral surface, and removing gonadal material from the fish. This gonadal material was weighed (.001g) and then GSI % produced (Gonad mass/somatic mass * 100). To visually determine the status of ovarian and oocyte development, intact ovaries (if present) were dissected from females and digital images at 10X were captured with a Wolfe Digivu TM CVM Stereo digital dissecting microscope using the software package Motic Images 2000 version 1.2. Ovaries were then teased apart to separate developing oocytes from ovarian connective tissues, and digital images were made of all of the oocytes spread in a single layer the size

of the image field. For Hurricane Creek fish, ovaries and eggs were characterized by developmental stages 1-4, using a combination of size and color in a modified Heinz and Rabito (1986) classification scheme. Figures 4 and 5 display these developmental stages. For further elaboration, see Holmes 2009.



2009)

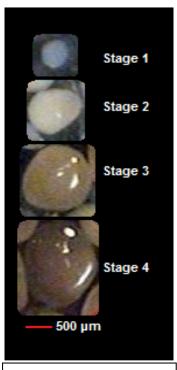


Fig 5. Oocyte developmental stages. (Holmes 2009)

Gill Inspection

Visual inspections were performed on removed gill arches from the collected fish. To remove gill arches, the operculum was forced anteriorly and each gill arch was carefully removed with forceps. The gill arches were stained in a 1% acetocarmine solution for at least 24 hours before being inspected under a dissecting microscope at 30x magnification. Gill arches were feathered through with a micro dissecting needle, counted, and then flipped and counted again to ensure all of the parasites on a given arch were counted. Figure 3 displays *Dactylogyrus* attached to a gill filament.



Fig. 6. *Dactylogyrus* on a partial gill arch of N. telescopus. The parasite is apx $300\mu m$ in length. w.m. 1% acetocarmine. 30x magnification. Photo by Andrew Adrian.

ELISA for 11-ketotestosterone

Blood samples (between 6.5 and 86 µl) were taken from anesthetized subjects using a heparinized capillary tube. Blood was collected within 5 minutes of removal from the tank by means of a puncture to the caudal sinus. A sharp, pointed scalpel was used to puncture the sinus causing blood to quickly pool at the skin's surface. As blood pooled at this site, it was quickly collected using a heparinized capillary tube (80 µl capacity), and then transferred to a chilled 1.5-ml centrifuge tube containing 20 µl of sample buffer with protease inhibitor (1%) and kept on ice. All blood samples were then centrifuged for 3 minutes at 12,000 rpm to separate plasma from red blood cells. The plasma was isolated and stored at -80°C until assayed. Plasma 11KT concentrations were measured using an enzyme-linked immunosorbent assay, or ELISA (Cayman 2003). Cold-spike extraction (Cayman 2003) using diethyl ether was used with slight modification to purify the samples. Plasma samples were transferred to glass tubes containing 200 µl of EIA buffer. Samples were extracted three times using diethyl ether. The diethyl ether was evaporated by heating to 30°C under a gentle stream of nitrogen, and then samples were stored overnight under desiccation at -20° C. Purified plasma samples were thawed and reconstituted in a 1:10 solution with assay buffer. Samples were then assayed at two dilutions (1:50 and 1:100) in triplicate. The ELISA kit protocol was then followed, which had a specificity of 100% for 11KT and \leq 0.01% for other similar analytes. All 11-KT assays were performed by Jennifer Schade.

<u>Description of a New Species</u>

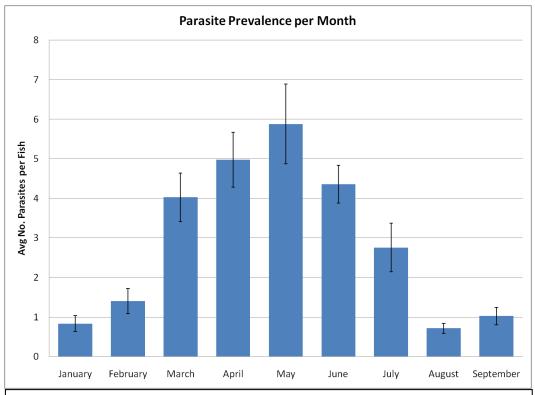
Morphological features such as average length, reproductive complex length and shape, and haptoral hook arrangement, size, and shape differentiate species of Dactylogyrus from one another. Stained and unstained specimens were removed from gill filaments using a microprobe and mounted in 1% glycerin jelly. The specimens were then observed under a brightfield microscope at 100, 400, and 1000x magnification—capturing images using Motic Imaging software. This software also allowed measurements to be made. Outlines of each sclerite were made and compiled to create an image for descriptive purposes.

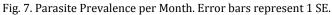
III. Results and Discussion

In the following section I address each major portion of my investigation, presenting data and supporting it with discussion.

<u>Dactylogyrus Seasonality</u>

Seasonality data from Hurricane Creek were collected from *D. spatulus* observed on the gills of 281 fish from eight months, February to September. To provide a more complete picture, we supplemented this data with Telescope shiners from January that originated from Estill Fork, at a collection site less than 5 miles away from the original Hurricane Creek site. We found that average parasite load peaked in May and was lowest in August (Fig. 7). Parasite infections reached highest severity per fish in May, with some fish possessing as many as 17 *Dactylogyrus* while dropping dramatically in August, September, January, and Feburary(Fig. 8).





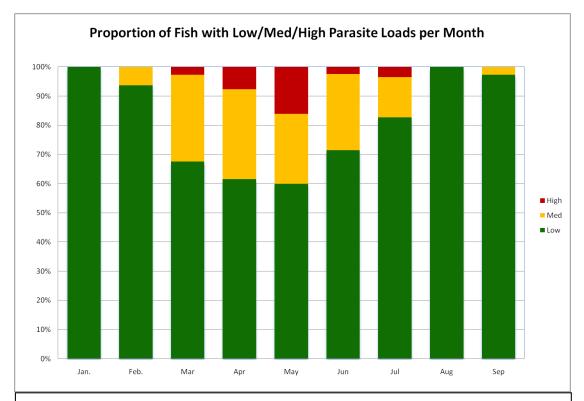


Fig.~8.~Proportion~of~infection~per~month.~Low/Med/High~classes~are~defined~as~possessing~a~number~of~parasites~in~the~bottom,~mid,~or~top~33%~of~total~infection~counts,~respectively.

The observed seasonality profile shows a declined period of activity from August to Feburary, until a rapid peak from March to July. Water temperature at the collection site varied from a low of 9 $^{\circ}$ C in February to a high of 24 $^{\circ}$ C in July and while the *Dactylogyrus* life cycle is known to be somewhat temperature dependant (Olsen 1986), the temperature peak in July does not correspond with increased infection. ANOVA of data from Figure 7 supports that the peak months be grouped together, resulting in a prevalence peak from March to July. What is especially interesting is that this peak corresponds with the reproductive peak of the host, *N. telescopus*, as observed through Gonadosomatic Index and ovarian condition (Figs. 9 & 10).

This close association in reproductive timing may be attributable to coevolutionary processes whereby the parasites have matched up their reproduction at the time when the host is least capable of fighting off the infection due to the dramatic energy costs of reproduction. (Simkova and others 2008) Synchronous reproduction would also lend more potential host subjects for the parasites' young.

We also observed, as expected, a significant correlation with fish size and the number of parasites present per fish (fig. 11) which is likely attributable to higher gill surface area and older age of larger fish.

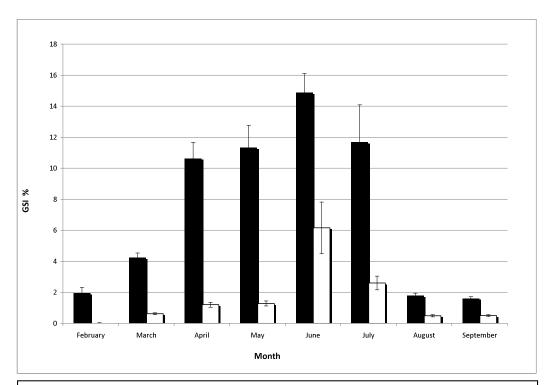


Fig. 9. Average monthly GSI % for females (black bars) and males (white bars). Error bars represent 1 SE. (Holmes 2010)

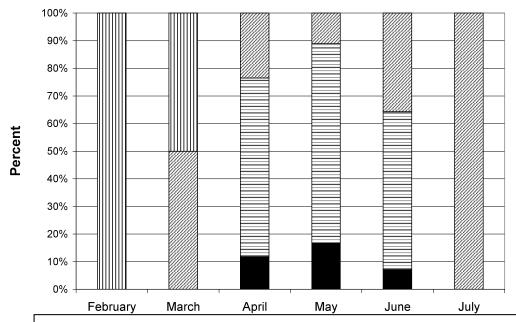


Fig. 10. Ovarian developmental stages by percentage for months with observable development. Stage 1=vertical shading, stage 2=diagonal shading, stage 3=horizontal shading, stage 4=solid shading (Holmes 2010)

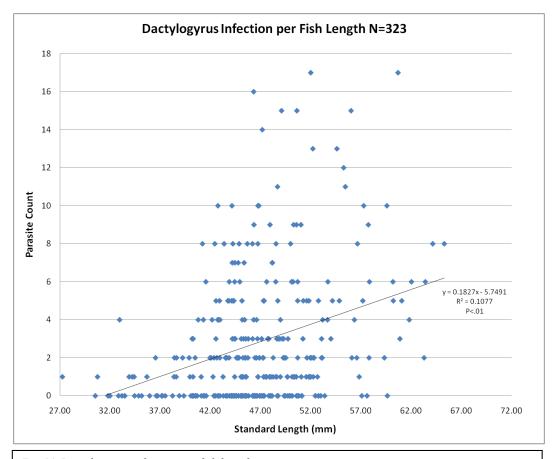


Fig. 11. Dactylogyrus infection per fish length.

Host Reproductive Impact

While the effect is not linear, higher parasite loads tended to result in decreased mass of gonadal tissue relative to a fishes' total mass. This decrease in mass is most apparent in females (Fig 12). Males also exhibited reduced GSI at above average parasite loads, but the effect was less drastic yet still significant. Statistical analysis concluded that the two columns in Figure 12 are indeed significantly different with P < .01, indicating that the difference is most likely not attributable to random chance. Regression analysis also corroborates this correlation.

It would then appear that *Dactylogyrus* do have an effect upon the reproductive effort of *N. telescopus* or that *Dactylogyrus* are infecting less fit individuals to begin with. During May at the peak of N. telescopus' gonadal development, this effect upon GSI becomes increasingly prominent (Fig. 13).

% of Avg GSI per Parasite Load, Females.

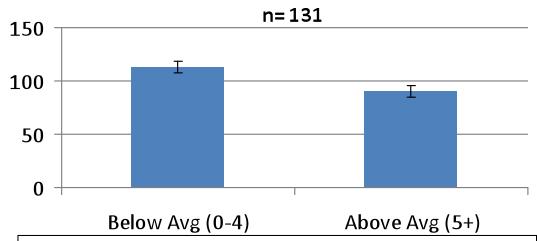


Fig. 12. % of Average GSI per Below and Above average parasite loads. Data is restricted to female hosts. N=131. Error bars are 1 SE.

% of Average GSI per month at Below and Above Average Parasite Loads

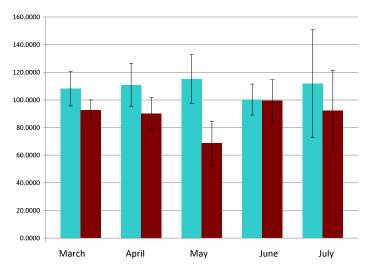
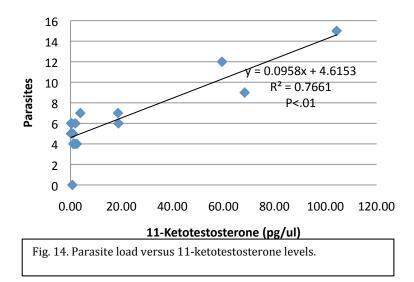


Fig. 13. % of Average GSI per month at Below (Blue) and Above (Red) Average Parasite Loads. Average = 4 Error bars represent one standard error.

<u>Influence of 11-KT on Parasite Load</u>

11-ketotestosterone is an androgen present in fishes that functions similarly to testosterone in humans, increasing sexual behavior, influencing aggression, and promoting muscle growth. It is present at highest concentrations during breeding phases (Pall 2002) and has been shown to have a negative correlation with immunocompetence (Kurtz and others 2007)(Kortet and others 2003)(Duffy and others 2000). We found a positive correlation with parasite infection and increasing 11-KT levels (Fig 14). This is likely attributable to the immunosuppressant qualities of 11-KT or is a product of alpha males, who generally possess higher 11-KT levels, being larger (Schade 2009). The data is strengthened by the fact that both males and females are included in our analysis, and that there were several beta males at sizes equivalent to that of alpha males, yet the trend remains. Due to the difficulty involved with obtaining 11-KT measurements, we were only able to obtain accurate levels for 16 fish. Despite small sample size, the trend is significant enough to warrant further research.



<u>Description of Two New Species</u>

Herin I describe two new species found during this study, *Dactylogyrus lythruri* and *Octomacrum lamiaruthis*.

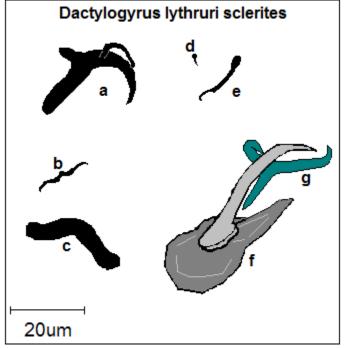
Dactylogyrus lythruri

Two species of *Dactylogyrus* were found on the gills of *Lythrurus fasciolaris*, one described but unknown to the host, and another undescribed. *Dactylogyrus crucis* (Rogers 1967) has been found on species of *Lythrurus* and several other minnows, especially *Notropis crysocephalus* (Cloutman 2009). The finding of *D. crucis* from *L. fasciolaris* and other *Lythrurus* suggests *Lythrurus* monophyly. This is the first record of *D. crucis* found upon *L. fasciolaris*.

Description

Hosts: Lythrurus fasciolaris, Lythrurus bellus, Lythrurus atrapiculus.

With characters of the genus as emended by Mizelle and McDougal (1970). Two pairs of anterior cephalic lobes. Head organs not observed. Two pair of eyes approximately equal in size, anterior pair usually father apart than posterior pair. Pharynx circular to ovate (dorsal view). The novel species found in this study seems to be most closely related to *D. beckeri* (Cloutman 1987), sharing a similar set of haptoral anchors and cirrus (Fig 15). It has an average length of 350µm with vitellaria sometimes present throughout the body without extending into the foremost anterior and haptoral regions. Achors with deep, elongated dorsal roots. Dorsal bar present and obvious, ventral bar indistinct. Fourteen marginal hooks of similar size and shape and a pair of ventral anchor (4A) hooks present when viewed under high contrast. Differs from *D. beckeri* in size of male reproductive organ: 45um compared to 58um in *D. beckeri*. *Dactylogyrus lythruri* may be seen in Fig. 16. Etymology: *Dactylogyrus lythruri* is named after the genus of its host (*Lythrurus*).



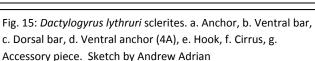




Fig. 16. *Dactylogyrus lythruri* at 400x. w.m. unstained. Haptor and cirrus obvious. Photo by Andrew Adrian

Octomacrum lamiaruthis

The genus *Octomacrum* includes five described species that are host specific to fishes in the families Castomidae and Cyprinidae. The species tentatively described herein was found from the gills of *Notropis telescopus* from Estill Fork, Jackson County, Alabama. No *Octomacrum* has been found on any fishes in Alabama and has only been found on two *Notropis* species, *N. cornutus*, and *N. crysoleucas* (Hoffman 2000). *Octomacrum lamiaruthis* is shown in figure 16.

Description

Host: *Notropis telescopus*

With characters of the genus as described by Mueller (1934). ~1mm in length. Unarmed genital sucker with no vagina present. Vitellaria dense and confined to lateral fields, not entering the haptor. Eight clamps in pairs, most posterior pair narrower in diameter.



Fig. 16. *Octomacrum lamiaruthis*. W.m. unstained. 40x brightfield. Photo by Andrew Adrian

IV. Conclusion

From the data presented, I have unequivocally demonstrated that *Dactylogyrus spatulus* displays a clear seasonality profile, exhibiting a peak in average prevalence from March to July—nearing six parasites per fish in the peak month of May. This observed seasonality is likely a result of several sources: breeding aggregations where fish are in close contact with one another throughout the breeding season facilitating parasite transmission (Heins and Rabito 1986), the dramatic cost of reproduction which in turn reduces the host's ability to fight off infection (Šimková and others 2008), and coevolutionary processes between the parasite and the host where the parasite selectively exploits its host when successful infection is most probable. Water temperature is known to affect the life cycle of *Dactylogyrus* (Olsen 1986), but not to a linear effect—which was also observed in our study.

After noting that D. spatulus is at peak prevalence during spawning of the host, we next examined the impact *Dactylogyrus* has upon the reproductive effort of the host, measured via GSI. We found that at high parasite load (5+ parasites per individual) there was an average 21% decrease in GSI from the monthly average in females (P<.01). While this could be an artifact of multiple spawnings in a given breeding season, (Heins and Rabito 1986) the same pattern was observed in males to a less drastic extent but nonetheless at significant levels. It is supposed that *Dactylogyrus* introduces lesions in gill material promoting microbial infection, which along with subtle decrease in respiration efficiency may perhaps compromise the host's ability to devote energy to reproduction.

We were also curious as to what factors were influencing heightened parasite loads. This led us to examine parasite load as a function of 11-KT concentration. 11-KT, like its testosterone relative, is an immunosuppressant. While we did not anticipate finding a strong correlation with parasite load, we found that the relationship is almost linear.

Despite small sample size due to the difficulty in obtaining 11-KT quantification, the relationship is statistically significant. Further research is needed to determine if this is an artifact of sampling or outside effects, but our preliminary analysis suggests that 11-KT may indeed have an impact on parasite load.

In the midst of examining over 1000 parasites, we stumbled upon two new species. One *Dactylogyrus* and another *Octomacrum*. *Dactylogyrus luthruri* has been found on all examined species of *Lythrurus* and seems to be closely related to *D. beckeri* in shape of haptoral and reproductive sclerites. It is distinct, though, by possessing a cirrus that is more than 10um smaller than the cirrus of *D. beckeri* and by its preference for *Lythrurus* hosts. *Octomacrum lamiaruthis* is proposed as a new species due to its presence on *N. telescopus* in Alabama—a site and fish that have never before yielded any specimen of *Octomacrum*. To formalize the description of *Octomacrum lamiaruthis*, clamp shape and length measurements should be made and compared to the five described species. Also discovered in our study was the presence of *D. crucis* upon *L. fasciolaris*. These two species, *D. lythruri* and *D. crucis* have been found on all examined *Lythrurus* species, and is perhaps a factor supporting the monophyly of *Lythrurus*.

V. Acknowledgements

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VI. References

- Cayman Chemical. 2003. Cold-spike extraction literature citation obtained from Cayman Tech Support.
- Cloutman, Donald G. 1987. *Dactylogyrus* (Monogenea: Dactylogyridae) from *Hybopsis* and *Notropis* (Cyprinella) (Pisces: Cyprinidae) from the Tennessee River drainage, with descriptions of three new species and remarks on host relationships. Proceedings of the Helminthological Society of Washington 54:78-73.
- Cloutman, Donald G. 1988. *Dactylogyrus* (Monogenea: Dactylogyridae) from seven species of *Notropis* (Pisces: Cyprinidae) from the Tennessee River drainage: descriptions of four new species and remarks on host relationships. Proceedings of the Helminthological Society of Washington 55:214-223.
- Cloutman, Donald G; Rogers, Wilmer A. 2005. Determination of the *Dactylogyrus Banghami* complex (Monogenea: Dactylogyridae) from North American Gulf of Mexico coastal drainages with descriptions of three new species. Comparative Parasitology 72(1):10-16.
- Cloutman, Donald G; Adrian, Andrew B. 2009. Description of *Dactylogyrus lythruri n. sp.* and new records of *Datylogyrus crucis* (Monogenoidea: Dactylogyridae) parasitizing shiners of the genus *Lythrurus* (Pisces: Cyprinidae). Comparative Parasitology. In preparation.
- Duffy, Deborah L; Bentley, George E; Drazen, Deborah L; Ball, Gregory F. 2000. Effects of testosterone on cell-mediated and humoral immunity in non-breeding adult European starlings. Behavioral Ecology 11(6):654-662
- [EPA] Environmental Protection Agency. 2008 Nov. An introduction to freshwater fishes as biological indicators. Washington D.C.: U.S. Environmental Protection Agency Office of Environmental Information Office of Information Analysis and Access.
- Heins, D; Rabito F. 1986. Spawning performance in North American minnows: direct evidence of the occurrence of multiple clutches in the genus Notropis. Journal of Fish Biology 28:343–357.
- Hoffman, G. L; 1999. Parasites of North American freshwater fishes. 2nd ed. New York: Comstock Publishing Associates.

- Holmes, Britanny; Whitington, Lindsay; Marino, Loren; Adrian Andrew B; Stallsmith, Bruce W. 2009 Reproductive timing of the telescope shiner, *Notropis telescopus*, in Alabama, USA. The American Midland Naturalist. In Press.
- Kortet, Raine; Vainkka, Anssi; Rantala, Markus J; Jokinen, Ilmari; Taskinen, Jouni. 2007. Sexual ornamentation, androgens and papillomatosis in male roach (*rutilus rutilus*). Evolutionary Ecology Research 5:411–419
- Kurtz, Joachim; Kalbe, Martin; Langefors, Asa; Mayer, Ian; Milinski, Manfred; Hasselquist, Dennis. 2007. An experimental test of the immunocompetence handicap hypothesis in a teleost fish: 11-ketotestosterone suppresses innate immunity in three-spined sticklebacks. American Naturalist 170(4):509–519
- Mettee, Maurice F; O'Neil, Patrick E; Pierson, Malcolm J. 1996. Fishes of Alabama and the Mobile Basin. Birmingham: Oxmoor House.
- Mizelle, J; McDougal, H. 1970. Studies on monogenetic trematodes. xlv. the genus Dactylogyrus in North America. key to species, host-parasite and parasite-host lists, localities, emendations, and description of D. kritskyi sp. n. The American Midland Naturalist 84(2):444-462
- Mueller, Justus F. 1936. Studies on North American gyrodactyloidea. Transactions of the American Microscopical Society 55:55-72.
- Olsen, Oliver W. 1986. Animal parasites: their life cycles and ecology. 3rd ed. New York: Courier Dover Publications.
- Pall, Miklos K; Mayer, Ian; Borg, Bertil. 2002. Androgen and behavior in the male three-spined stickleback, *Gasterosteus aculeatus* i.—changes in 11-ketotestosterone levels during the nesting cycle. Hormones and Behavior 41(4):377-383
- Rogers, Wilmer A; 1967. Studies on Dactylogyrinae (Monogenea) with descriptions of 24 new species of *Dactylogyrus*, 5 new species of *Pellucidhaptor*, and the proposal of the *Aplodiscus* gen. n. Journal of Parasitology 53:501-524.
- Schade, Jennifer. 2009. Role of 11-KT in sexual dimorphism of the scarlet shiner. Unpublished master's thesis.
- Šimková, Andrea; Lafond, Thomas; Ondračková, Markéta; Jurajda, Pavel; Ottová, Eva; Morand, Serge. 2008. Parasitism, life history traits and immune defence in cyprinid fish from central Europe. BMC Evolutionary Biology 8:29.

VII. Annotated Bibliography

If you are interested in our local fishes and their parasites, the following sources will give you a good starting knowledge to pursue any fascination you may have.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes. 2nd ed. New York: Comstock Publishing Associates. 539 p.

This book, written by one of the top authorities on fish diseases, is a reference work providing morphological descriptions and information about most North American fish parasites. It provides information on public health concerns about fish parasites, methods for sampling and examination, and known species of each genus. More importantly, this work contains reference citations that enables readers to find literature pertinent to species identification, life cycle, and control. Each chapter covers a major phyla of parasites, its relevant families and genera, followed by a list of known species, and in most cases a drawing or photograph for the lay parasitologist to compare their specimens to.

Mettee, Maurice F; O'Neil, Patrick E; Pierson, Malcolm J. 1996. Fishes of Alabama and the Mobile Basin. Birmingham: Oxmoor House. 819p.

Mettee, O'Neil, and Pierson's book, Fishes of Alabama and the Mobile Basin provides readers with an overview of almost all of the freshwater fishes present in Alabama—an amazing feat considering Alabama is host to over 300 species of freshwater fishes. This book contains a dichotomous key to the families of fishes and another key within each family that leads to species. Each chapter on the 29 families gives a brief morphological and evolutionary description of the family. Within each chapter readers find each species, with common name, scientific, etymology of the name, characteristics, distribution, and a short section on habitat and biology. Each species page is complimented with a map of Alabama with collection sites marked. Citations occur within each species page that leads readers to the associated articles for more information.

Olsen, Oliver W. 1986. Animal parasites: their life cycles and ecology. 3rd ed. New York: Courier Dover Publications. 564p.

One of the great works in parasitology, this book presents readers with an overview of the life cycles and known biology of parasites of North American wild and domesticated animals. It contains a wealth of knowledge about the various parasitic phyla with numerous illustrations detailing morphology and life history. The first part of this book provides a primer for parasitology, describing parasite ecology and several methods of infection that exist. Each parasite is accompanied by a selected reference section allowing readers to follow up on any material presented in the book.