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Laurel Dace (Chrosomus saylori) reproductive behaviors

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Laurel Dace (*Chrosomus saylori*) Reproductive Behaviors

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Summary

This thesis focuses on observations of the reproductive behaviors of the Laurel Dace (*Chrosomus saylori*), an endangered fish species in which reproductive behaviors have not been previously documented in a scientific publication. I worked with the Tennessee Aquarium Conservation Institute (TNACI) to determine the reproductive behaviors for the Laurel Dace. The results from this study will be used to inform the U.S. Fish and Wildlife Service (USFWS) and the Tennessee Wildlife Resources Agency (TWRA) on the likely reproductive behaviors of Laurel Dace and to help these agencies determine the best protocols for a future propagation program to facilitate captive breeding by this species. These results will potentially be used to better inform the USFWS and the TWRA on how to best manage this federally endangered species. Along with this study, this thesis includes a literature review on the Laurel Dace.

Table of Contents

Chapter 1: *Chrosomus saylori* Background Information

Taxonomy

The Laurel Dace (*Chrosomus saylori*) is in the kingdom Animalia, phylum Chordata, class Actinopterygii, order Cypriniformes, family Cyprinidae, subfamily Leuciscine, and is part of the genus *Chrosomus* (NatureServe 2013). *Chrosomus* is made up of seven recognized and one undescribed species in North America. *Chrosomus saylori* was originally described in the genus *Phoxinus*, but Strange and Mayden (2009) reassigned it and all other North American *Phoxinus* species to *Chrosomus* based on an analysis of the mitochondrial cytochrome *b* gene. *Chrosomus saylori* is the sister species of the Clinch Dace (*Chrosomus* sp. cf. *saylori*) based on an analysis of the cytochrome *b* gene (Strange and Skelton 2005). Given their evolutionary history, it is possible that the Laurel Dace and Clinch Dace could have once been the same species that subsequently evolved independently and speciated in allopatry. The Laurel and Clinch Dace are sister to the federally threatened Blackside Dace (*C. cumberlandensis*). These three species are nested within a larger group of daces, including the Southern Redbelly Dace (*C. erythrogaster*), Finescale Dace (*C. neogaeus*), Tennessee Dace (*C. tennesseensis*), and Mountain Redbelly Dace (*C. oreas*) (Strange and Mayden 2009, George et al. 2016). *Chrosomus saylori* received its common name, Laurel Dace, from Christopher Skelton, who described this species because of the existence of mountain laurel (*Kalmia latifolia*) shrubs at a majority of the locations where this species is found (Skelton 2001). The specific epithet was given in honor of TVA ichthyologist Charles F. Saylor, who was a member of the team that first collected this species (Skelton 2001).

Diet

Chrosomus saylori has a large terminal mouth relative to most Cyprinid species, a relatively short digestive tract, which is common in fish species that feed on many insect species, a reduced number of pharyngeal teeth, and primitively shaped basioccipital bone (Skelton 2001). These characteristics are in line with an organism that mainly feeds on animal material. Skelton (2001) has observed *C. saylori* in loose schools feeding on insects and plant material at the water's surface. To better understand the diet of *C. saylori*, it is helpful to examine the Clinch Dace's diet given that the Clinch Dace is the sister species to *C. saylori* (George et al. 2016). The Clinch Dace feeds on many types of macroinvertebrates, including dobsonfly (any insect in the subfamily Corydalinae), beetle (Coleoptera), fly (Diptera), and wasp (Hymenoptera) larvae, as well as ticks (Ixodida); however, small amounts of algae, other plant material, and sand grains have been identified as dietary components as well (George et al. 2016). During field observations, the Clinch Dace was primarily observed drift feeding, while also sometimes feeding on attached algae and periphyton (George et al. 2016).

Habitat

Chrosomus saylori has mainly been collected from pools, slow runs from undercut banks, or beneath slab boulders (George et al. 2016). This species is usually found in clear first or second order streams with a maximum temperature of 26 °C or 78.8 °F (George et al. 2016). *Chrosomus saylori* habitat consists of a mix of substrates such as cobble, rubble, and boulders, usually with a dense riparian zone composed of mountain laurel (*Kalmia latifolia*), eastern hemlock (*Tsuga canadensis*), mixed hardwoods, and pines (*Pinus* spp.) (George et al. 2016).

Distribution

Chropsomus saylori is historically known to be found in only seven streams in the Walden Ridge portion of the Cumberland Plateau (George et al. 2016). These seven streams are split into three independent systems. These three systems are: 1) the Soddy Creek system; 2) the Sale Creek system, which includes the Horn branch tributary and Laurel branch tributary to Rock Creek, and the Cupp Creek tributary to Roaring Creek; and 3) the Piney River system, which includes Youngs Creek, Bumbee Creek, Moccasin Creek, and Lick Branch, which is a tributary to Moccasin Creek (Figure 1) (George et al. 2016). *Chrosomus saylori* is historically known from Laurel Branch; however, it is thought that it was extirpated at its time of description. *Chrosomus saylori* is believed to be extirpated from Soddy Creek, since it has not been observed there since 2004 (Personal Communication, Bernard Kuhajda, July 19, 2018). For the last several years, *C. saylori* has also not been collected in Moccasin Creek or its tributary Lick Branch, Cupp Creek, or Horn Branch (Personal Communication, Bernard Kuhajda, July 19, 2018). Given this, *C. saylori* is believed to be extirpated from these creeks as well (Personal Communication, Bernard Kuhajda, July 19, 2018). *Chrosomus saylori* is now believed to be found only in Bumbee Creek and Youngs Creek (Personal Communication, Bernard Kuhajda, July 19, 2018). *Chrosomus saylori* sampling survey results are illustrated in Figure 2.

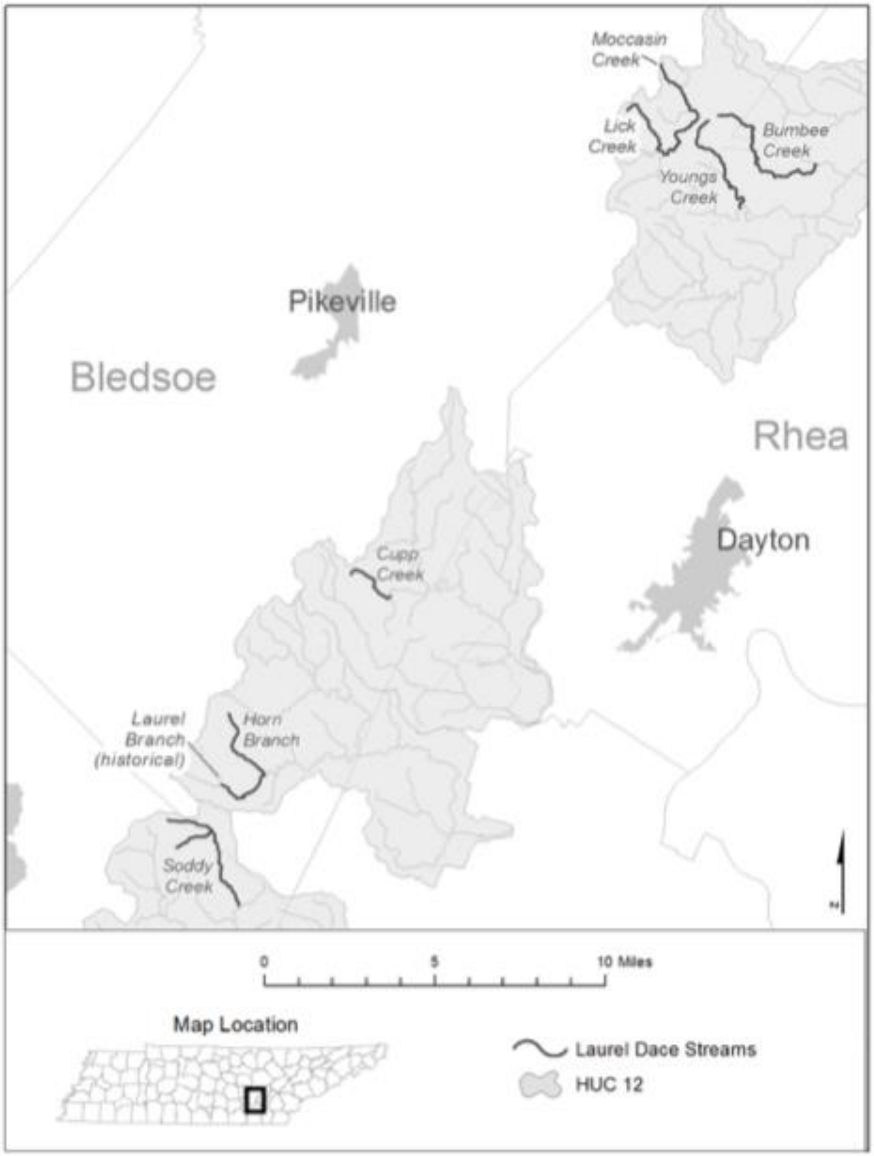

Figure 1: The seven stream systems where *C. saylori* are either currently or have historically been extant. This image was previously published in "Recovery Plan for the Laurel Dace (*Chrosomus saylori*)." Written permission to use this image in the present document was granted by Bernard Kuhajda.

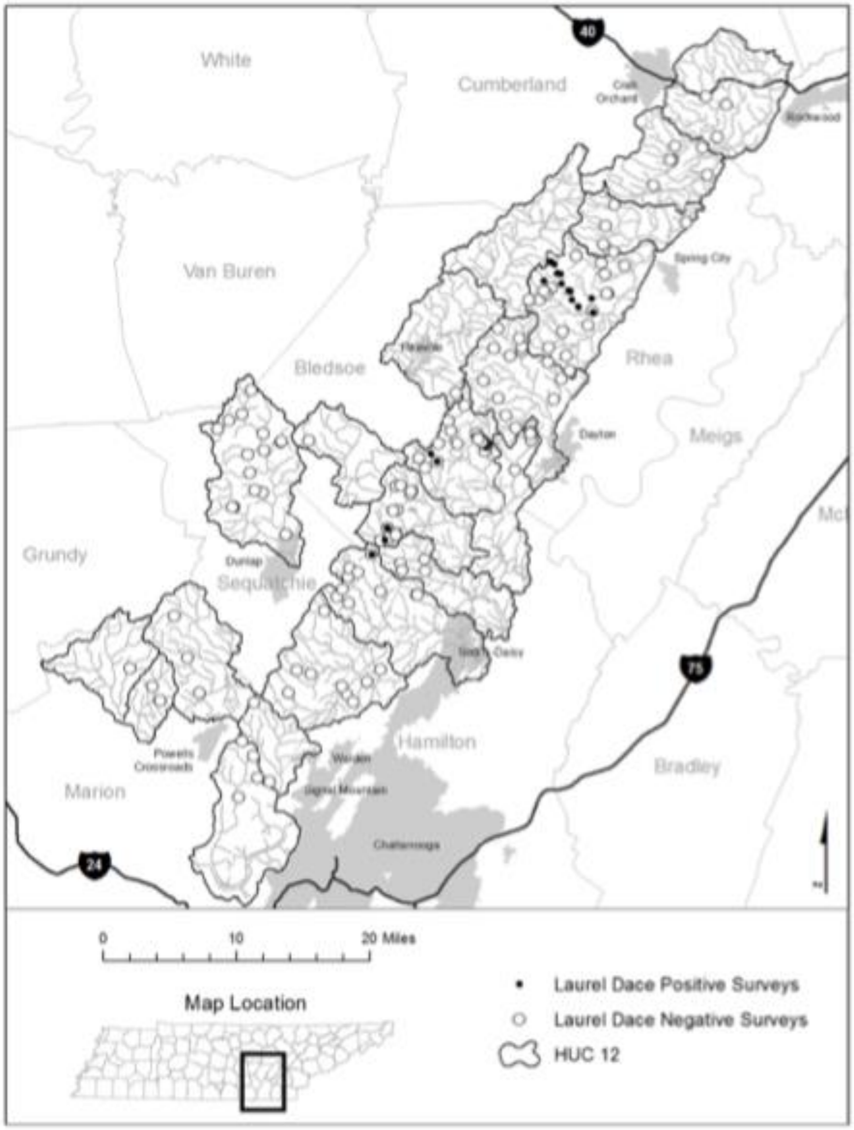

Figure 2: *Chrosomus saylori* distribution map (1991-2013). Solid black circles indicate *C. saylori* were at that location during at least one sampling event that occurred at that location. Solid white circles indicate that no *C. saylori* were present at that location. This image was previously published in "Recovery Plan for the Laurel Dace (*Chrosomus saylori*)." Written permission to use this image in the present document was granted by Bernard Kuhajda.

<u>Life Span</u>

Chrosomus saylori becomes sexually mature at two years of age (which is its 3rd summer of life), exhibits broadcast spawning from late March until mid-June (the peak spawning season), and has a lifespan of approximately three years or more (George et al. 2016). Likely predators of *C. saylori* include introduced sunfishes and basses (family

Centrarchidae) (George et al. 2016). One example that provides evidence that these species prey on *C. saylori* can be found in surveys that were done in 2013 at Cupp Creek, where *C. saylori* populations are in danger of extirpation (George et al. 2016). The surveys revealed that there were high numbers of Green Sunfish (*Lepomis cyanellus*), Bluegill (*L. macrochirus*), and Largemouth Bass (*Micropterus salmoides*) (George et al. 2016). However, one landowner stated that these species listed above were in Cupp Creek due to the frequent flooding of his farm pond (George et al. 2016). In the early 1990s, Skelton noted that there had been an increase in sunfishes and basses in pools in Cupp Creek, which falls in line with a decline in the numbers of *C. saylori* (field notes described in George et al. 2016). Along with predators, water temperature could also be a limiting factor in the number of *C. saylori* that are present (George et al. 2016). However, the biggest cause of *C. saylori* endangerment is due to tomato fields (George et al. 2016). Tomato fields have had a major impact on the headwaters of Soddy Creek and Youngs Creek (George et al. 2016). When it rains, these tomato fields have fungicides, herbicides, and fertilizer that runoff into these waters (George et al. 2016). This causes water pollution, which ends up killing many plants and animals in these waters, including *C. saylori*.

Population Genetics

The study of variations in mitochondrial DNA of *C. saylori* by Strange and Skelton (2005) showed that there were two different groups of *C. saylori*. There are the northern populations in tributaries of the Piney River, and the southern populations in Soddy Creek and tributaries to Sale Creek (Strange and Skelton 2005). Out of thirty individuals in the northern populations, there were six haplotypes recovered. Out of these six haplotypes, only one was shared across all of the populations in the three streams (George et al. 2016). In the southern populations, there was only one haplotype recovered from seven *C. saylori* specimens (George et al. 2016). This haplotype was not found in the northern populations (George et al. 2016). Molecular analysis showed that 72% of the genetic variation was recovered between the northern and southern populations, rather than between populations in either system (10%) or within populations (17%) (George et al. 2016).

Non-breeding Characteristics and Sexual Dimorphism

According to measurements and counts found in Skelton (2001), male and female *C. saylori* are relatively similar. However, there are a few differences. Males tend to have a longer pectoral and pelvic fin. On the other hand, females attain a larger size (length) than do males. Non-breeding male *C. saylori* have two broadly rounded pectoral fins, while the females have two pectoral fins that are narrow and diminish to a point (Skelton 2001). Both sexes of non-breeding *C. saylori* have two black stripes that can vary in color from dark black to barely visible (Skelton 2001).

The upper stripe starts near to where the occiput and the posterodorsal part of the operculum merge (Skelton 2001). Anteriorly, the upper stripe is usually three scale rows deep and the stripe steadily narrows caudally (Skelton 2001). The upper stripe of *C. saylori* is different from the upper stripe of other *Chrosomus* species because in *C. saylori* it is usually continual from the beginning to the end, instead of breaking into various spots on the caudal peduncle like other *Chrosomus* species (Skelton 2001).

The lower stripe starts on the snout and goes through the eye and onto the body at a downward angle from the shoulder girdle toward the belly (Skelton 2001). This lower stripe most often dips slightly, but abruptly, over the anal fin, creating a small notch (Skelton 2001). The lower stripe then partially narrows and continuous uninterrupted to the base of the caudal fin (Skelton 2001). Generally, there is also a continuation of dispersed black pigment down the middle of the caudal fin (Skelton 2001). At the base of the caudal fin, there is usually a small black spot or narrow bar that is approximately the same depth as the lower black stripe (Skelton 2001).

A black line, varying in completeness and intensity, runs along the middle of the dorsum, and there is a row of small irregular spots that are on either side of the midline (Skelton 2001). The number of these small spots can vary from less than 5 to about 20 (Skelton 2001).

The color of the dorsum can vary from olive to a very pale tan (Skelton 2001). The area between the upper and lower stripe has a silvery-white shine (Skelton 2001). The belly, breast, and lower half of the head are whitish-silvery; however, at any time during the year, *C. saylori* can develop red coloration below the lower lateral stripe from the beginning of the pectoral fins to the base of the caudal fin (Skelton 2001). All of the non-breeding characteristics mentioned can be seen below in Figure 3.

Figure 3: A non-breeding *C. saylori*. This picture was taken by Bernard Kuhajda. Written permission to use this image in the present document was granted by Bernard Kuhajda.

Breeding Coloration and Sexual Dimorphism

With the exception of the breeding males' thickened pectoral fin rays and the distal edges of the fins appearing almost straight, breeding male *C. saylori* have the same morphological characteristics as the non-breeding males (Skelton 2001). The breeding males' upper and lower lateral stripes, the entire bottom of their head, and their breast turn profoundly black (Skelton 2001). The black coloration can cover anywhere from one-half to the entire breast, and can sometimes be found on parts of the belly (Skelton 2001).

The whole ventral portion of the body develops a deep scarlet coloration that is adjacent to the lower black strip and black breast (Skelton 2001). However, a slender strip of red coloration goes askew through the lower black stripe behind the start of the pectoral fin, and the red coloration follows the gill openings to the upper connection of the operculum cover and the head (Skelton 2001). The red coloration is most vivid on the caudal peduncle (Skelton 2001). Usually about 20-30% of the base of the dorsal fin develops the red coloration, with the exception of the first ray (Skelton 2001). The red coloration also forms on the lips and around the black caudal spot, diverging out toward the edges of the caudal fin (Skelton 2001). However, red is not the only breeding color that *C. saylori* develop.

The cheeks, the space between the upper and lower lateral stripes, the upper and lower portions of the operculum, and the upper and lower parts of the iris develop a metallic gold coloration (Skelton 2001). The metallic gold coloration is most extreme in the space between the upper and lower lateral stripes (Skelton 2001). The pectoral and pelvic fins turn a bright yellow, while the dorsal fin (above the red coloration), anal fin, and caudal fin develop a lighter yellow coloration (Skelton 2001).

The bases of the pectoral and pelvic fins acquire a little pearly-white patch of coloration (Skelton 2001). The pearly-white coloration on the base of the pectoral fins is two-three times larger than the coloration on the base of the pelvic fins (Skelton 2001).

Breeding female *C. saylori* acquire all of the same colorations as the breeding males, although, the females' colorations are generally less fervent (Skelton 2001). Another difference between the breeding males and females is that the females' anterior part of the upper lateral stripe becomes wider (Skelton 2001). All of the breeding characteristics can be seen below in Figure 4.

Figure 4: A breeding *C. saylori*. This picture was taken by Bernard Kuhajda. Written permission to use this image in the present document was granted by Bernard Kuhajda.

Breeding Tubercles and Sexual Dimorphism

During breeding season, male *C. saylori* develop breeding tubercles on the head and the posterior edge of every scale, except for the scales on the belly and breast (Skelton 2001). Most of the tubercles on the anterior of the body are uniconic (having only one cone on each tubercle) and become progressively multiconic (having more than one cone on each tubercle) towards the posterior of the body (Skelton 2001). Clusters of keratinized cells form the cones on the tubercles (Personal Communication, Bernard Kuhajda, July 19, 2018). One or two rows of uniconic tubercles also form on the dorsal part of pectoral rays 2-5 or 2-6 (Skelton 2001). Most often, the majority of the multiconic tubercles (up to 6 or 7 points) are found around the anal fin and the ventral part of the caudal peduncle (Skelton 2001). *C. saylori* also develops 7-10 rows of comb-like tubercles on the breast that are anterior to each pectoral fin (Skelton 2001).

The tubercles located on the head are small, uniconic, and are usually spread over the entire surface of the head (Skelton 2001). Other than a thick patch of rear-facing tubercles on the posterodorsal part of the operculum, the head tubercles are randomly distributed (Skelton 2001). A row of tubercles is also often present on the posterior edges of the operculum and sub-operculum (Skelton 2001). Female *C. saylori* develop similar tubercle patterns as the males; however, the females' tubercles are not as well developed (Skelton 2001).

C. saylori also has another horned structure that forms during breeding season (Skelton 2001). Without extra magnification, the structures appear as small, raised dots, found in the center of the scales along the dorsal, pelvic, anal, and caudal rays (Skelton 2001). These structures are also randomly dispersed all over the head and are theorized to function as a type of sensory structure (Skelton 2001).

Chapter 2: The Importance of *Chrosomus saylori* Conservation Efforts

Conservation efforts for *C. saylori* are crucial since it is on the verge of extinction. In most of its historical and current range, *C. saylori* is one of two native fish species in these aquatic ecosystems that help keep the water clean and usable (Personal Communication, Bernard Kuhajda, May 21, 2018). The aquatic ecosystem is like a building made of bricks. Once you start removing bricks from the building, it is only a matter of time before the entire building crumbles. Therefore, it is crucial for *C. saylori*, and other plant and animal species, to be protected; otherwise, the entire aquatic ecosystem could eventually crumble if enough organisms are removed (Personal Communication, Bernard Kuhajda, May 21, 2018).

The information presented in this thesis on the potential reproductive behavior of *C. saylori* in a captive setting is part of the conservation efforts specifically designed to assist in keeping this species from extinction. As such, it is critical that we better understand and document the potential reproductive behavior of this species as part of the captive propagation efforts at the Tennessee Aquarium Conservation Institute (TNACI).

Chapter 3: Reproductive Patterns and Behaviors of Closely Related Species

Since researchers have not yet explicitly documented the reproductive behaviors of *C. saylori*, one of the best ways to have a better understanding of its likely reproductive behaviors is to analyze the reproductive behaviors of species that are closely related to *C. saylori*. Below, is an overview of the reproductive behaviors of these closely related species.

<u>Clinch Dace (*Chrosomus* sp. cf. *saylori*)</u>

Orth et al. (2014) studied the Clinch Dace (*Chrosomus* sp. cf. *saylori*) over a two-day span during its breeding season, which peaks around late May and early June. The Clinch Dace were located in a stream in southwest Virginia and were gathered over a gravel pit during this time (Orth et al. 2014). Within the two-day span, the Clinch Dace showed a decrease in off-nest male-male chases and a decrease in on-nest male-male chases (Orth et al. 2014). This could be a sign of increased spawning behaviors as males become more interested in going after females (Orth et al. 2014). During the two-day span, there was also an increase in 1) off-nest male-female chases, 2) on-nest male-female chases, 3) females on a nest not being chased at all, 4) Clinch Dace benthic feeding, and 5) nest construction by a Central Stoneroller (*Campostoma anomalum*) (Orth et al. 2014). This could suggest that the Clinch Dace uses other species' nests to spawn (Orth et al. 2014).

<u>Southern Redbelly Dace (*Chrosomus erythrogaster*)</u>

The Southern Redbelly Dace (*Chrosomus erythrogaster*) spawns in large groups during late April or early May (Ohio Dept of Nat Resources 2012). Like many other smaller species in the family Cyprinidae, the Southern Redbelly Dace spawns by using the nests of larger minnows or suckers, such as Creek Chub (*Semotilus atromaculatus*), Striped Shiner (*Luxilus chrysocephalus*), Common Shiner (*Luxilus cornutus*), and White Sucker (*Catostomus commersonii*) (Ohio Dept of Nat Resources 2012). Nests from these species are usually found just above or below fast riffles in course sand or fine gravel (Ohio Dept of Nat Resources 2012). The larger Southern Redbelly Dace parent will guard the eggs on the nest but will not provide parental care after the eggs have hatched (Ohio Dept of Nat Resources 2012).

Tennessee Dace (*Chrosomus tennesseensis*)

The Tennessee Dace (*Chrosomus tennesseensis*) starts to spawn in late spring, from early April to about mid-June (Skelton 2008). Spawning usually happens over small, clean gravel substrates at the head of small riffles (Skelton 2008). The Tennessee Dace will sometimes spawn over the nests of other minnows, such as the Creek Chub (*Semotiles atromacelates*), stoneroller species (*Campostoma* sp.), and Saffron Shiner (*Notropis rubricroceus*) (Skelton 2008). The Tennessee Dace does not provide parental care for their offspring (Skelton 2008). Hamed et al. (2009) have observed spawning behavior in the Tennessee Dace during late April and early May, in which males would follow a single female throughout pool and run areas (Hamed et al. 2009). There would often be as many as 20 males following a single female, with males forming a straight line with the snout of one male just behind the caudal fin of the male before it, and the males do not pass each other (Hamed et al. 2009).

Blackside Dace (*Chrosomus cumberlandensis*)

The Blackside Dace (*Chrosomus cumberlandensis*) spawns from April-June (Starnes and Starnes 1981). This species exhibits a polygynandrous mating system in which males gather in small groups during spawning, and multiple males externally fertilize the eggs of each female as she deposits them (Starnes and Starnes 1981). The Blackside Dace usually spawns over silt-free gravel in the nests of other cyprinid species (Starnes and Starnes 1981). However, when nests are not available, they will use riffle areas (Starnes and Starnes 1981). Once the eggs are fertilized, there is not any parental care (Starnes and Starnes 1981).

Chapter 4: *Chrosomus saylori* Reproductive Behavior Study

Study Overview

Thus far, there has not been any documented research that aims to identify and describe the reproductive behaviors of *C. saylori*. My aim was to describe the reproductive behavior of *C. saylori.* To do this, I made video recordings of a captive group of *C. saylori* during their breeding season. I watched these recordings and I noted any potential reproductive behaviors including courtship behaviors and behaviors that occurred after egg fertilization. I then made a summary for every behavior that I observed. Finally, I analyzed my data and drew conclusions about the reproductive behaviors for *C. saylori*.

Study Subjects

The fish species being studied is the Laurel Dace (*Chrosomus saylori*). There were 25 wild adult *C. saylori* caught in Bumbee Creek (Rhea County, Tennessee) by biologists at the Tennessee Aquarium Conservation Institute (TNACI). These biologists started catching *C. saylori* on April 10, 2018. The number of males and females obtained was unknown because the sex of most individual *C. saylori* cannot be externally identified with complete confidence because coloration can vary daily and males and females visually appear relatively similar. The frequent color change within individuals is most likely due to factors such as water quality and hormonal changes.

Tank Design, Lighting, and Upkeep

A single tank housed all 25 *C. saylori* and was placed on a rack that was 1.83 meters high. The tank was 242.27 L and 76.20 cm long x 76.20 cm wide x 45.72 cm high. The tank was setup with a recirculating water flow system so that there was always filtered water entering the tank and dirty water leaving the tank. Due to water continuously flowing into the tank, dissolved oxygen was continually being added to the water. The bottom of the tank was covered in a cobble-sand mix substrate. There was also a thick piece of a tree limb in the tank that was approximately 63.50 cm long and 12.70 cm wide. The cobble-sand substrate and tree limb gave *C. saylori* certain aspects of their natural environment within the tank. Approximately two weeks after the fish were introduced to the tank, but prior to data collection, a biologist at TNACI built a mound using the substrate. This mound was intended to resemble the nest of other minnows, such as the Creek Chub (*Semotiles atromaculatus*) or stoneroller species (*Campostoma* sp.). This mound was added to determine if the nests of other fish species had any potential effect on *C. saylori* behaviors. Natural lighting was provided to *C. saylori* through windows on two walls, allowing for approximately 13 hours of light to 11 hours of dark during the study. Biologists at TNACI did all water quality monitoring, fish monitoring, fish feeding, and tank upkeep for the entire study duration.

<u>Video Recording Process</u>

I recorded *C. saylori* 2-3 times per week for approximately 20-minutes per recording session. Table 1 shows the recording sessions, device used, dates, times, and durations of all of my recordings. However, the first recording day consisted of two 15-minute recordings, and one 11-minute recording. This was because I did not have all of the equipment for the recordings. The recordings were made between 12 noon and 4 pm. This was done between April 27th and June 14th, which corresponds with the majority of their expected breeding season. In order to record the fish, I used two GoPro Hero 5 Blacks mounted on two 190.50 cm tall tripods. However, on two recording days I used a GoPro Hero 3. For each recording, I used each of the two GoPros to record one half of the tank, and the combination of recordings from each GoPro allowed me to have a detailed and complete recording of the entire tank for each 20-minute recording session; this, in turn, subsequently allowed me to accurately see all individuals in the tank and record detailed behavioral data. During each recording, I remained out of sight so as to not disturb *C. saylori* individuals while they were being recorded.

Table 1: A summary of all of recording sessions.

Recording Session	Device Used	Date	Time	Duration (minutes)
1	(GoPro Hero 3)	4-27-18	3:00 pm - 3:15 pm	15
2	(GoPro Hero 3)	4-27-18	3:20 pm - 3:35 pm	15
3	(GoPro Hero 3)	4-27-18	3:40 pm - 3:51 pm	11
4	(GoPro Hero 3)	4-30-18	1:00 pm - 1:20 pm	20
5	(GoPro Hero 5)	4-30-18	1:00 pm - 1:20 pm	20
6	(GoPro Hero 5)	4-30-18	1:00 pm - 1:20 pm	20
7	(GoPro Hero 5)	5-8-18	3:00 pm - 3:20 pm	20
8	(GoPro Hero 5)	5-8-18	3:00 pm - 3:20 pm	20
9	(GoPro Hero 5)	5-9-18	1:00 pm - 1:20 pm	20
10	(GoPro Hero 5)	5-9-18	1:00 pm - 1:20 pm	20
11	(GoPro Hero 5)	5-15-18	12:15 pm - 12:35 pm	20
12	(GoPro Hero 5)	5-15-18	12:15 pm - 12:35 pm	20
13	(GoPro Hero 5)	5-21-18	12:40 pm - 1:00 pm	20
14	(GoPro Hero 5)	5-21-18	12:40 pm - 1:00 pm	20
15	(GoPro Hero 5)	5-22-18	2:40 pm - 3:00 pm	20
16	(GoPro Hero 5)	5-22-18	2:40 pm - 3:00 pm	20
17	(GoPro Hero 5)	5-30-18	3:40 pm - 4:00 pm	20
18	(GoPro Hero 5)	5-30-18	3:40 pm - 4:00 pm	20
19	(GoPro Hero 5)	6-8-18	2:20 pm - 2:40 pm	20
20	(GoPro Hero 5)	6-8-18	2:20 pm - 2:40 pm	20
21	(GoPro Hero 5)	6-14-18	3:30 pm - 3:50 pm	20
22	(GoPro Hero 5)	6-14-18	3:30 pm - 3:50 pm	20

Data Collection

I downloaded and watched all recordings on my computer in order to identify any potential reproductive behaviors that occurred among any individuals in the tank during each recording period. There were 13 possible behaviors that I looked for. These behaviors were based on the behaviors of other smaller fish species, such as the Clinch Dace (*C.* sp. cf. *saylori*) (Orth et al. 2014), Southern Redbelly Dace (*C. erythrogaster*) (Ohio Dept of Nat Resources 2012), Tennessee Dace (*C. tennesseensis*) (Skelton 2008), Blackside Dace (*C. cumberlandensis*) (Starnes and Starnes 1981), and the Guppy (*Poecilia reticulata*) (Huber 2003). Out of the 13 potential behaviors that I looked for in the recordings, I observed 9 of these behaviors. All 13 behaviors are described below:

- **Attacking** behavior occurs when one individual swims up to another individual and bites or nudges the chest, head or caudal fin of the other individual.
- **Biting of the cloaca** occurs when one individual bites the cloacal region of another individual. This is not an aggressive bite that would be seen in attacking.
- **Chafing** occurs when one individual rubs any part of its body against another individual.
- **Chasing** occurs when one fish rapidly accelerates towards another individual.
- **Cloacal contact** occurs when one individual briefly touches another individual's cloaca with its cloaca.
- **Cloacal swing** occurs when one individual brings their cloaca forward from a resting position without directing it towards another individual's cloaca.
- **Cloacal thrust** occurs when one individual swings their cloaca toward another

individual’s cloaca, without making contact.

- **Dance** occurs when one individual would swim close to another individual. The first individual would then position itself upright and rapidly “flap” its pectoral fins.
- **Following** occurs when one individual or multiple individuals are following another individual, either from behind or from the side.
- **Nest takeover** occurs when an individual uses another species’ nest for spawning.
- **Shoaling** occurs when a group of fish are gathered together for social reasons.
- **Sigmoid display** is when a fish, usually a male directed towards a female, flexes its body into a strong "C" or "S" shape and vibrates (Huber 2003). The fish in the sigmoid display may be at various angles to the other fish, or even facing away (Huber 2003). The degree of intensity (i.e., high, medium or low) can depend on either the duration or the amount of flex in the body of the fish in the sigmoid display (Huber 2003).
- **Territorial behavior** occurs when one individual is showing signs of aggression while staying close to a specific area or spot. This aggression can be demonstrated through behaviors such as attacking and chasing any other fish that come too close to the specific area or spot. Because biting, nudging, and chasing tended to occur with territoriality, I reported both the overall and total frequency of biting, nudging, and chasing, as well as the frequency of these behaviors during territoriality.

I used Microsoft Word to gather data while watching the recordings. In every video, I frequently stopped and re-watched the recordings, often times in slow motion, to ensure that I had an accurate observation of each behavior that occurred in a given tank. In order to record behaviors, I would keep track of a few fish throughout the entire video and/or watch for any signs of rapid movement among the fish in a given tank depending on activity level in the tank. Because I frequently re-watched the videos and because I watched only one-half of a tank at a time, I am confident that I documented all relevant behaviors that occurred within a given tank.

I kept track of the behaviors described above by noting the time stamp for the beginning and end of the relevant behaviors that occurred in the videos. I then recorded a brief summary of what was happening for each behavioral observation. After watching each recording, I then identified the total number of times a behavior occurred during the 20-minute period within the tank. I used these data to record the relative frequency of each behavior observed over all of my observation periods.

Results: Observed Behaviors

In the 421 minutes of recording across 22 recording episodes, I identified a total of nine relevant behaviors. The total number of times that I observed the nine behaviors was 146. There was one behavior that was recorded and observed, but it was not included in the data totals because I was not completely confident in identifying which behavior it was. Below, I provided a description of the relative frequency of each behavior. Additionally, I provided a complete summary of the relative frequencies of each behavior (Figure 5) and a complete summary of the percentages of each behavior (Figure 6).

1) The behavior that occurred most often was chasing, which occurred 52 times and

accounted for 35.62% of the behaviors observed. However, this does not include the numerous chases that most likely occurred behind the tree branch while *C. saylori* were shoaling. If all of the chasing during the shoaling could have been observed and included, the total number of chasing behavior exhibited is expected to be greater than what was observed. Regardless, chasing appears to be very frequent among breeding-season *C. saylori*. Chasing was often done when one territorial *C. saylori* chased other *C. saylori* away from a specific spot. Numerous episodes of chasing also occurred when *C. saylori* were gathered under the tree limb. When one or more *C. saylori* would swim away from the tree limb, multiple *C. saylori* would chase after that individual and chase them back to the group under the tree limb. While *C. saylori* were gathered under the tree limb, multiple *C. saylori* would chase each other around the tree limb and back to the group. In other instances, chasing would occur when all *C. saylori* were casually swimming. This chasing only lasted approximately 5 seconds, and then *C. saylori* would disperse.

2) Attacking behavior was observed the second most frequently, occurring 43 times and accounted for 29.45% of the behaviors observed. The attacking behavior usually involved one territorial *C. saylori* that would bite or nudge the body or caudal fin of another *C. saylori*. In a few cases, one *C. saylori* would attack another individual while it was not being territorial. The most extreme case of this attacking behavior occurred only in one recording, where two *C. saylori* would attack each other every 15 seconds over approximately a two minute period.

3) A relatively short variation of a sigmoid display occurred 30 times and accounted for 20.55% of the behaviors observed. When the sigmoid behavior was observed, a *C. saylori* would flex its body into a strong "S" position and rapidly flop its body once either

at another *C. saylori*, on the substrate close to another *C. saylori*, or on the substrate without another *C. saylori* present. Fifteen of the total 30 sigmoid displays (50%) were exhibited on the substrate. While I did not directly observe spawning, it is possible that this behavior occurred during spawning given that it involved two individuals and occurred on the substrate.

4) Chafing occurred 5 times and accounted for 3.42% of the behaviors observed. However, this is not including all of the chafing that most likely occurred while the *C. saylori* were shoaling, which is very difficult to observe in the middle of a group during shoaling. If chafing during shoaling could have been counted, the total number of chafing behavior is expected to be greater than what is reported here. Chafing was typically observed when *C. saylori* were gathered under the tree branch. Chafing was occasionally observed when *C. saylori* were swimming.

5) Territoriality occurred at a similar frequency as chafing, occurring 4 times and accounting for 2.74% of the behaviors observed. Territoriality was always exhibited by one *C. saylori* each time the behavior was observed. During territoriality, a *C. saylori* would appear to guard a specific spot. Behaviors that frequently occurred with territoriality included biting, nudging, and chasing off all other *C. saylori* that came in close proximity to the specific area appearing to be guarded. These associated behaviors (biting, nudging, and chasing) were observed for a total of 81 times during territoriality. As mentioned above, biting and nudging were observed a total of 43 times during all recordings, and 34 (of these 43) instances occurred during territoriality. Biting and nudging accounted for 41.98% of the behaviors observed during territoriality. These two associated behaviors were grouped together because it was not possible to identify which

action was occurring during each instance while a fish was exhibiting territoriality. As mentioned above, chasing was observed a total of 52 times during all recordings, and 47 of these occurred during territoriality and accounted for 58.02% of the behaviors that I observed during territoriality. Thus, it appears that biting, nudging, and chasing are typically associated with and related to territoriality in this species. When observed, territoriality and its associated behaviors would generally persist for the entire recording. It is not clear what the individuals are trying to protect; however, it is possible that they were guarding fertilized eggs that were deposited or buried in the substrate. If so, this would indicate that *C. saylori* exhibits parental care for fertilized eggs. However, additional research would be needed to confirm this, as it is possible that they were simply territorial in the absence of fertilized eggs.

6) Following behind was a behavior that occurred 4 times and accounted for 2.74% of the behaviors observed. A single *C. saylori* would be followed for 10-15 seconds. Following behind usually only occurred when all of the *C. saylori* were swimming. I did not observe the following behind behavior when *C. saylori* were gathered under the tree limb.

7) Shoaling was a behavior that only occurred 3 times and accounted for 2.05% of the behaviors observed. In one instance, there were approximately 18-20 individuals that gathered tightly under a small portion of the tree limb. In the other instances, there were approximately 8-12 or 15-18 individuals grouped together. Thus, under the conditions of this study, it appears that shoaling groups range between eight and twenty individuals. While under the tree limb, chafing would frequently occur among these individuals. There was also frequent chasing around the tree limb. If a *C. saylori* left the group and

swam away, then multiple *C. saylori* would chase after it and chase it back to the group. There were also many *C. saylori* that would engage in chasing around the tree limb, but they would return back to the group after a very short time.

8) Biting of the cloaca occurred 3 times and accounted for 2.05% of the behaviors observed. One *C. saylori* would bite or nudge another *C. saylori* on or around the cloaca, and then either swim off or swim next to the individual that was bitten or nudged. This behavior was not observed while the *C. saylori* were gathered under the tree limb.

9) Dancing was the behavior that occurred the least, only twice, and accounted for 1.37% of the behaviors observed. One *C. saylori* would swim close to another *C. saylori*, position itself upright and rapidly "flap" its pectoral fins. This behavior only lasts a few seconds, and then the individual stops and either swims away or stays next to the other *C. saylori*. This behavior is potentially a reproductive behavior. However, this behavior needs to be analyzed in greater detail in order to fully confirm that this is a reproductive behavior.

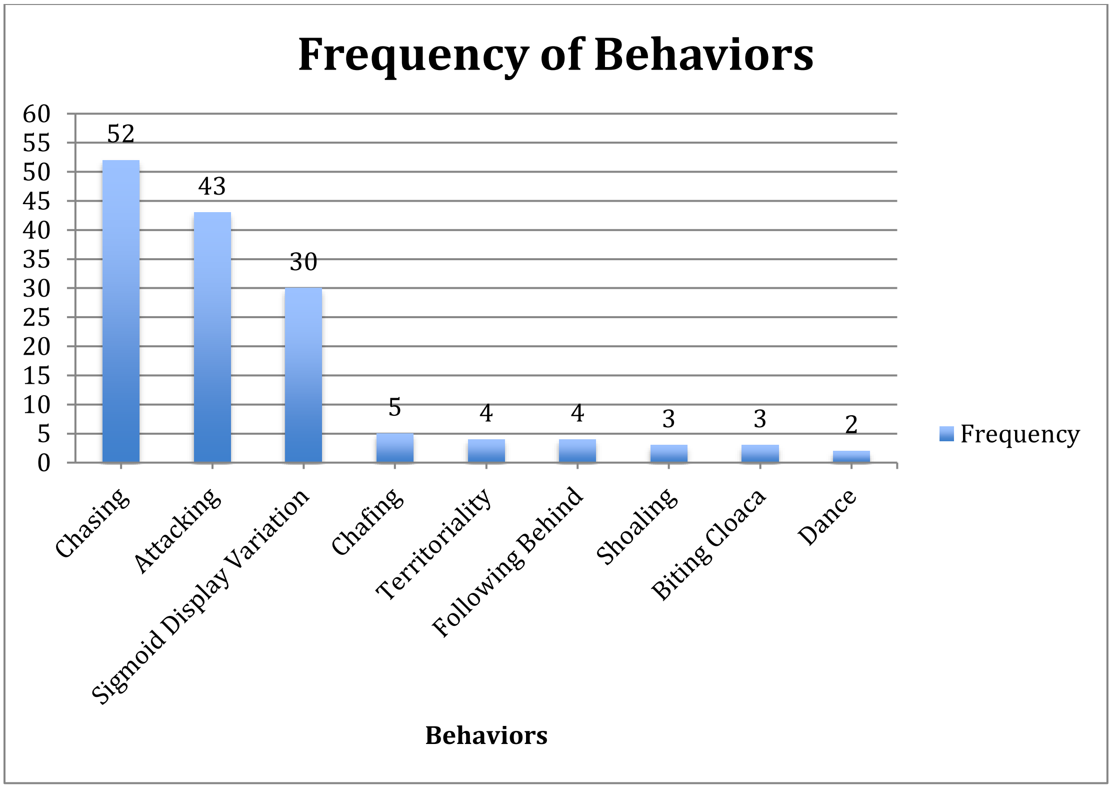

Figure 5: A summary of the frequency of each observed behavior.

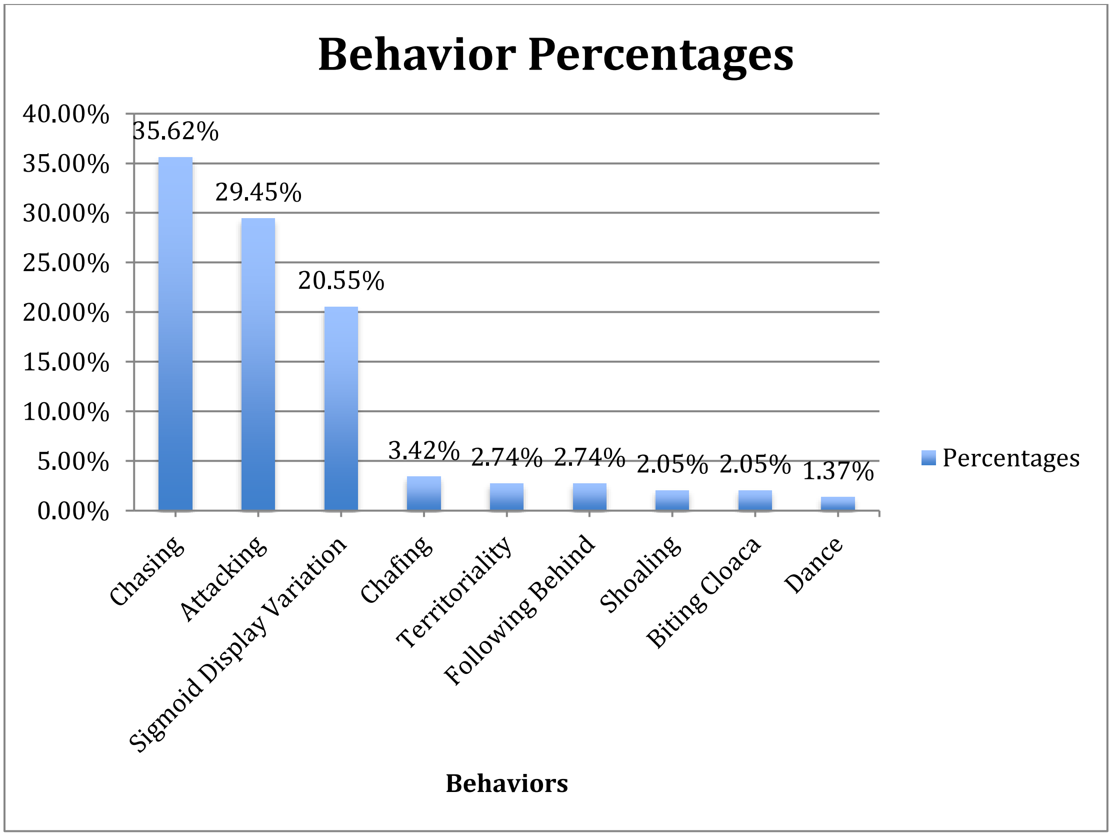

Figure 6: A summary of the percentage that each observed behavior occurred.

Cloacal contact, cloacal swings, cloacal thrusts, and nest takeovers were never observed during the observation period. Additionally**,** spawning was never directly observed during the recording periods. However, throughout the study period, TNACI biologists collected eggs on 5 occasions, and found a total of 987 eggs and larvae. Thus, breeding did occur during the 2018 mating season under the conditions at TNACI.

Discussion

Some of the observed behaviors of *C. saylori* are consistent with the behaviors that have been observed in other *Chrosomus* species, while other behaviors appear to be unique to *C. saylori*. Behaviors seen in other *Chrosomus* members that were also present in *C. saylori* are possible parental care of fertilized eggs (also seen in *C. erythrogaster*) and following (also seen in *C. tennesseensis*) (Hamed et al. 2009; Ohio Dept of Nat Resources 2012). Based on my observations and literature review, *C. saylori* appears to perform other behaviors not observed in other members of *Chrosomus* such as the sigmoid display and the dance.

Based on my recordings and informal observations of breeding *C. saylori*, TNACI researchers and I also discovered that the sex of an individual could not be visually determined from its coloration with complete confidence due to the frequent change in their color intensity. This is most likely due to factors such as water quality and hormonal changes. Another unexpected discovery was that the male *C. saylori* in our study did not seem to have tubercles (i.e., no tubercles were visually observed). Typically, male *C. saylori* will develop tubercles during the breeding season (Skelton 2001). However, in order to fully confirm the absence or presence of tubercles, a preserved specimen will need to be visually examined under a dissecting scope since the tubercles are so small.

The breeding season for *C. saylori* is normally between late March and mid June (George et al. 2016). However, in our lab population, individuals did not start breeding until late April and finished in mid June. Importantly, though, it is possible that the *C. saylori* might have already started spawning in the wild, since they were not collected until early April. Later breeding was expected due to the *C. saylori* being moved from their natural environment into a tank that mimicked their natural environment. The *C. saylori* started spawning soon after being brought in from the wild, with little time needed to become adapted to their new artificial surrounding. Additional research will need to be conducted to determine when *C. saylori* begin spawning in both the wild and in the lab.

Conclusion

Chrosomus saylori exhibited some reproductive behaviors that were expected and some that were not expected. Most of the observed behaviors are similar to behaviors that have been observed in other *Chrosomus* members, such as following and the possibility of parental care to buried fertilized eggs. In contrast, other behaviors seem to be relatively unique to *C. saylori*, such as a possible sigmoid display and dance. Other observations of note specific to *C. saylori* include the potential lack of breeding tubercles and their coloration intensity varied depending on factors that most likely include water quality and hormonal changes.

In summary, *C. saylori* reproduction in a lab setting can occur. Based on the current study, we suggest that the following conditions are conducive to and will potentially facilitate lab rearing of this species: 1) a tank that provides approximately 10 L of water per individual fish; 2) a cobble-sand mix substrate and a thick, long tree limb or other structure; 3) a mound in the substrate; 4) flow-through, recirculating water; and 5) natural lighting that provides approximately 13 hours of light and 11 hours of darkness. Breeding under these conditions are expected to occur from April through June based on our study; however, if *C. saylori* had been in the lab before early April, then spawning would have most likely occurred during their normal breeding season. It is possible that there are a range of other conditions that might facilitate lab rearing of *C. saylori*; given that we only focused on one set of conditions in the lab in this study, we cannot definitively conclude whether other conditions might also promote or improve upon breeding of this species.

During our study, successful reproduction occurred given that fry were frequently found in the tank. This indicates that individuals were in breeding condition and at least some of the behaviors observed were associated with spawning, including chasing, attacking, a variation of a sigmoid display, chafing, territoriality, following behind, shoaling, biting of the cloaca, and a dance. More research would be needed to determine if similar or different behaviors are exhibited during the non-breeding season.

Future Study Recommendations

Replication of this study is necessary in order to better understand the reproductive behaviors of *C. saylori*. However, there are a few possible changes that could allow us to dive deeper into their reproductive behaviors. Having *C. saylori* either in a bigger tank or split up into multiple tanks would be beneficial to the observer. This gives the *C. saylori* extra space, which would allow for easier tracking of certain behaviors such as following. When *C. saylori* are together in a small tank it makes it difficult to observe whether some of them are following one another or if they are all just closely swimming around one another. Another approach would be to find out what the sex is of each individual *C. saylori* that is being studied, either by conducting genetic testing or discovering a way to visually identify which are males and which are females. Once the sex of all individuals is determined, one could then split them up into multiple tanks. In these different tanks, there could potentially be different male to female ratios. This would allow us to observe what the ideal mating ratio is for *C. saylori*. Another recommendation would be to tag all of the *C. saylori* after the sexes have been determined so that the behavior of individuals could be followed.

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