

Behavioral and Early Developmental Biology of a Mouthbrooding Malaŵian Cichlid, *Melanochromis johanni*

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Abstract

This study aimed to characterize the reproductive biology of a little-studied, aquaria-raised mouthbrooding cichlid, *Melanochromis johanni*, endemic to Lake Malaŵi in the East African Rift Valley. Due to threats on lake conservation that are affecting genetic and biological diversity (Odada *et al.* 2003), it is important for this species, among many others, to be described as thoroughly as possible. The goals of this study were to characterize the reproductive biology of *M. johanni*, including behavioral aspects of dominance, territoriality, visual communication, courtship, breeding, and early-development. The questions tested are: does *M. johanni* form a linear hierarchy in a large group, and does the hierarchy remain stable and linear through time? Does territoriality in aquaria imply shared dominance between territorial holders, and to what spatial extent can that shared dominance occur? How does *M. johanni* use pigments to communicate visually? What is the courtship sequence of this species, and how often does it result in breeding? Does the female's fecundity have a relationship with her size, and what are the stages of early development?

Behavior was recorded using a camcorder and assessed in several aquaria. *M. johanni* was found to form a linear hierarchy, and only the *alpha* and *beta* males (the two most dominant positions in the hierarchy) maintained stable positions over time. Also, I found territoriality between males to imply shared dominance in larger aquaria. However, differing spatial extents of neighboring males were tolerated. The courtship sequence was identified and quantified using a flow diagram. Fecundity was positively correlated with female size, and the stages of early development were carefully documented, including the passage through cleavage, embryonic, and eleutheroembryonic (post-hatching) phases as outlined by Balon (1985).

Introduction

The cichlid species from Africa's Rift Valley Lakes have undergone tremendous adaptive radiation, and though much is known about their evolutionary background and systematics, much less is known about the reproductive biology of many species. In this study, I aim to characterize much of the behavioral and developmental biology of *Melanochromis johanni*, a mouthbrooding cichlid species endemic to Lake Malaŵi in the southern portion of the East African Rift Valley.

The Rift Valley Lakes of Africa provide the most dramatic case of vertebrate speciation known (Ribbink 1990), providing better evolutionary insights than Darwin's finches from the Galapagos (Fryer 1973). Due to intentional and unintentional species introductions, overfishing, pollution, and many other pressures on the lakes in the African Rift Valley (Odada *et al.* 2003), the native fish fauna of these lakes are being threatened (Odada *et al.* 2003). Thus it is important to characterize the biology of as many species as possible. We cannot leave species undescribed knowing that many environmental perturbations on the species occupying very specific niches could cause species to go extinct without us even having known they existed (Park and Cohen 2011), a fear expressed by conservationists (Ribbink 1990).

According to Kornfield and Smith (2000) and Konings and Stauffer (2012) a gap in the knowledge of the genus, *Melanochromis*, is a lack of a formal description of many of the species. *M. johanni* is one of these little-studied species (Eccles 1973; Andries and Nelissen 1990; Fishelson 1995). Also, *M. johanni* is a popular species among aquarists, but urged only to be kept by advanced cichlid owners due to its high aggressiveness in aquaria (Oliver 2013). Studying its behaviors in an aquarium setting could help aquarists keeping this species.

The goals of this study were to characterize the reproductive biology of *M. johanni*, including behavioral aspects of dominance, territoriality, visual communication, courtship, and early development.

Though reproductive biology studies on mouthbrooding cichlids have been performed in the Cruz lab (e.g. Roscow 2012, McCrea 2002) as well as in other studies (e.g. Balon 1985; Fleig 1993; Fishelson 1995; Fujimura and Okada 2007), similar studies have not been done on *M. johanni*. Also, complete early ontogeny (development) studies in African Cichlids are few and far between (Balon 1985).

Objectives

In this study, I addressed the following:

Dominance and Territoriality

1. Will *M. johanni* form a linear hierarchy in a large group, and does this hierarchy remain stable over an extended period of time? I hypothesized that they would form a linear hierarchy, and that this hierarchy would remain stable if the fish had been living together for several months to establish a rank order.
2. Does territoriality imply shared dominance between territory holding males, and, if so, will the dominant males avoid the territory of the other dominant male? I hypothesized that shared dominance could occur in a larger aquarium, and the dominant males would avoid the other dominant male's territory to reduce aggression. I further hypothesized that shared dominance would begin to break up once the territories got too close because the males would not tolerate the close proximity of another male that could take away chances for spawning events.

3. How does *M. johanni* use pigments to communicate visually? I hypothesized that the dominant males would have a unique coloring to show dominance as many African cichlids show this pattern (Seehausen 1996).

Courtship and Breeding

I characterized the courtship sequence of *M. johanni*. Since females are not always reproductively ready for spawning, I predicted that courtship displays would not lead to breeding very often. I hypothesized the breeding sequence to be a set pattern similar to many other African cichlids, and the eggs would be fertilized inside the mother's mouth rather than externally because this pattern is favored by evolution and will protect eggs better from egg predators (Ribbink 1990). I also hypothesized that only the territory holding males would display for females, and I expected to see floater (sneaker) males try to sneak in on spawning events in order to attempt to fertilize some of the eggs.

Reproductive and Developmental Biology

I characterized the early ontogeny of *M. johanni* and examined the relationship between fecundity and female size. I hypothesized that the fecundity (number of eggs) would be positively correlated with female size.

Background

Family *Cichlidae*

The family *Cichlidae* is native to tropical regions of the world in Central and South America, Africa, and Asia (Barlow 2000). Cichlids have two different forms of reproduction, substratum brooding and mouthbrooding (Ribbink 1990). While substratum brooders, the majority of the family *Cichlidae*, lay their eggs on a surface, mouth brooders carry their

developing young inside the buccal cavity where they are kept until they are independent and free swimming (Ribbink 1990). This breeding strategy is common among African cichlids, and it insures high survival rates in systems where predation pressures on eggs and juveniles are strong (Ribbink 1990). *Cichlidae* is not the sole family to have adapted this type of brood care; it is a rare occurrence comprising 10 families, only 2.4% of teleost fish, both from fresh and saltwater systems (Kuwamura 1985). Though most mouthbrooding is maternal, paternal mouthbrooding is also found among a few fish families, such as a family of cardinalfishes, *Apogonidae* (Barnett and Bellwood 2005), the sea catfish family *Ariidae* (Acero *et al.* 2007), and even some cichlids in the tribe tilapiine (Klett and Meyer 2002).

African Rift Valley

The East African Rift Valley is a system of lakes and volcanoes, caused by tectonic plate movements of the earth's crust, spanning 2000 km from Mozambique at its southern tip to the Red Sea in the north (Furman *et al.* 2004). Lakes formed by tectonic activity are among the oldest lakes on earth, outdating glacial lakes formed after the most recent ice age. The three largest lakes, Victoria, Tanganyika, and Malawi, are among the biggest lakes in the world, the latter two possessing an extremely deep relative depth (Odada *et al.* 2003). In the northern region of Lake Malaŵi, a maximum depth was recorded to be 800 m (Kornfield and Smith 2000). This region has a history of great evolution events; not only were the first humanoid species descendant of this area (Mancini *et al.* 2012), but within these lakes lies an unimaginable species diversity of cichlids.

Lake Malawi

Lake Malawi houses the greatest diversity of haplochromine (tribe of cichlids found in east Africa) cichlid species. Though there is some disagreement as to exactly how many cichlid

species inhabit Lake Malaŵi, there are estimates as high as 835 haplochromine cichlid species (Konings 2007), but only an estimated 535 have been documented formally (Oliver 2013). *M. johanni* is part of a rock-dwelling group of haplochromine cichlids, commonly called mbuna cichlids, which inhabit the rocky shorelines. They are all closely related, and their explosive speciation is due to the fact that each species is confined to microhabitats around the shorelines of the lake (Ribbink 1990). While the mbuna cichlids are so diverse, the open waters are inhabited by less specious fishes, including the tribe of tilapiine cichlids that comprise a mere six species in the lake (Ribbink 1990).

Conservation of the African Rift Lakes

The African Rift Lakes are currently experiencing numerous types of anthropogenic threats to their tremendous genetic and biological diversity (Odada *et al.* 2003). The three largest lakes, Victoria, Tanganyika, and Malaŵi, are each experiencing their own combinations of pressures. Lake Victoria is currently the most threatened because of the introduction of the Nile Perch, a fish that has indefinite growth, and eats any fish that can fit into its mouth (Kitchell *et al.* 1997). Lake Victoria serves as an example of how extreme certain threats on biodiversity can become (Kitchell *et al.* 1997).

Odada *et al.* ranked the major threats to Lake Malaŵi in 2003 to be “unsustainable exploitation of fisheries” and “habitat modification.” Overfishing, the capture of non-target species by fisheries, accidental contamination by humans, the modification of shoreline habitat, and invasive species introductions are changing ecosystems within the lake and contributing to a decline in genetic and biological diversity. Since Lake Malaŵi has the highest haplochromine cichlid adaptive radiation of the rift lakes, it is absolutely necessary that biologists describe and obtain life history information on as many cichlid species as possible. At present, just over half

of the species are estimated to be described with proper scientific researched in Lake Malaŵi (Oliver 2013), and it is possible species have already gone extinct within the past few years without anyone but natives even realizing their absence.

Study Species

The study species' genus, *Melanochromis*, belongs to the rock-dwelling group, mbuna, and contains relatively small, striped cichlids (Konings and Stauffer 2012). *M. johanni* is a sexually dichromatic species inhabiting the eastern shore of Lake Malaŵi between Makanjila point and the Mozambique border (Konings 1990). The females are bright yellow, and the males are blue with a black striping pattern. Since this species has only been mentioned in a couple studies (Eccles 1973; Andries and Nelissen 1990; Fishelson 1995), not much is known about their behavior.

Characterizing a Species

Characterizing the full spectrum of a fishes' behavioral and reproductive biology is a task that involves many different aspects. Though the present study does not cover the full spectrum of the behavior and life history of *M. johanni*, many aspects of its behavior were studied, including communication, dominance, territoriality, courtship and breeding behaviors, and the developmental staging.

Dominance. Studies of dominance behaviors have shown that some fish species form organized hierarchies in aquaria. There are several types of hierarchies formed by animals living in groups, but linear hierarchies have been found to be the most classic example (Chase *et al.* 2002). In a linear hierarchy, one fish (*alpha*) dominates all other individuals, the next dominant fish (*beta*) dominates all but *alpha*, and so on (Chase *et al.* 2002). Complexities in this hierarchical structure are called intransitive triads (a triangular structure in which three

individuals in the group deviate from this linear hierarchy; Chase *et al.* 2002). Instead of A dominating B and B dominating C, who is the least dominant individual, A dominates B and B dominates C, but C dominates A. The more intransitive triads a hierarchy contains, the less linear it is; a perfectly linear hierarchy contains no intransitive triads (Chase *et al.* 2002).

Hypotheses have been formed regarding the social ascent and decent involved in an individual's attempt to situate itself in a hierarchy. The winner effect states that the winner of an interaction with one member of the hierarchy can continue winning and moving on up the hierarchy caused by unknown effects that may be a release of hormones or adrenaline due to the good feeling of winning (Dijkstra *et al.* 2012). The loser effect is just the opposite, stating that a loser of a social encounter will continue losing, causing an individual to fall considerably from its previous position within the hierarchy (Dijkstra *et al.* 2012).

Most dominance studies performed on fish involve pairing fish and recording winners of one-on-one trials. Fewer studies involve observing a hierarchy among a group of captive fish (Nelissen 1985). It has been concluded that group dynamics can differ from the outcomes of one-on-one trials. In group trials, like their relatives *M. auratus*, *M. johanni* have been found to establish linear rank orders in groups of three to six individuals in aquaria (Andries and Nelissen 1990).

Territoriality. Territoriality is an important factor for mbuna cichlids who occupy rather small niches. There is only enough territory for a small proportion of males in the rocks, so many males without territories (deemed “floaters”) cannot attract females, and thus many rely on a tactic of “sneaking” in on mating of territorial males and females (Haesler *et al.* 2009). Territorial males of several cichlid species have been found to differ significantly in their color patterning, compared to other fish of their species (Andries and Nelissen 1990; Seehausen 1996).

Courtship and breeding. As many haplochromine cichlid species are closely related and can hybridize, many questions arise about why this does not occur in nature. One answer may lie in the courtship behaviors of these fishes. Though most courtship sequences that lead a male to attract a female to mate with him resemble closely among cichlid species, it has been found that there are subtle differences among species (Ribbink 1990). It is important that the courtship sequences of many species be very closely scrutinized to find differences among the sequences that may show exactly which reproductive and behavioral barriers keep these species from hybridizing.

Reproductive and developmental biology. When describing a fish species in detail, the reproductive biology and development are very important subjects. In mouthbrooding cichlids, the reproductive biology and early development is especially imperative. Expending so much energy in parental care is very time and energy consuming for the female, who sacrifices eating for the 21 to 31 days that she carries her young in her mouth (Balon 1985). The young are also provisioned with large yolk sacs during the time they are in the buccal cavity (mouth), thus the young develop much differently and at different rates than most fish species (Fishelson 1995).

Many fishes' early developments have been formerly described (Balon 1985; Fleig 1993; Fishelson 1995; Fujimura and Okada 2007). Studying the early development of the haplochromine cichlids provides another way to define evolutionary pathways among these closely related fish (Yanze *et al.* 2001; Coates 1994). The mouth-brooding African cichlid's early development differs from the development of many different types of fish. In comparison to most other fishes, the eggs of mouthbrooders are very large and irregular shaped (Fleig 1993). This different egg structure is an important perception into certain stages of development (Fleig

1993). Also, early development is slower in mouthbrooding cichlids since the young will be securely protected by the mother inside the buccal cavity until independence (Fishelson 1995).

Methods

Four breeding tanks were used in this study. Tank A and B were both 76 L. Tank A housed seven fish, five females and two males. Tank B held ten fish, three females and seven males. Tank C was 208 L and contained ten fish, five females and five males. Tank D was 378 L and contained eight females and seven males. Fish were sexed based on appearance, as the females are lighter in color than males.

All tanks were kept between 24 and 26°C. They also all contained natural crushed shell as a substrate and clay flower pots for establishing territories, breeding, and hiding in. Foam and/or under gravel filtration was used, and the water was changed every two weeks. The fish were fed a high quality flake food once daily. A salt mixture was used to mimic natural water parameters in order to promote breeding.

Behavioral Experiments

General observations. African cichlids have been found to communicate in different ways. Auditory communication has been studied (Maruska *et al.* 2012) as well as visual communication (Nelissen 1974; Smith and Staaden 2009). In order to evaluate the way the fish communicate physically, it was necessary to observe the species for hours and create a detailed ethogram of behaviors. The behaviors were then divided into the categories: dominance, submission, courtship, and territorial behaviors. This ethogram allowed me to perform the behavioral experiments in this study.

After noticing that each *M. johanni* possesses a unique pattern, I decided to look into forms of visual and physical communication of this species. Since the territorial males in each tank were larger than the other fish in the tank, all fish were measured and sizes were compared by assigning the territorial males a score of one and the subordinate fish a score of zero. Female and male sizes were both included in the subordinate fish count because *M. johanni* is not sexually size dimorphic. I plotted a linear model for each tank as a visual, and to test for significance of the difference in sizes of the territorial males and the subordinate fish across tanks of differing size, I performed a Randomized Block-Design Anova. The block design compared all of my linear models while controlling for the large discrepancies in sizes of fish that are in smaller aquaria compared with the fish kept in large aquaria.

Dominance observation. This observation tested the linearity of the hierarchy formed by a group of *M. johanni*. For this experiment, tank B, a 76 L tank, with a well-established group of *M. johanni* was used. This tank housed ten fish, seven males and three females. The fish had been kept together in this tank for four months without any new individuals introduced or any individuals removed. I used the ethogram I made for this experiment.

Six trials of 15 minutes each were performed on the *johanni* group. The tapes were recorded roughly once a week for one month to test the stability of the hierarchy over time. During these trials, the tank was recorded for a 15 minute period. The tape was reviewed, following each fish until the tape had been reviewed as many times as there were fish in the tank. Thus every individual was observed for a total of 90 minutes with 10 total individuals. Each fish was given a name using M or F for sex and a number corresponding to the numerical order in which the individual was observed in the first recording. During these reviews, all interactions and behaviors were recorded as well as their coordinating times (such as M4 chase: 3:35). After

all fish were viewed for the complete 15 minutes and all interactions and times were recorded, each interaction was matched with its subsequent interaction corresponding to the interaction of another individual (such as M4 chases M7: 3:35). This allowed for each fish to be identified.

In order to distinguish the individuals and to give them their proper titles in the subsequent videos, details about their appearance that differentiated them from others were recorded or sketched, including coloring and the pattern of their left and right lateral sides, as they are not the same on both sides.

After each video was finished being reviewed, a quick analysis was performed that indicated the number of dominance interactions, subordinate interactions, and courtship and/or territorial behaviors of each individual. Then each fish was ranked against each individual with which interactions were observed. They were found to be either dominant or subordinate to the other fish based on the number of dominant and subordinate interactions with that specific fish. Then all fish were ranked in each tank assuming a linear dominance hierarchy. The rank orders from all six trials were compared to answer the question about the stability of the hierarchy overtime. Next each trial's rank order was tested for its linearity with Landau's equation, $H=12/(n^3-n)\sum(v_a-(n-1)/2)^2$, as adapted by Kendall's index (De Vries 1995) for rank orders involving tied individuals. The mean of the outcomes of the six trials was recorded to test the hierarchy's linearity.

Territoriality and shared dominance observation. Territoriality in an aquarium setting is a good insight into the dispersal pattern of territories in the wild. It also may complicate a dominance hierarchy in a large aquarium. To test whether two territorial males in an aquarium both share the dominant *alpha* status, I performed an experiment testing space allocation. If one male dominates over the other, they are not truly sharing the *alpha* status. This observation was

performed on an aquarium containing two large males, each occupying a territory. The observation tests how territoriality and shared dominance functions in *M. johanni*. For this experiment, I observed tank D containing 15 *M. johanni*. The décor in the tank was fashioned into two territories used for breeding (formed by a circle of three flower pots on either side of the tank). The aquarium was divided into three equal sections using thin strips of tape. The left section contained the territory inhabited by “Male Left,” while the right section contained the territory inhabited by “Male Right.” Each side also contained a large sponge filter, so that both territories were identical. The middle section of the tank was referred to as the neutral zone as it was the section of the tank in between the two territories (Figure 1).

The aquarium was observed for ten trials of ten minutes each. During each trial, the two dominant males were observed and the times they spent in each section of the tank were recorded. The times spent in each zone of the aquarium for both males for all trials were averaged. The average times in ten minutes that each male spent in the three zones of the tank were plotted on a bar graph for a visual comparison of the results. If the two males were truly sharing dominance, I expected them to stay away from each other’s sections of the aquarium to avoid persistent aggression.

Territoriality and shared dominance manipulation. In order to test the spatial limits of this shared dominance, an experiment was performed testing the hypothesis: shared dominance can only exist given enough space between territories of the *alpha* males. There are potentially many other variables that affect the existence of territorial-driven shared dominance, but due to time parameters, space was the only variable tested.

For this experiment two tanks were used. One tank was D (a 378 L tank housing 15 fish on which shared dominance trial one was performed), and the second tank was C (a 208 L tank

housing 10 fish). I chose these tanks because shared dominance had already been established between two large, territory-holding males, each displaying the vertical stripping pattern of a dominant male. The décor in each tank was fashioned into two identical territories, each comprised of three clay flower pots. Both tanks were divided into six equal sections (numbered 1-6) using tape (Figure 2). The sections in tank C were 20.3 cm in width and in tank D were 30.5 cm wide. Three different spatial distances of the *alpha* males' territories were tested using two repetitions of 20 minutes each. In the first trial, the territories were placed in zones one and six; that is one territory was in zone one and the other was in zone six. As these two zones were at the farthest sections of the tanks, the territories were as far apart as possible (81.2 cm apart in tank C and 122 cm apart in tank D). After the two repetitions of 20 minute recordings on each tank had been performed, the territories were both moved to zones two and five (40.6 cm apart in tank C and 61 cm apart in tank D). I waited 48 hours before recording the two 20 minute trials with the territories in the new zones to let the fish adjust to the spatial movement of the territories. Last, the territories were moved to the closest position possible, zones three and four (at this point the two territories were touching). After 48 hours two repetitions of 20 minutes were recorded.

After the recordings had been gathered, the tapes were reviewed following each of the two dominant males for both tanks. The males were named after the side of the tank in which their territory was kept (Male Left and Male Right). The times the males spent in each of the six zones was recorded for each video. In this way it could be discerned how much time the male spent in or near his territory since the territories took up an entire zone.

To analyze the data, the average times spent in each zone for both tanks was calculated for Male Left and Male Right for each of the three spatial positioning of the territories. A

histogram was made describing the results of each tank for the three trials. The overall times each Male Left and Male Right spent in the zone containing his own territory were compared among trials as the territories were moved ever closer to tell if or when the territory and shared *alpha* status were given up.

Courtship observation. This observational study involved recording aquaria and reviewing the tapes, specifically looking for courtship and breeding behavior. Some of the data were found in the recordings of other experiments, and these data were recorded as well. The courtship behaviors are defined in the ethogram I made. The main behavior that I searched for was the male's display. Once a good courtship sequence was found, it was used to produce a list of behaviors that were likely to lead to other steps in the courtship to breeding process. All courtship events were compared to this ordered list of behaviors:

1. Male approaches female- The male swims up behind a female and tries to get her to notice his presence.
2. Male displays- Two types of displays were observed but not differentiated in this experiment.

In one display (lateral display), the male swims up quickly and back down in a large movement, while waving the caudal fin. In the other display (quiver), the male stops swimming and begins to quiver or vibrate his body very quickly.
3. Male leads female to territory- The male swims to a territory, trying to coax the female to follow him.
4. Male enters territory- The male swims into the territory to signal his will to spawn.
5. Male displays- (same as above)
6. Female enters territory- A willing female enters the territory.

7. Male exits territory- The male quickly swims back out of the entrance to the territory, seemingly to trap the female in.

8. Male displays- (same as above)

9. Female exits territory- A female who is not gravid (ready to breed) leaves the territory.

OR

10. Mutual T-position- The male performs his vibrating display as the female approaches perpendicular to his body and nibbles his genital region.

11. Breeding- The male and female perform a series of T-positions, in which the pair take turns nibbling each other's genitalia. Once the female drops a few eggs, she immediately takes them into her mouth, and they are fertilized during her next T-position with the male.

This cycle continues until all her eggs have been laid, taken into her mouth, and fertilized by the male.

To analyze the data, all courtship events were evaluated, and the sequence that occurred was recorded. These data were fashioned into a flow diagram, showing how many times each behavior occurred and how likely one behavior would lead to a subsequent behavior on the list.

Reproductive and Developmental Biology

The fecundity and development studies required identifying carrying females. The term "carrying" refers to a female that has mated and is holding her brood of eggs in her buccal cavity (mouth). These females were stripped immediately once they were noticed to be carrying.

Stripping the mothers is a task that involves catching the carrying female in a net and placing her into a shallow tub filled with water from her aquarium. There, she is gently grasped with the hand, and her mouth is carefully opened using the fore-finger. By stroking her throat, water current is formed that begins to sweep the eggs from her mouth into the tub. Once all eggs have

been removed, they are counted and placed into a tumbler, a tube with a screen on the bottom and an aerator on the top portion, to gently pull fresh water in and tumble the eggs, mimicking the environment of the female's mouth to avoid fungus growth. The female is measured in millimeters and replaced back into her aquaria.

Fecundity vs. female size comparison. For this experiment I compared female size with fecundity (number of eggs). Size was the only variable tested against female fecundity though a few others were controlled for by feeding all fish the same high-quality diet and keeping water parameters ideal in all tanks. Our lab records the date, tank, size of the carrying female, and the number of eggs she is carrying. The data points were plotted on a scatter plot, comparing the size of the female in millimeters and the number of eggs she was found to be carrying. In order to determine if the results were significant, a trend-line was fit to the scatterplot, and its corresponding r^2 value and p-value were recorded.

Developmental stages. In this study I characterized the early ontogeny of *M. johanni*. It was important to closely monitor the females in order to insure that any eggs were stripped from the females as soon as possible after fertilization. Once the eggs were obtained, an egg was chosen and brought to the microscope each day. The microscope used was a Carl Zeiss Stem SV II. It was connected to an Axio camera, model Hrc. Using the camera and the computer program AxioVision, images of the developing *M. johanni* were captured daily. A sequence of images with days from conception (conception being day 0) was compiled to compare the development of many individuals on the same day of development. Once the sequence was created, the images were scrutinized to determine different structures developing at certain days of development. Stages of development were documented as outlined by Balon (1985) and Fujimura and Okada (2007)

Results

Behavioral Experiments

General observations. It became apparent that one or two males in each tank had a radically different striping pattern than the other fish in the aquarium. While most of the fish all have a very distinct horizontal bar running along the lateral line, these other males were observed to have lost the horizontal bar, displaying very pronounced vertical bars and making the blue color stand out vividly on each side of the individual (Figure 3).

After performing the dominance review, it became apparent that these specially colored fish, displaying the vertical bars, were in fact the dominant, territory-holding males. These males could be observed to be the most aggressive and territorial males in the aquarium. It appears that this coloration is used as a form of visual communication among *M. johanni*. In this case, the dominant male makes his status known in the form of a unique color pattern. I observed that the dominant males were the ones to enter the territories in the tanks and chase other males away from them. I noted that the larger aquaria housing *M. johanni* contained more than one territory-owning male, while the smaller aquaria only contained one territorial male. The Randomized Block-Design Anova that I ran showed that the territorial males were significantly larger than the other fish in the aquaria (P-value= $9.643e-10$ and R-squared= 0.6875; Figure 5).

Behaviors observed were compiled into an ethogram (Figure 6). The aggressive interactions were categorized under “dominance” and were observed among both males and females. These behaviors included non-physical interactions, such as “full display,” “vibrate,” and “chase,” as well as physical interactions that varied in intensity, beginning with “bite” and moving to very aggressive interactions, such as “circle fight” and “lip lock”. Though both sexes were observed to perform these behaviors, I found the very physical interactions to be sex-

specific, that is males were observed to circle fight and lip lock with other males, and females were observed to perform these interactions with other females, but I never observed male-female interactions of this intensity. Sometimes I saw the dominant males try to break up circle fights in the aquaria.

Submissive behaviors included: “run,” “turn-away,” and “hide.” Individuals very low in a dominance hierarchy were found to perform these behaviors numerous times, whereas they seldom or (in the case of the *omega* individual) never performed dominance behaviors.

Courtship behaviors could be broken down by sex. Males performed the majority of the courtship interactions, including: “lead,” where he would attempt to lead a female to his territory, as well as two types of displays. One display was slow and included very big deliberate “fanning” movements of his caudal (rear) fin. I called this display the “lateral display” and observed it to be a males’ attention-grabber to a female. The other display, “quiver,” was a smaller movement but very vigorous and pronounced. The male would stay relatively still and vibrate very quickly. This behavior was also seen during breeding events. The male would perform “quiver” while the female nibbled near his anal fin to insure better fertilization of her brood, already taken up into her mouth. The females’ courtship behavior only consisted of “follow,” where she would follow a courting male back to his territory.

Dominance observation. After creating a dominance matrix that included the number of dominant interactions and the recipients of the aggression, the rank orders could be mapped. It became clear that the hierarchy did not remain stable over the course of the month long observation period (Figure 7). Just as outlined by the study by Nelissen (1985), when an individual’s rank was lost, the result was not only an exchange of rank between two fish; rather many fishes’ rank orders were completely reshuffled. Only the dominant (*alpha*) male was

observed to hold his title of alpha male throughout every trial. The *beta* male also kept a stable position once it was attained.

As previously mentioned, to conclude whether the hierarchies illustrated above were linear, Landau's equation as perfected by Kendall's index was used. The equation is $H = 12 / (n^3 - n) \sum (v_a - (n-1)/2)^2$, and it yields an answer between 0 and 1; a score of 0 means the hierarchy is not at all linear, and a score of 1 means that the hierarchy is perfectly linear. The individual outcomes for each trial were averaged, and the outcome was 0.965. Based on this value, I can conclude that the hierarchies formed by *M. johanni* in this dominance study are almost perfectly linear.

Territorial and shared dominance observation. Based on the graph (Figure 8) contrasting the times each fish spent in each of the three sections of the tank, the times each male spent on their own sides of the aquarium were much greater than the times spent on the side containing the territory of the other male. Male Left hardly ever crossed into the right section of the tank, and Male Right rarely entered the left section, however he spent the majority of his time in the neutral zone rather than the right side of the tank containing his territory. This could be because the other individuals in the aquarium spent most of the time on the left side of the tank. He did enter his allotted territory several times during each trial, so I know he claims that territory. By examining the graph, it becomes obvious that Male Left spent more time on the left side of the tank than Male Right. Out of 600 seconds, Male Left spent an average of 594.1 seconds on the left side of the tank, while Male Right only spent an average of 27.4 seconds on the left side of the tank, proving that Male Left spent much more time in tank left where his territory was located. Male Right spent significantly more time in the neutral zone (an average of 409.1 seconds) than Male Left (an average of 5.7 seconds), and Male Right also spent more

time in tank right (an average of 163.5 seconds) than Male Left (an average of 0.2 seconds; Figure 5).

Territorial and shared dominance manipulation. This experiment testing the tolerance of spatial proximity between two neighboring territorial males yielded differing results (Figure 9). While shared dominance dissipated on the third trial (the closest spatial proximity of territories) in tank C (208 L), shared dominance remained in the larger tank D (378 L). During trial one when the territories were 81.2 cm apart in tank C and 122 cm apart in tank D, both Male Left and Male Right were seen to enter their own territories and spend a considerable amount of time on their own side of the aquarium, while rarely entering the zone containing the territory of the neighboring male and never entering the other territory. In trial two when the territories were 41.6 cm apart in tank C and 61 cm apart in tank D, shared dominance still held up in both tanks. The same pattern of space allocation was seen in trial two as in trial one, but during one moment in one of the tank C repetitions, Male Right was seen to leave his territory and enter the territory of Male Left, remaining in the territory for almost 15 seconds before Male Left realized and chased him away. Trial three, in which the neighboring territories were touching, yielded very different results in tank C than in tank D. In tank C, Male Left was observed to be hiding behind the heater in the tank, spending an average of 93.2% of his time across repetitions in this location. Male Right was observed to spend an equal amount of time in zones 3 and 4, the zones containing the two territories. In tank D, on the other-hand, I observed Male Left and Male Right to continue occupying their own territories.

Courtship observation. Overall, 132 courtship events were observed and fit to the steps of the previously described sequence (Figure 10). In all 132 events, the male was observed to approach a female before moving on to any subsequent steps. In 114 instances, the male led

directly into step two, a display, this step was skipped in 11 instances where the male went straight from approaching the female to attempting to lead her to his territory. After the display, 74 courting events led to the male leading the female to his territory. Out of these 74 leads, in 35 events the male entered into the territory and 13 of these events led to a display, whereas in nine events the male displayed again without having entered the territory. Sixteen of these displays led the female to enter the territory. In only three instances, I observed many steps of the courtship sequence to be skipped. The male approached a female and displayed. Then the female entered the territory without having been led by the male. Directly after the female entered the territory, many different steps were observed to occur. In eight instances, the male who had been in the territory left, either to trap her in, or to chase off potential predators on the eggs or other fish lingering too close for comfort. In two instances the male displayed for her again, whereas in five instances the female entering the territory led straight into a mutual T-position. In four instances the female exited the territory, just after entering, before any other steps could occur. Out of the total 16 instances where the female entered the territory, she was observed to exit and leave the courting event in eight instances. In all, 11 courtship sequences led to a mutual T-position between a gravid female and a courting male, who were all dominant-territorial holding males. Ten of these T-positions led to breeding.

To summarize these results according to the status of the courting male, I observed results that differed substantially depending on whether the courting male was subordinate or dominant as only the dominant males in the aquaria are territory-holders. The subordinate males courting events accounted for 30 out of 132 total events; that is 22.7% of total courtship events. Their success of progression past step three of the sequence (lead female to territory) was only 10% as only three events out of their total of 30 progressed past step three. Their breeding

success was 0%; I never observed a subdominant male to breed in any observation. The failure of the courtship attempts by subdominant males was mainly due to their lack of a territory. These males were forced to lead the female to a random territory that they did not have the status to defend in the aquarium. When the dominant male would observe an attempted courtship event by a subdominant male, he would chase and nip the male to interrupt. As for the dominant males in the aquaria, they had much more success in their courting events. Out of the total 132 events, 102 were performed by a dominant, territory-holding male. These males had a 46.1% success rate of progression past step three (lead female to territory); that is 47 of their 102 events surpassed step three. All ten breeding events observed were with dominant males in their own territories. That makes their breeding success rate 9.8%. Of the total events performed by dominant and subdominant males, 7.58% led to breeding.

Through observation of breeding events, it appeared that the female's eggs were fertilized once taken up into her mouth and not before. The female would lay her eggs during the circular breeding sequence while her mouth was on the male's genital region. Then she would turn around and take her eggs up off the ground into her mouth. After, she would continue the circular breeding sequence with the male, nibbling at the male's genital region until she laid more eggs. This sequence was repeated until all eggs had been laid and fertilized. In one instance, a female was observed to leave the territory of the male during spawning and attempt to finish mating with another territorial male.

Reproductive and Developmental Biology

Fecundity vs. female size comparison. This experiment yielded highly significant results ($P=9.409e-10$). Brood sizes observed during my study ranged from around ten eggs to as high as nearly 100 eggs, while female sizes ranged from around 50 mm to over 90 mm. I

observed a positive correlation between the size of the female and the number of eggs produced. The corresponding R-squared value was 0.6835, illustrating a fairly close-fitting relationship between the two variables (Figure 11).

Developmental staging. The developmental staging of *M. johanni* was divided into three phases as outlined by Balon (1985): cleavage phase, embryonic phase, and eleutheroembryonic phase. The cleavage phase is the period of cell division of the first cells, the embryonic phase is the development of the young that occurs before hatching, and the eleutheroembryonic phase begins with hatching (the shedding of the chorion) and continues through the development of the yolk-sac juvenile.

The cleavage phase (Figure 12.1) begins with the first cell division, which was observed to occur within around one to three hours post-fertilization (day zero). Above the dividing cells, in the space between the cells and the chorion (membrane shell; Balon 1985), is a space called the perivitelline membrane (Balon 1985) that serves as a cushion, shielding the delicate cells from any subtle impacts that occur while tumbling in the female's mouth. The cells divide over and over passing through a 16 cell, 32 cell, 64 cell, 128 cell blastomere, and they keep dividing until the cells of the blastomere collapse into the yolk cell and lay flat against the yolk (Wood and Timmermans 1988). Under the dividing cells, located at the blastoderm pole of the egg, lies the large yolk sac. The cleavage phase was observed to last through day one, when the blastodisc (a cloudy cap) was observed at the blastoderm pole, and the germ line began to form over the top (Kimmel *et al.* 1995).

The beginning of the embryonic phase (Figure 12.2) was observed on day two when cephalization (the formation of a head) and gastrulation (the posterior or tail end of the embryo has grown) were observed. The germ ring called the mesoderm was observed at the far posterior

end of the tail. This ring will follow down the yolk as the tail of the embryo elongates (Wood and Timmermans 1988). This stage is called epiboly (Kimmel *et al.* 1995). This day was also the first day when pigment spots, called melanophores, were observed on the yolk of the egg. On day three, the heart and the notochord can be identified as well as segmentation of the brain into two hemispheres. The auditory and optic vesicles are present, but the eye has not yet appeared. The beginnings of nostrils were observed. Also, segmentation in the form of somites can be seen on the tail. By day four, the vertebrae in the tail region are beginning to develop. The heart and blood vessel network is developing further. Arteries can be seen running from the heart in a network all across the yolk sac and to the developing embryo. The optic vesicle is darkening as the eye begins to develop within it. By day five the eye is nearly completely developed. It is blue in color as it still has a small number of melanophores. A sparkly pigment called guanin can also be seen in the eye.

The commencement of the eleutheroembryonic (post-hatching) stage occurred at different days depending on water temperatures (Figure 12.3). It was observed to occur as early as day three and as late as day six, but the structures developing remained relatively constant regardless of the day of hatching. By day six, hatching has occurred. The chorion has been shed, and the tail is freed. The yolk-sac juvenile is now capable of movement, but is not capable of free swimming. Vertebrae are further developing, and the caudal and pectoral fins have developed with several fin rays each. Gills have begun to develop, and the mouth is closed as the jaw has just begun to develop. Yellow pigmentation can be seen on the head region. On day six, the fins are growing larger, and the heart is perceived to be stronger. Pigmentation is darkening the head, and the gills are further developing. On day seven the eyes are changing from blue to brown, and the juvenile continues to grow in total length as well as its fins and

melanophore development. The jaw has developed, and the mouth opens and closes. On day eight, the heart and gills look fully developed, and the pigmentation has spread across the entire body. After this day of development, the juvenile continues to grow, and the yolk sac diminishes day by day. The juveniles begin to be able to swim fairly efficiently at this age. However, the still large yolk-sac is very cumbersome. The pelvic fins are the last fins to develop on the juveniles. They were seen to appear as early as day 15 and as late as day 18. I observed exogenous feeding (consumption of food: yolk sac is gone) to occur around day 19, which is just two days before the female *M. johanni* normally releases her brood of young into the wild to fend for themselves.

Discussion

Behavioral Experiments

General observations. The result showing that territorial males were the largest fish shows that territorial status is a good predictor of size among *M. johanni*. A male that defends a territory is expected to be larger than the other fish and to possess the dominant, vertical-stripping pattern. Territorial males among other haplochromine cichlid species have been found to be uniquely colored as well. They will generally be much more vibrantly colored or possess a coloring completely different than the subordinate fish (Seehausen 1996).

Dominance observation. Based on the results of the dominance observation, my hypothesis stating that the hierarchy of a larger group of *M. johanni* would be linear was supported by the data, and this finding supports the study by Andries and Nelissen (1990), showing *M. johanni* form linear hierarchies in groups of three to six. Since *M. johanni* was found to form linear hierarchies by the present study as well as the study by Andries and

Nelissen (1990), it can be predicted that this species will always form linear hierarchies in aquaria. This is a conclusion that is also observed among *M. auratus* (Andries and Nelissen 1990; Nelissen 1985), a close relative of *M. johanni*.

My hypothesis predicting that the hierarchy would remain stable over time was not supported. Drawing upon figure 3, it does not appear that the hierarchy in tank B remains unchanged over time. Only the individuals that are more dominant in the aquaria held fairly stable positions, and only the alpha male did not ever change rank status. The subordinate end of the hierarchy was not stable. The members at this end of the hierarchy changed around quite a bit in their status. The loser effect (Dijkstra *et al.* 2012) was seen in action when Male 7 was observed to be fighting for dominancy in the first tape, and when he lost he continued falling down the rank orders, until he occupied a position far from his *beta* position in the first trial. Similar results were found by Andries and Nelissen (1985) when they found that a change in position of one individual usually led to a dramatic reshuffling of many members.

This experiment should be followed by a removal of the dominant male and an observation of the subsequent changes undergone in his absence. When he is reintroduced into the tank, another observation should be made. Also, an experiment should be performed to test whether the females fit into the hierarchy or whether they have their own hierarchy because that was an aspect of the hierarchy that was unclear to me as the males were observed to mouth fight, and the females were observed to mouth fight, but females were never observed to mouth fight with males. Since it has been found that female African cichlids form their own hierarchies in the absence of males (Renn *et al.* 2012), it could very well be the case that females have their own hierarchy separate from the males, connected into the hierarchy of the males by group factors.

My experiment also supported an experiment that showed that size is important in linear hierarchies, but it is not everything (Nelissen 1992). I found that the dominant males were always the largest males in the aquaria, but the rank orders did not necessarily correspond directly with size. For instance, the smallest female was usually the omega individual, but this was not always the case. This finding was also observed in *M. johanni* by Andries and Nelissen (1990).

Territoriality and shared dominance observation. The significant results of the shared dominance observation support my hypothesis that shared dominance can occur in territorial *M. johanni* males. Each of the two males definitely preferred spending time on the side of the tank with their own territory rather than the side containing the territory of the other dominant male. The two males were never observed to interact physically with one another besides a few times when one male would perform ritualistic aggression (aggression that avoids physical harm), swimming back and forth in full display (fins erect) when the other dominant male got close to its territory. However, each male was observed to chase and bite other subdominant individuals.

Since mbuna cichlids live in close proximities among the rocks in the wild (Ribbink 1990), it is not surprising that shared dominance between territory-holding males would be established in aquaria. The avoidance pattern between the two males can probably be attributed to different olfactory and visual signals, defining the presence of another territorial male from afar. Because it has been shown among cichlids that territorial males are more sensitive to chemosensory of hormones in the water, this assumption is most likely valid (Huntingford 2012).

The observation that shared dominance only occurs in a 208 L tank and a 378 L tank in the lab, but not in the two 76 L tanks, suggests that there can only be more than one male sharing

the *alpha* status in the aquarium if there is enough space and territory to support more than one dominant male.

Territorial and shared dominance manipulation. Because the results of this experiment were so ambiguous between the two tanks, this is an experiment that should be followed up by an experiment testing more variables besides space that may affect the stability of shared dominance. *M. johanni* have been noted to be minimally territorial in the wild (Eccles 1973), so this is something that should be looked into in aquaria. Perhaps if more territories were available, dominant males would move around territories, or more territory-holding males would be tolerated in the aquarium. The observation that the males in tank C (in which shared dominance dissipated when the territories were touching) had injuries on their mouths shows that these males had a grand fight to decide who would take over the space. This supports the findings of Turner (1994) where he showed that among mouthbrooding cichlids, residence males would fight intruder males to protect their territories.

It is highly important that this study be followed by more studies testing dispersal patterns of African cichlid territorial males. Because habitat modification was ranked as a severe risk to the biodiversity of Lake Malaŵi, studies showing the limits of space for territorial males can be a great insight into how to amend or find a solution to the risk of habitat modification along the rocky shorelines where mbuna cichlids dwell.

Courtship observation. The courtship sequence of *M. johanni* that I observed was very similar to the courtship sequences of many other haplochromine cichlids. The male *Astatotilapia nubila* has been found to perform a sequence consisting of an approach, a lateral display, a quiver display, and a fast lead to the territory, which is ultimately followed by a mutual-T position and spawning (Seehausen *et al.* 1996). A similar sequence was found by

another student working with *Ctenochromis horei*, another African cichlid, in the Cruz lab in her honors thesis (McCrea 2002). In both of our studies, the number of events occurring decreases as the males' progress through the courtship sequence, and breeding was the rarest event.

The finding that subdominant males courted females less and were less likely to succeed in their courtship attempts parallels a study by Desjardins *et al.* (2012), in which it was shown that subdominant males were less likely to court females in the presence of the dominant male. In my study as well as in theirs, dominant males were observed to break up courtship events performed by subdominant males. Another possible reason why non-territorial males do not court as often is because non-territorial males have been found to differ in gonadal morphology compared to territorial males (Huntingford 2012). Whereas territorial males have larger gonads and higher sperm motility, so they are always ready to spawn, non-territorial males have been found to lack these two morphologies that make spontaneous spawning impractical (Huntingford 2012).

An observation that a female was observed attempting to breed with two different males by leaving the territory of one male during spawning and entering the territory of another male is synonymous with a study by Kellogg *et al.* (2012). They found that multiple paternity events were very common among the lekking cichlids from Lake Malaŵi. In a lek mating system, many territorial males are located in the same area where females are able to compare and choose the male with which they would like to mate. Apparently, it is a natural occurrence for a female to mate with multiple males among lek systems in Malaŵian cichlids.

Reproductive and Developmental Biology

Fecundity vs. female size comparison. The highly significant positive correlation found between fecundity and size of females supports my hypothesis that the size of the female would

directly correlate with the number of eggs she was found to be carrying. This data shows that larger females will be able to generate more gametes than smaller females of the same species. As the females grow, they will be able to raise more young. This pattern has been found among other cichlid species, including *Sarotherodon galilaeus* from Nigeria (Fawole and Arawomo 2000). This study raised the question: do males prefer to mate with larger females over smaller females? Since the size of the mother usually conveys her age, another question this experiment raised is at what age are *M. johanni* able to produce gametes and engage in reproduction? The findings of this experiment and others like it are crucial for the conservation of the African Rift Valley Lakes. If larger fish are being selectively removed from the lakes by fishermen, this is contributing to the declines of fish abundance in the lakes. It is important to have facts that can be used to educate the people of this region in order to try to ameliorate cichlid population deteriorations.

Developmental staging. The early development of *M. johanni* is similar to that of many other African Cichlids, such as *Labeotropheus ahl* (Balon 1985), *Oreochromis niloticus* (Fujimura and Okada 2007), and *C. horei* (Roscow 2012). A future study that should be done with *M. johanni* is studying the early development of the young that are raised naturally, in the buccal cavity of the mother. Differing rates of development were noted in broods that were carried by the females in the lab and the broods that were artificially incubated. It has been found by Balon (1985) that externally incubated *L. ahl* hatched 18 hours earlier than internally incubated siblings. I am also interested by the coloring of the *M. johanni* juveniles in the lab. Groups of artificially incubated young developed much more pigment than naturally raised young.

Implications

Because of anthropogenic threats on the lakes of Africa's Rift Valley, including pollution, overfishing, accidental and intentional species introductions, and many more (Odada *et al.* 2003; Kitchell *et al.* 1997), it is extremely important that an effort be made to describe as many species as possible. Genetic and biological diversity in these lakes is being threatened, and if we do not act now, we may miss out on the chance to fully describe the diversity that exists now in these lakes. With the introduction of the Nile Perch in Lake Victoria, an example of the worst possible scenario for these lakes is being witnessed. Efforts should be made to make sure nothing like this happens to Lake Malaŵi because it has the most cichlid diversity of all the African Rift Valley Lakes. There are many important implications for the characterization of *M. johanni*. Its close relative *M. auratus* has been described in several studies (e.g., Andries and Nelissen 1985; Andries and Nelissen 1990), and differences in behavior between these two species could be a good insight into why species that can readily hybridize and produce viable offspring do not hybridize in the wild. In addition, as a popular aquarium breed, a full understanding of the species behavior, especially dominance and territoriality is pertinent.

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experimental design. Also, I owe thanks to the entire Cruz lab for their insight and support, and the BURST Program for funding the developmental portion of the study.

Tables and Figures

Tank A	Tank B	Tank C	Tank D
58.6	56.6	55.4	69.4
58.8	57.5	56.7	71.2
62.1	58.2	57.7	72.9
64.4	64.3	59.5	73.3
67.4	64.4	61.8	73.3
68.3	65	65	73.8
72	66.6	71.8	75.1
	67.9	72.7	76.9
	70	73.5	77.7
	73.4	74.6	78.4
			92
			92.5
			92.6
			94.4
			99.6

Figure 1: The sizes of all fish in each study tank in millimeters. The fish are order from largest to smallest. Male and female sizes are not differentiated between as the sizes of the sexes are not considerably different. Some of the smaller fish are males and some of the larger fish are females. The fish highlighted in yellow are fish displaying the dominant coloration and occupying territories.

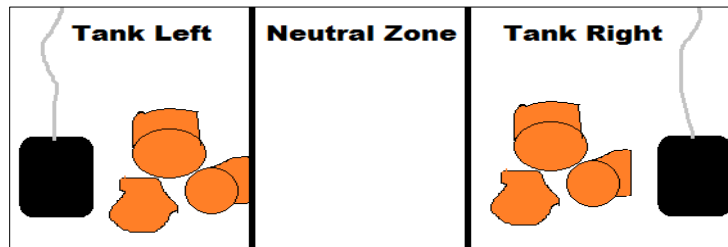


Figure 2:

The setup of the aquarium for the territorial and shared dominance experiment. The two territories were exactly the same with three clay pots in a circle and a large sponge filter.

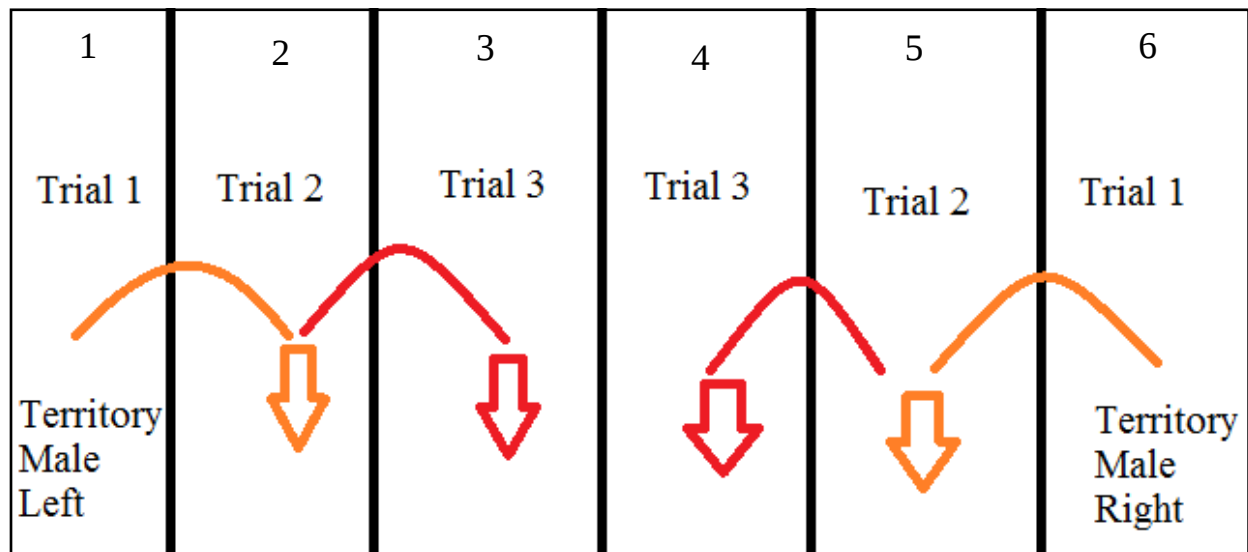


Figure 3: The set-up of the aquaria in the territorial and shared dominance manipulation experiment. In the first trial the territories were in zones 1 and 6. In the second trial the territories were in zones 2 and 5. In the third trial the territories were in zones 3 and 4.

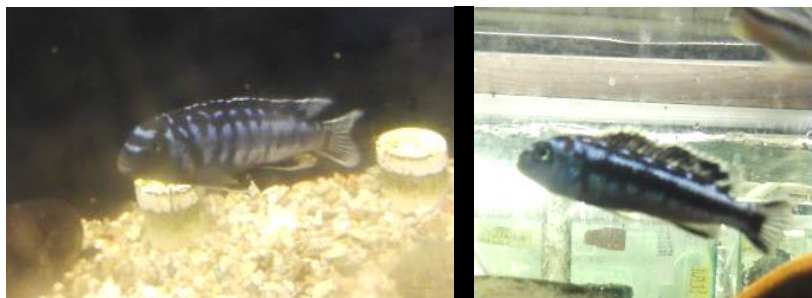


Figure 4: The photo on the left shows the dominant coloration of a male *M. johanni*. His horizontal bar is very faint in comparison to the subdominant individual in the picture on the right.

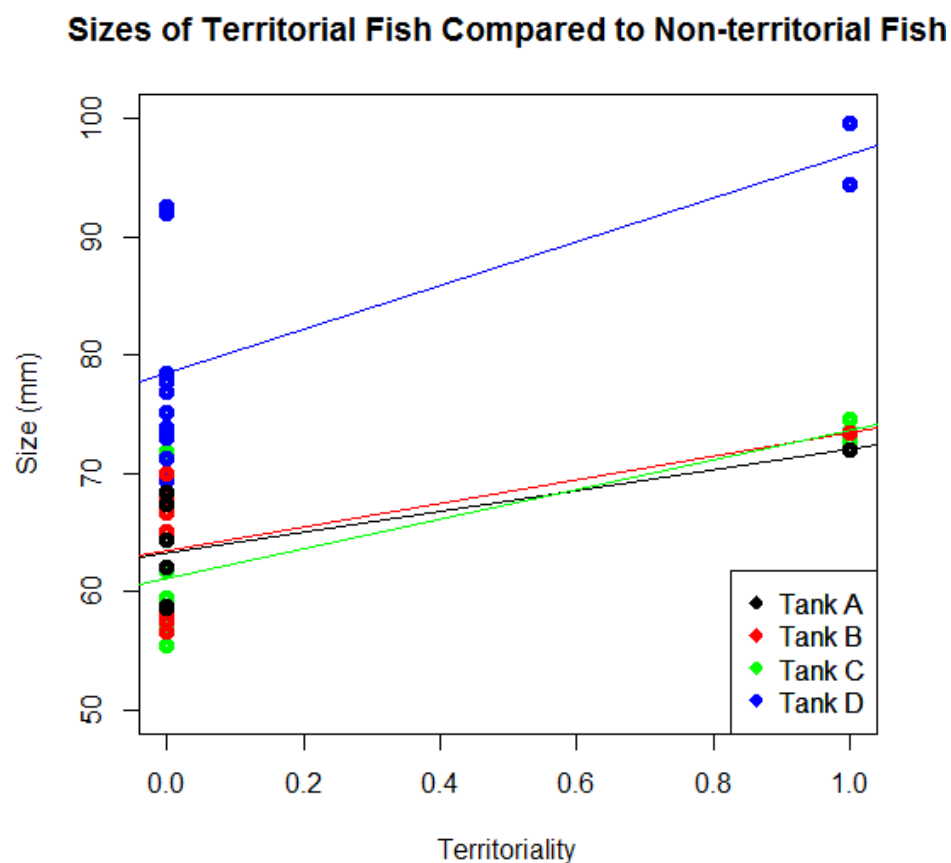


Figure 5: The relationship of size between the non-territorial fish in the tank (ranked 0 in territoriality) and dominant, territory-holding males (ranked 1 in territoriality). These four linear models were compared and controlled for size of different tanks using a Randomized Block Design Anova (P-value= $9.643e-10$ and R-squared= 0.6875).

Figure 6: Ethogram used in the behavioral experiments.

Ethogram of Behaviors	
Behavior	Interpretation
Dominance	
C- chase	Fish swims after another fish
B- bite	Fish bites or nips at another fish
CF- circle fight	Two fish circle around each other very quickly when fighting
LL- lip lock	Two fish lock lips when fighting
FD- full display	Fish fans out all fins to look bigger
Submission	
R- flee	Fish flees when being chased
T- turn away	Fish turns perpendicular to the attacker to give up
H- hide	Fish hides to avoid aggression
Courtship	
Q- quiver	Male shakes vigorously to a potential mate or a threatening behavior to a male
L- lead	Male leads a female into his territory
F- follow	Female follows males lead
LD- lateral display	Male darts and wiggles caudal and anal fins, this behavior was also observed as an intimidation technique among females
Territorial	
E- enter flower pot	Fish enters a territory (flower pot)
D- dig	Fish digs out a space of territory

Figure 7: The rank orders observed in each trial of the dominance observation. It can be noticed that while some group members' positions remain fairly constant, some members move around quite a bit, such as fish 7. Fish 1, 2, and 3 are females and fish 4, 5, 6, 7, 8, 9, and 10 are males.

Date	Rank Order									
10/27/2012	1 →	2 →	3 →	5 →	10 →	9 →	6 →	7 →	4	
	8 →									
11/4/2012	5 →	3 →	9 →	1 →	2 →	10 →	8 →	7 →	6 →	4
11/9/2012	3 →	5 →	7 →	1 →	2 →	9 →	8 →	6 →	4	
					→ 10 →					
11/14/2012	3 →	1 →	2 →	5 →	7 →	10 →	8 →	9 →	6 →	4
11/16/2012	3 →	1 →	5 →	7 →	10 →	8 →	9 →	6 →	4	
		→ 2 →								
11/24/2012	5 →	3 →	7 →	2 →	10 →	8 →	9 →	6 →	4	
			→ 1 →							

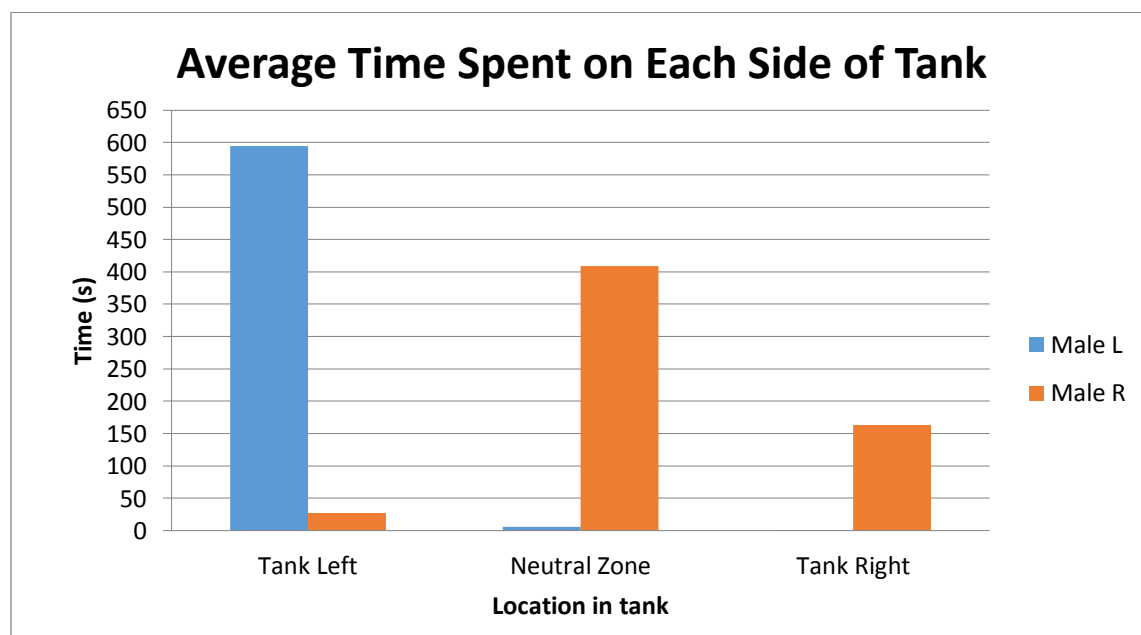


Figure 8: The average amount of time in seconds that Male Left and Male Right spent in each zone of the aquarium in the territorial and shared dominance observation.

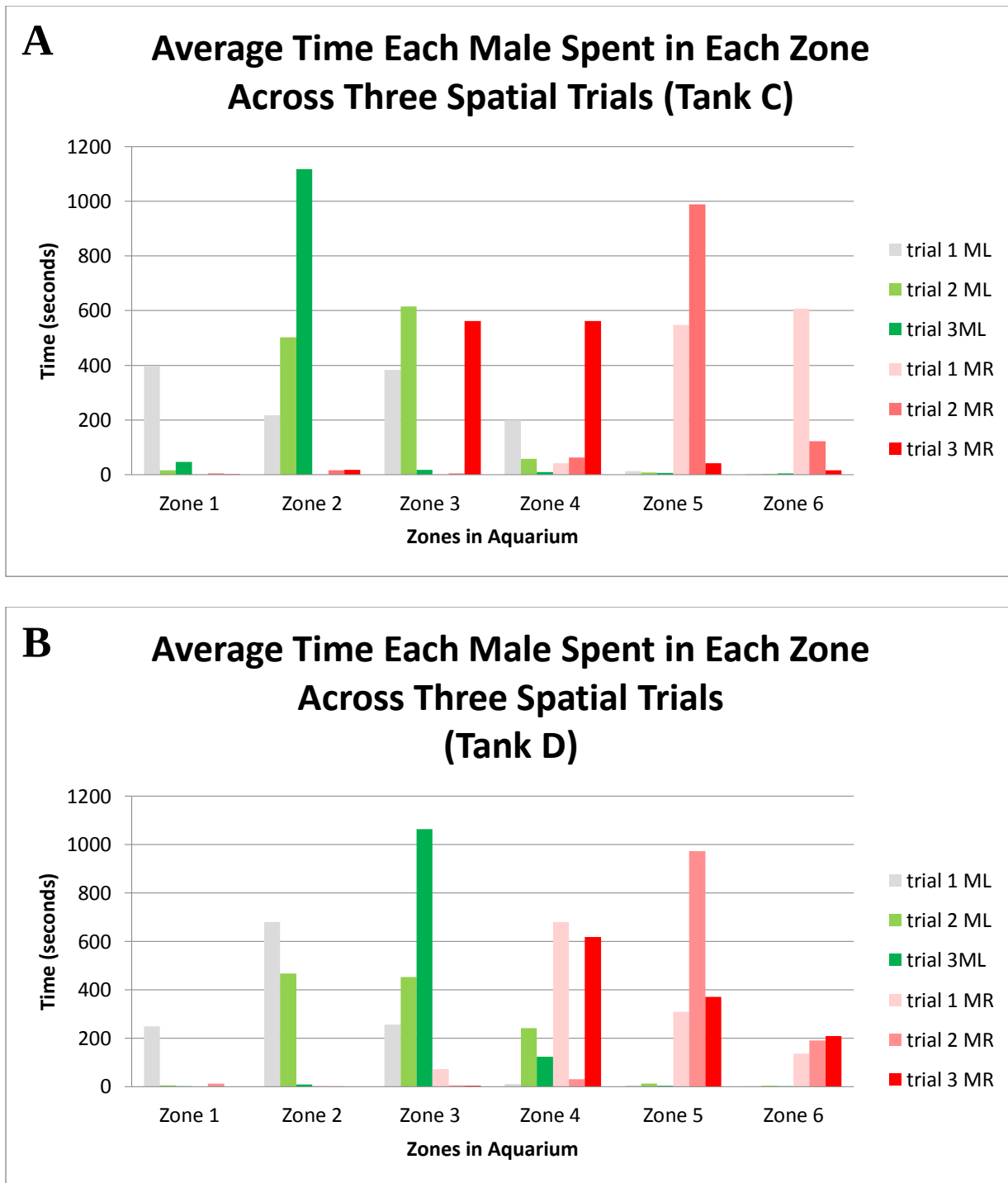


Figure 9: The average amount of time spent in each zone of the aquarium by Male Left and Male Right for each trial for the two tanks in the territorial and shared dominance manipulation. Note the zones containing the territories across trials: trial 1 (Male Left- zone 1 and Male Right- zone 6), trial 2 (Male Left- zone 2 and Male Right- zone 5), and trial 3 (Male Left- zone 3 and Male Right- zone 4). Male Left is all green shades and Male Right is all red shades. Shades go from light to dark across trials. **A** shows the results for tank C, and **B** shows the results for tank D. It is expected that if a male owns a territory, he will spend a good amount of time in that zone.

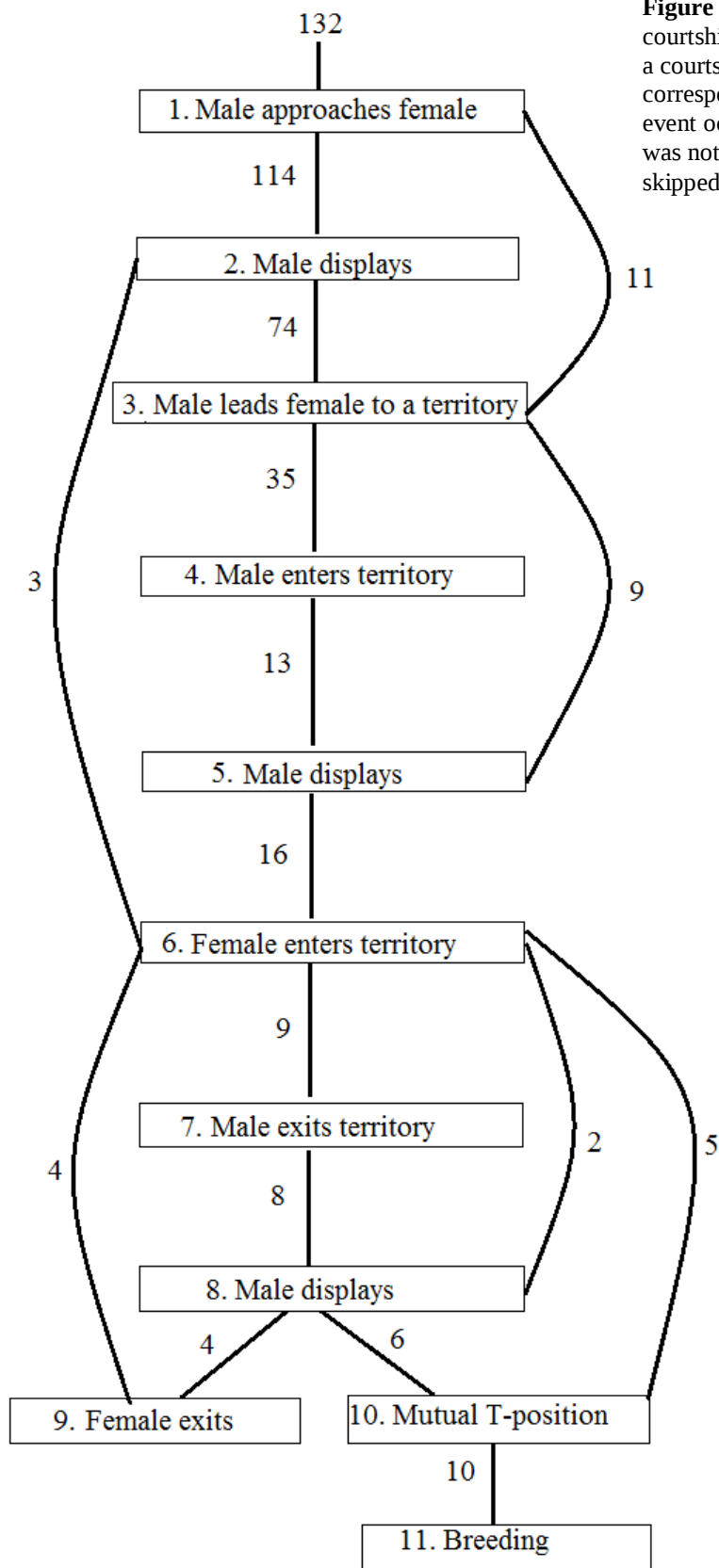


Figure 10: This flow diagram charts the results of the courtship observation. Each numbered box indicates a courtship event in the sequence. The numbers correspond to the number of times each courtship event occurred. The straight lines indicate the step was not skipped, whereas the curved lines show skipped steps.

Female Fecundity and Size

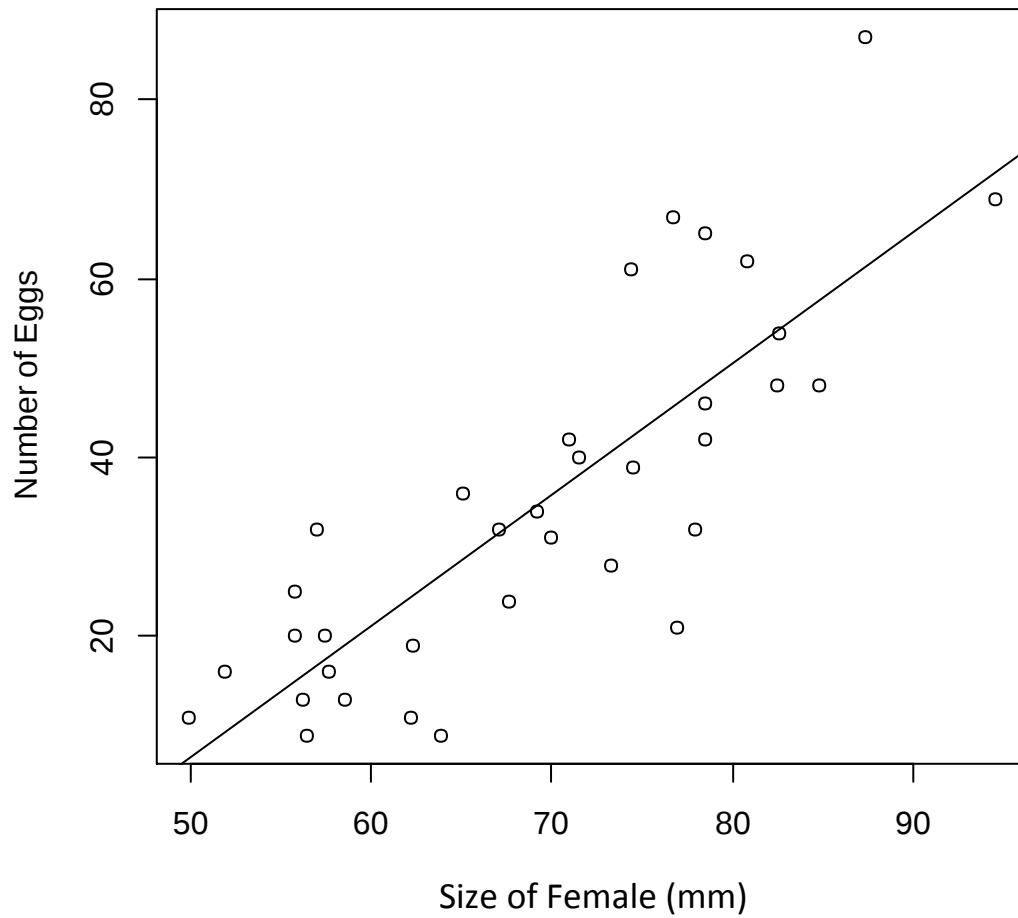


Figure 11:

Scatter plot with a trend-line illustrating the correlation between the size of the female and the number of eggs she was found to be carrying in the fecundity vs. female size comparison. P-value= 9.409×10^{-10} . $R^2 = 0.6835$.

Cleavage Phase

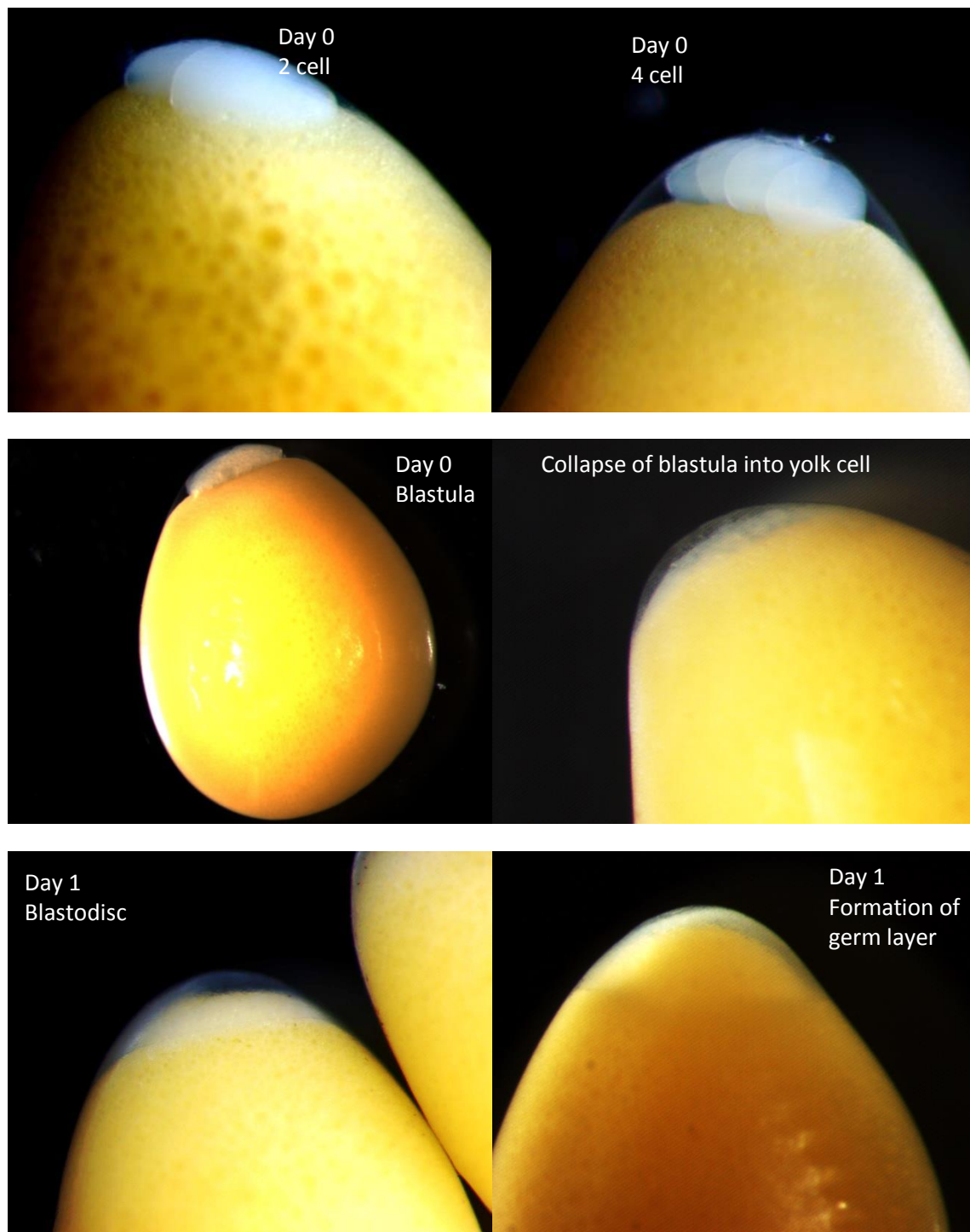


Figure 12.1: These images show the multiple stages that define the cleavage phase, lasting from day zero through day one. In this phase the first cells divide until they form a blastula. This stage is followed by the cells of the blastula collapsing into the yolk cell. The cells continue to divide, eventually covering the animal-pole of the egg, forming the blastodisc. The formation of the germ layer that covers the blastodisc is the last step in the cleavage phase.

Embryonic Phase

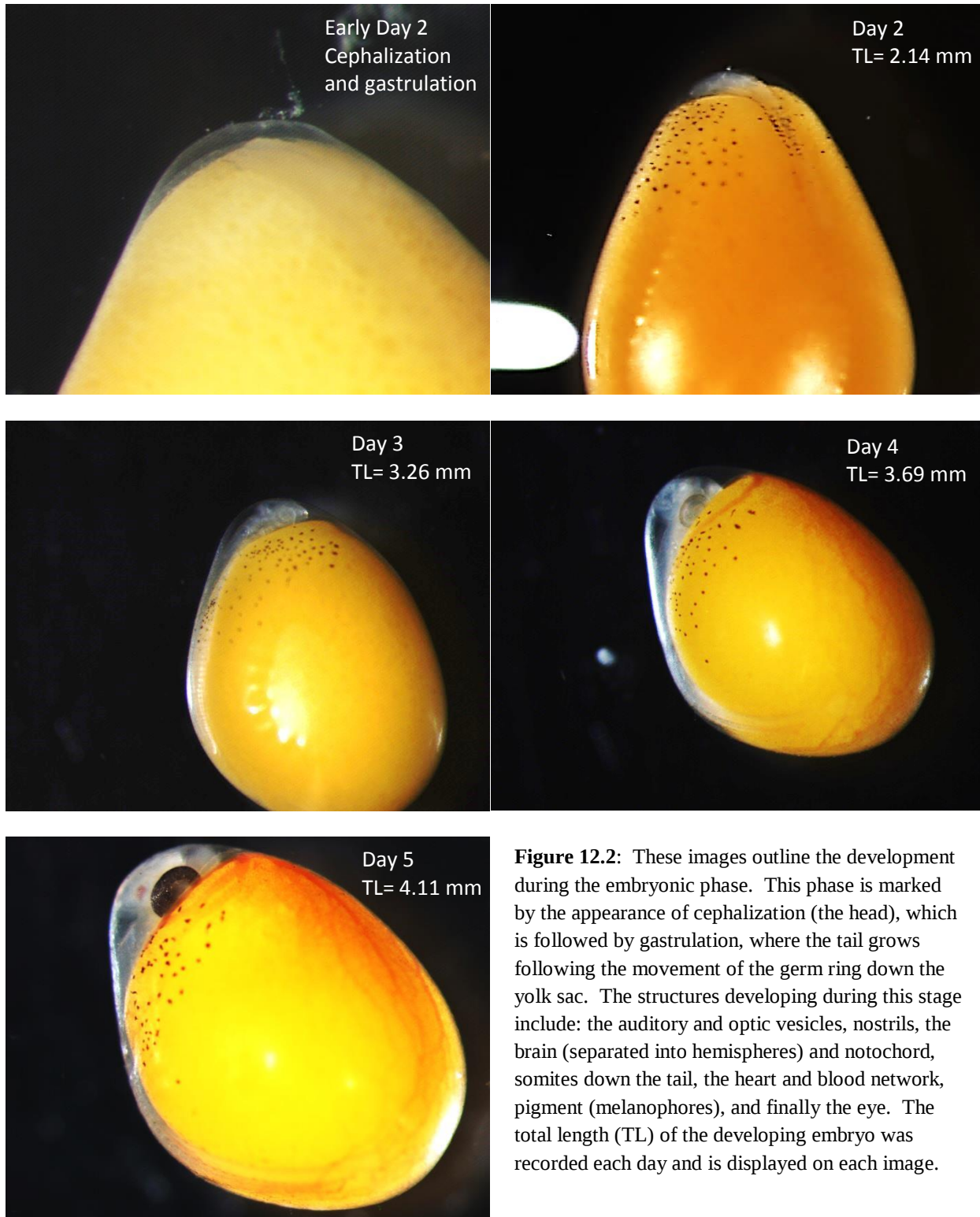


Figure 12.2: These images outline the development during the embryonic phase. This phase is marked by the appearance of cephalization (the head), which is followed by gastrulation, where the tail grows following the movement of the germ ring down the yolk sac. The structures developing during this stage include: the auditory and optic vesicles, nostrils, the brain (separated into hemispheres) and notochord, somites down the tail, the heart and blood network, pigment (melanophores), and finally the eye. The total length (TL) of the developing embryo was recorded each day and is displayed on each image.

Eleutheroembryonic phase

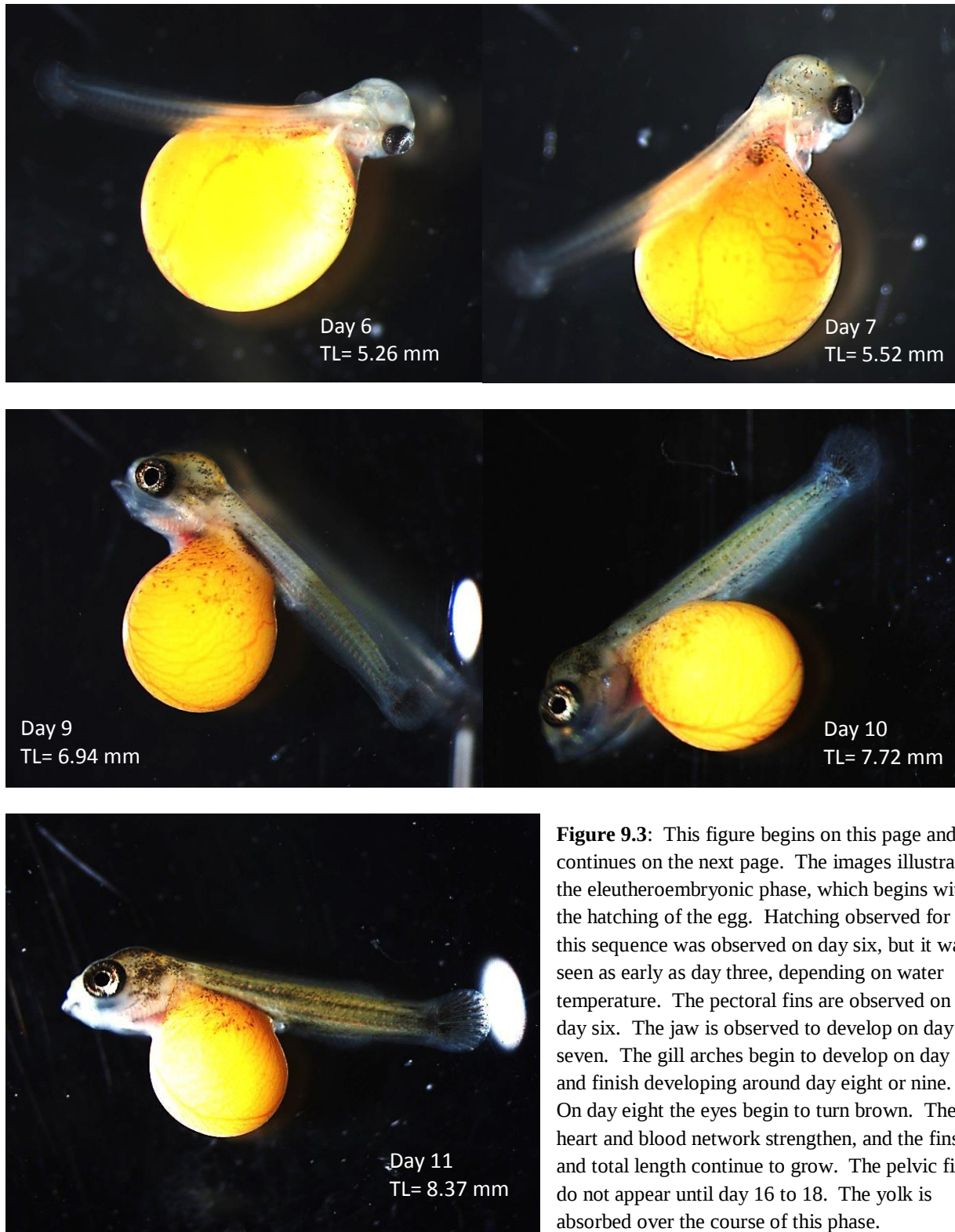
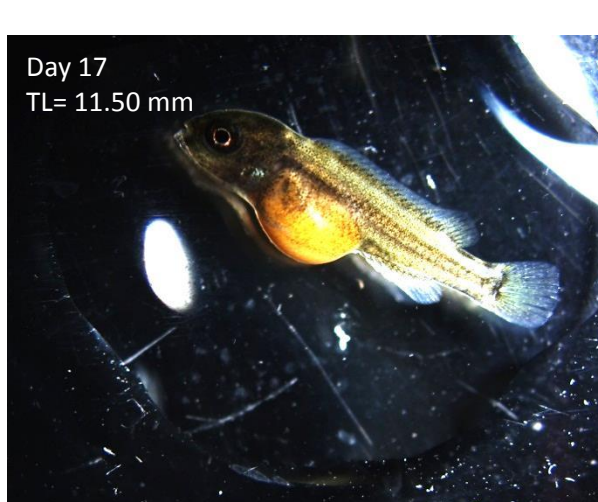
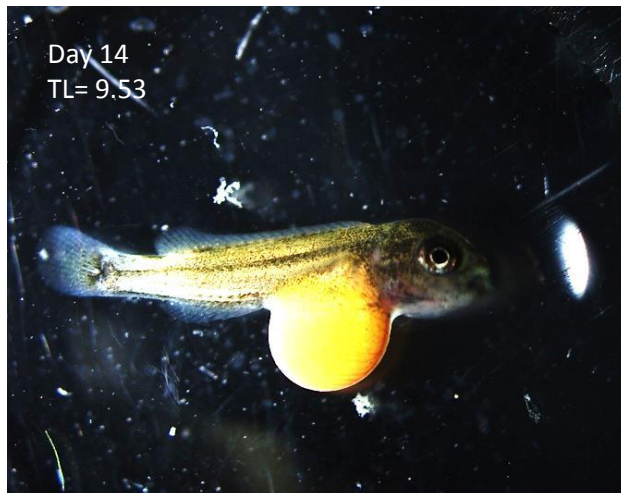
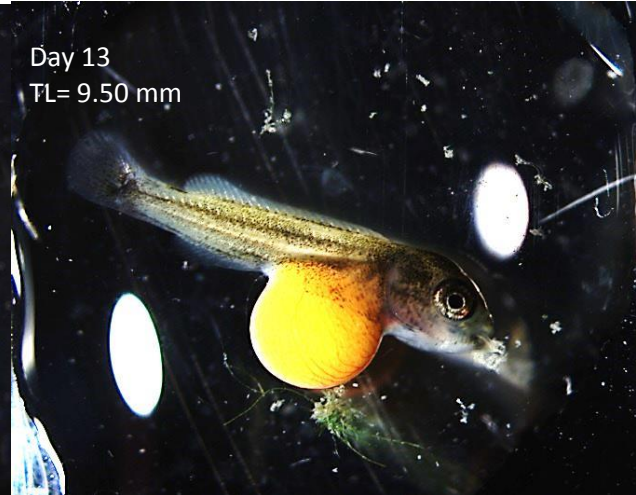


Figure 9.3: This figure begins on this page and continues on the next page. The images illustrate the eleutheroembryonic phase, which begins with the hatching of the egg. Hatching observed for this sequence was observed on day six, but it was seen as early as day three, depending on water temperature. The pectoral fins are observed on day six. The jaw is observed to develop on day seven. The gill arches begin to develop on day six and finish developing around day eight or nine. On day eight the eyes begin to turn brown. The heart and blood network strengthen, and the fins and total length continue to grow. The pelvic fins do not appear until day 16 to 18. The yolk is absorbed over the course of this phase.

Eleutheroembryonic phase



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