Host-Parasite Interactions: The Cuckoo Catfish and Non-Sympatric Hosts

Anna C. Vinton

Ecology and Evolutionary Biology, University of Colorado at Boulder

Defense Date April 9, 2014

Thesis Advisor:
Dr. Alexander Cruz, Ecology and Evolutionary Biology

Defense Committee:
Dr. Alexander Cruz, Ecology and Evolutionary Biology
Dr. Barbara Demmig-Adams, Ecology and Evolutionary Biology
Dr. Holly Barnard, Geography
# Table of Contents

Abstract .......................................................................................................................... 4

Introduction ................................................................................................................... 4-6

Objectives and Significance .......................................................................................... 6-7

Background

| Cichlid Fish of the Great Lakes in the East African Rift Valley | 8 |
| Lake Tanganyika | 8-9 |
| Lake Malawi | 9 |
| Conservation Concerns | 10-11 |

*Maylandia estherae* (red zebra) and *Maylandia zebra* (albino zebra) ............ 11-13

*Ctenochromis horei* .................................................................................................. 13

*Synodontis multipunctatus* (cuckoo catfish), a brood parasite ......................... 14

Methods

| Rate of parasitism | 15 |
| Courtship behavior observations | 15-16 |
| Visual documentation of early development | 16-17 |
| Statistical Analysis | 17-18 |

Results

| Red Zebra Length and brood size | 18 |
| Rate of Parasitism | 18 |
Courtship Observations .................................................................................. 18-19
Developmental Staging .................................................................................. 19
Cuckoo Catfish Host Preference ..................................................................... 20

Discussion

Red Zebra Length and Brood Size ................................................................. 20-21
Parasitism Avoidance Strategies ................................................................. 21
Red Zebra Rate of Parasitism and Comparison with other Host Species .... 21-22

Parasitism of the Red Zebra vs. Albino Zebra ........................................... 22-23
Early Developmental Stages ......................................................................... 23
Courtship Observations ................................................................................. 23
Cuckoo Catfish Host Preferences .................................................................. 24

Conclusion ..................................................................................................... 24-25
Acknowledgements ......................................................................................... 25
References ....................................................................................................... 26-33
Figures and Tables .......................................................................................... 34-44
Abstract

The cuckoo catfish (*Synodontis multipunctatus*) from Lake Tanganyika in Africa parasitizes mouth-brooding cichlid fishes (catfish eggs get picked up and incubated in the mouths of cichlid females, where the catfish young feed on cichlid eggs). I examined the impact of parasitism by the cuckoo catfish on an allopatric (not naturally co-occurring) mouthbrooding cichlid, the red zebra (*Maylandia estherae*) relative to that of a previously characterized sympatric (naturally co-occurring with the parasite) host species (*Ctenochromis horei*). The red zebra was parasitized significantly more than the sympatric *C. horei*, which suggests that the sympatric host has evolved strategies to minimize cuckoo catfish parasitism. Early development of the red zebra did not differ significantly from that of the *C. horei*, indicating no early developmental adaptations to deter the catfish young from eating the host young. While the courtship behaviors of *C. horei* were also similar to that of the red zebra, *C. horei* was clearly more aggressive, which may be an adaptation to avoid brood parasitism. I also examined levels of parasitism between the red zebra and a closely related, also sympatric albino zebra cichlid (*Maylandia zebra*). Due possibly to its lower visual acuity, the albino zebra morph was parasitized significantly more than the red zebra. Lastly, the catfish was found not to parasitize hosts at random, but to prefer to parasitize female cichlids of an intermediate size.

Introduction

Obligate brood parasitism (use of hosts for providing care to the parasitic progeny) has great evolutionary and ecological significance. Because of the close interactions between parasite and host, brood parasitism can reveal much about coevolutionary mechanisms (Soler
et al. 2000, Cichon 1996, Rothstein 1990). These mechanisms underlie adaptations that increase the success of the parasite, as well as host adaptations to avoid brood parasitism. It was initially thought that such brood parasitic interactions occurred only in birds and insects (Sato 1986, Fanelli et al. 2005). However, Sato (1986) found that the cuckoo catfish (Synodontis multipunctatus) from Lake Tanganyika in East Africa parasitizes mouth-brooding cichlid fishes that incubate their newly laid eggs in their mouths where their young develop.

African cichlids are well known in evolutionary biology due to their adaptive capabilities. They vary in color, morphology, eating habits, and much more in order to fill the numerous niches available in the East African great lakes. Because African cichlids are model organisms to study adaptive radiation, their response to a brood parasite provide insight into the mechanisms underlying their abilities to develop defenses against brood parasitism.

In order for the cuckoo catfish to parasitize a cichlid brood, it must spawn in synchrony with the cichlids. Catfish eggs are laid for the cichlid to pick up along with their own eggs. The catfish eggs are thus mouth-brooded together with the host eggs, but hatch earlier and feed upon the developing larvae of the cichlid host. The fry (young) of the parasitic catfish not only depend upon the host for protection, but also exploit parental investment of the host (i.e., receive nutrients from the parent host by feeding on the host’s own fry). The feeding on cichlid fry by the catfish leaves few or no surviving cichlid offspring. Cruz et al. (2004) found that, under laboratory conditions, the catfish can also parasitize cichlids from Lake Victoria and Lake Malawi, hosts that do not naturally co-occur with the cuckoo catfish. This finding is important as it provides a system for studying parasitism in a laboratory, where questions can be rigorously addressed. Since natural selection (causing traits to become more or less common in a population) acts on the breeding biology of these fishes, factors affecting
development of host-parasite interactions can be studied in this system as a model system in evolutionary, behavioral, and developmental biology. Through studying this unique interaction, we can also observe to what extent the cuckoo catfish can parasitize a new host with which it has not coevolved by examining its reproductive success. The study of the interaction between such a naïve host (red zebra) and the parasitic cuckoo catfish provides insight into the adaptive capabilities of the red zebra, i.e., whether or not the red zebra reacts to being parasitized. These results will allow inferences to be made about what behaviors may be established in order to defend against brood parasitism.

**Objectives and Significance**

The objectives of my study were to:

1. characterize the breeding biology of the red zebra in order to better understand the interaction between the brood parasitic catfish and naïve cichlid hosts. This study provided insight into the early developmental biology of the red zebra, as well as the courtship behaviors of the spawning cichlids. The courtship behaviors and early developmental biology were compared to other mouthbrooding cichlids that did (*Ctenochromis horei*) and did not (*Maylandia zebra*) coevolve with the parasitic catfish to see if there are any differences. My null hypothesis in this case was that the courtship and early development of the red zebra did not clearly differ from that of the sympatric *Ctenochromis horei*.

2. better understand the non-sympatric host (red zebra) relationship with the parasitic cuckoo catfish, and experimentally compare the rates of parasitism of the red zebra versus species that coevolved with the cuckoo catfish under laboratory conditions (*Ctenochromis horei – A. Cruz, M. Cohen, Cruz lab unpublished data*). My study investigates the interaction of the cuckoo catfish
with a naïve host. No prior study has investigated this brood parasite-naïve host interaction in fish. Studies of the interaction of avian brood parasites with naïve hosts (Rothstein 1990, Soler 2000, Cruz et al. 2008) have shown that avian brood parasites were able to parasitize naïve hosts, and some of the naïve host populations were able to develop the ability to distinguish their own eggs from foreign eggs, exhibiting parasite-egg rejection behavior. Therefore, an objective of the present study is to increase our understanding of the naïve host and parasite relationship between cichlids and the cuckoo catfish. Reproductive success of each species in this study is measured by the number of her own young each host female incubates in her mouth. The null hypothesis for this objective was that the rate of parasitism of the red zebra did not significantly differ from that of the sympatric C. horei.

(3) compare the rates of parasitism between the red zebra and the albino zebra. The present study compared the response and susceptibility to parasitism of a wild-type line of a non-sympatric host (red zebra, M. estherae) and a mutant morph (albino zebra, M. zebra) particularly to determine if vision is an important factor for successful parasitism avoidance. How eyesight plays a role in the success of the parasitic catfish has not yet been investigated in the literature. The null hypothesis to address this objective was that the rate of parasitism of the red zebra did not significantly differ from that of the albino zebra.

(4) gain understanding about how the cuckoo catfish chooses its hosts by investigating what class sizes of cichlids are parasitized. I asked the question of whether or not the cuckoo catfish parasitizes spawning mouth-brooding cichlids at random. Because parasites have close relationships with their host species, it is possible for the parasite to be selected by evolution to specialize on one or a few host species. Among insects, only one paper wasp has been found to parasitize as many as four host species (Fanelli et al. 2005). But, like many avian brood parasites
(Rothstein 1990, Soler 2000, Cruz et al. 2008), the cuckoo catfish can parasitize many hosts. Studying the behaviors that allow the cuckoo catfish to parasitize so many hosts can give us insight into what makes brood parasites so successful. The specific null hypothesis to investigate this objective was that the cuckoo catfish parasitized cichlid hosts randomly, with no host size preference.

**Background**

**Cichlid Fish of the Great Lakes in the East African Rift Valley**

The subjects of this study are native to the East African Great Lakes, located in the African Rift Valley. The African Rift Valley consists of a geographic rift in Eastern Africa that is over 4,828 kilometers long, and was formed about 25 million years ago when Africa and Eurasia collided (Roberts et al. 2012). A rift is a fracture in the earth's surface that widens over time. The oldest rift in this valley (Ethiopian Rift) occurs in the Afar region of Ethiopia (Roberts et al. 2012). South of this is a series of rifts that include the Western branch or the "Albertine Rift" which contains the East African Great Lakes (Figure 1) (Plumptre 2007).

The cichlid fishes of the East African Great Lakes are a widely used source of study for adaptive radiation and sympatric speciation (Lowe-Mcconnell 2003, Allender et al. 2003, Genner et al. 2005). The East African Great Lakes differ in age, which allows different stages of adaptive radiation of the cichlid fish to be studied at the same time (Sturmbauer 1998). These lakes include Lake Malawi, where the red zebra cichlid and albino zebra cichlid are native, Lake Tanganyika where the cuckoo catfish and *C. horei* are native, and Lake Victoria.
Lake Tanganyika

Lake Tanganyika is the oldest of the three East African Great Lakes as it was formed 9-12 MYA (Sturmbauer et al. 2001). It is divided between Tanzania, the Democratic Republic of the Congo, Burundi, and Zambia (Figure 1). Likely because of its great age, the cichlid species of Lake Tanganyika are more diverse morphologically and behaviorally than the cichlids in the other two great lakes (Fryer and Iles 1972). There are about 23 species of cichlids in the lake (Konings 1998). Although there are fewer endemic cichlid species in Lake Tanganyika than in Lake Malawi and Lake Victoria, the former lake’s species are more genetically distinct from each other, making them better suited for phylogenetic studies (Meyer et al. 1990). Due to lake level fluctuations, Lake Tanganyika was once composed of three smaller bodies of water (Sturmbauer et al. 2001). The bodies of water separating and coming together presumably favoring allopatric speciation of the cichlid fishes that inhabited the lake (Sturmbauer and Meyer 1992, Verheyen et al. 1996).

Lake Malawi

Lake Malawi (Lake Nyasa) is located between Malawi, Tanzania, and Mozambique (Figures 1 and 2). It is the ninth largest lake in the world, approximately 13974 sq. km, and 701 m deep and home to over 500 species of cichlids that presumably evolved from a few common ancestors within the past million years (Albertson et al. 1999). This rapid diversification has been the subject of studies of speciation, morphological adaptation, sexual selection and breeding behavior (Kornfield et al. 2000, Allender 2003, Kocher 1993). The pH of Lake Malawi ranges from 7.7-8.5 with the variation caused by the level of carbon dioxide dissolved in the water (Muller et al. 1973). In areas where the water is more turbulent, the pH
is more basic, while in calmer waters the pH is lower. This is because in the more turbulent areas, the water is more aerated, and in the calm areas of the lake, there is a higher level of dissolved carbon dioxide. About one third of the coast of Lake Malawi is rocky, and this shoreline is where rock-dwelling cichlids (mbuna) live, including the red zebra and albino zebra (Lowe-McConnell 1993).

**Conservation Concerns**

The Rift Valley cichlids are under tremendous human pressure and many species are vulnerable to extinction (Odada et al. 2003). The rock-dwelling cichlids of Lake Malawi (mbuna) which includes the red and albino zebras, are endemic (not found elsewhere) and localized in distribution, some of which only live in a single confined rock group (Genner 2005, Stauffer et al. 1997). This makes the risk of extinction of these cichlids greater than that of more widely distributed species (Stauffer et al. 1997).

The mbuna include many species with limited ranges and habitats. They also do not have a pelagic dispersal phase, have slow growth rates, and low fecundity (Genner 2005). Because of these traits, the mbuna are especially vulnerable to species loss. There are some no-fishing zones within Lake Malawi, but overfishing may remain a serious threat to some fish species. Although according to Genner et al. (2005), the mbuna are not under a direct threat from overfishing due to their lack of a commercial value. To date, scientists have not found any introductions of invasive species into Lake Malawi (unlike that of Lake Victoria). A potential threat may be posed by the aquarium trade (Ribbink et al. 1983). Due to this trade, introduced species have been added to the lake, and although they have not been found to compete with the native mbuna, there have been instances of hybridization (Stauffer et al. 1996). Although scientists agree that the most significant
threat to the mbuna stems from habitat modification due to pollution, little is still known about the effects of the changes related to pollution. Because both Lake Tanganyika and Lake Malawi all have long flushing times because of their large volumes and small outflow, pollution retention could be a serious threat. Genner et al. (2005) suggests a conservation focus on specific taxa rather than attempting to conserve the mbuna as a whole. To conserve species in general, knowledge about their breeding biology is vital. This knowledge about the breeding biology is valuable in large part because of the conservation concerns posed in their native habitat (Lake Malawi). More specifically, this information can be applied to implement habitat rehabilitation/creation using knowledge about optimum breeding environments.

**Maylandia estherae** (red zebra) and **Maylandia zebra** (Albino Zebra)

The rocky shoreline of Lake Malawi is inhabited by the mbuna - made up of 200 species. The establishment of some species of mbuna presumably occurred within the last 200-300 years (Owen et al., 1990). **Maylandia** is a genus of mbuna cichlids endemic to (only found in) Lake Malawi (Stauffer et al. 1997, Oliver, 2009). Although it was previously proposed that these cichlids speciated from a single ancestor (Kocher et al. 1993, Moran et al. 1994), recent studies show that these populations arose as a hybrid population from at least two different Haplochromine (dominant cichlid group in East Africa comprised of the genus *Haplochromis* and other closely related genera) ancestors (Joyce et al., 2011). It is hypothesized that the common ancestor is a riverine generalist closely related to Lake Tanganyika’s haplochromine tribe 700,000 years ago (Kocher et al. 2001). The exact drivers of cichlid speciation are unknown, but it is suggested (Genner and Turner 2005) that either allopatric or sympatric speciation was involved. Allopatric speciation is hypothesized to have
occurred due to fluctuations in the lake level (Owen et al. 1990) that can cause temporary
formation of smaller water bodies, (causing fish populations to become geographically
isolated) and subsequent rejoining with the larger body of water, (causing fish populations to
become reunited). Sympatric speciation may have been driven by “intrinsic isolating
mechanisms” (mechanisms with a genetic basis, e.g., behavioral post-mating isolations)
(Genner and Turner 2005). However, researchers speculate that, in view of the relatively
young age of the mbuna (0.3-0.5 million years (Genner et al. 2007)), a fast-acting form of
species divergence must have occurred in Lake Malawi. Speciation by sexual selection has
been suggested as the mechanism (Albertson et al. 1999) with variation in male breeding
coloration leading to rapid evolution of haplochromine cichlids (Kidd et al. 2006). Since
coloration and breeding behavior may thus separate closely related species by causing them not
to breed (Stauffer et al. 1997). Due to this hypothesis, differences in coloration have recently
been used to identify species; coloration may be vital in mate recognition and may act as a
prezygotic barrier to gene flow (Konings 2007). That is, the mating pair must be a specific
color for mating, and ultimately gene transfer to occur.

One of the subjects of the present study is *Maylandia estherae*, the red zebra. The red
zebra is typically solid orange and sexually dimorphic. Males of this species, like many cichlid
species, are highly territorial (Amorim et al. 2008). There are 40-50 species in the genus
Maylandia (Ciccotto 2008, Stauffer et al. 2013). The species in the genus are relatively small
(less than 25 cm) in length, are brightly colored and often strongly sexually dimorphic
(phenotypic differences between males and females are present) and all members of this genus
exhibit mouthbrooding (Konings 2001). Mouthbrooding is a mechanism whereby one (or
both) of the parents carries the eggs and fry in the mouth (Barlow 2000), although most cichlid
mouthbrooders are maternal (including the subjects of this study) (Kuwamura 1986). The offspring develop using their yolk sac as a nutrition source and use the parent’s mouth for protection as young juveniles, before becoming independent and leaving the mouth, large enough to survive on their own (Genner et al. 2005).

The albino zebra, an albino morph of zebra cichlid, is similar to the red zebra and was once considered the same species. Like the red zebra, the Albino zebra is also a maternal mouthbrooder. *Maylandia* is one of the most species rich genera of the Lake Malawi mbuna (Knight and Turner 2004). Currently *Maylandia* (formerly *Pseudotropheus*) is comprised of 13 described species, with 20 species recognized by aquarists but not formally described (Konings 1990). Eyesight in albino organisms relative to wild organisms is well documented. Lack of pigment in the eyes of albino morph cause reduced visual acuity (Lashley 1930, Wilson et al. 1988, Abadi and Pascal 1991, Ren et al. 2002). Because visual aspects, including the ability to see color, has been shown to be important in cichlid mating (Seehausen and van Alphen 1998), having reduced visual capabilities may impact the breeding success of a species. The present study poses the question of whether or not reduced visual capabilities increase the rate of brood parasitism by the cuckoo catfish on a cichlid host.

**Ctenochromis horei**

*C. horei* is the species in this study used as a model for cichlids that coevolved with the cuckoo catfish. Native to the shallow (1m-4m) (Sefc et al. 2009) vegetated zone of Lake Tanganyika, like the red and albino zebras, *C. horei* is a haplochromine cichlid. *C. horei*, like the other species in this study, is a sexually dimorphic, maternal mouthbrooder (Ochi 1993). *C. horei* is a carnivorous cichlid (Taborsky and Foerster 2004) feeding predominantly on fish.
In the wild, *C. horei* establish a strict hierarchy between the males, the largest and most colorful usually the most dominant (Ochi 1993). Unlike other mouthbrooding cichlids, *C. horei* mates by defending a group of females, meaning that reproductive success is dependent on male to male combat as opposed to female mate choice (Reichard et al. 2007).

*Synodontis multipunctatus* (cuckoo catfish), a brood parasite

Brood parasitism (use of hosts for providing care to parasitic progeny) evolved in insects, birds, and fish (Soler et al. 2000, Cichon 1996, Rothstein 1990). Recent studies documented the presence in Lake Tanganyika of an obligate brood parasite, the cuckoo catfish (*Synodontis multipunctatus*) that uses mouthbrooding cichlids as hosts (Sato 1986, Cruz 2004). Prior to the discovery of obligate brood parasitism in the cuckoo catfish, it was thought that this form of social parasitism occurred only in birds and insects.

As an obligate brood parasite, the cuckoo catfish can only reproduce by prompting a mouthbrooding cichlid host to take care of its young. During cichlid spawning, the cuckoo catfish enters the spawning area of the cichlids and breeds simultaneously. With each successive intrusion into the breeding area, the catfish lay and fertilize their eggs while eating some host eggs. Concurrently, the female cichlid inadvertently picks up some of the catfish eggs along with its own, even though the catfish eggs are of different size and color. Eggs of the catfish are incubated in the mouth of the host species together with host eggs. Catfish eggs hatch earlier than those of the host cichlid. Following absorption of their yolk sac, the catfish fry feed upon the host fry while still in the cichlid’s mouth, and consequently take advantage of the complete maternal effort (Cruz et al. 2004). Early stages of this catfish are therefore dependent on the host for food and
protection. When parasitized, the host cichlid is left with few to no young as they are eaten by the catfish young.

**Methods**

**Rate of Parasitism**

Experimental aquaria used ranged in size from 110 to 568 liters, and contained breeding groups of red zebra cichlids and cuckoo catfish in ratios of about 3:1 female to male cichlids and 1:1 female to male catfish. The fish I used in this study were located in the Cruz lab in the University of Colorado at Boulder. In this lab, the tanks are kept at a consistent temperature of 24-26° C. Tanks were also buffered in order to maintain a pH close to 8.5, which is close to that of the pH in the East African Great Lakes. I placed flowerpots inside the tanks to serve as breeding territories for the male red zebras; this is where spawning occurred.

I monitored tanks daily for female cichlids carrying embryos, as evidenced by distended buccal (oral) cavities. Following identification, I carefully removed these females from the tank and recorded the standard length of the female cichlid. Then, I obtained the embryos by gently holding the female’s mouth open in a small volume of tank water and squirting water into the oral cavity to dislodge the eggs from the mouth. I then recorded the number of cichlid eggs and catfish eggs that were present. I put these embryos into incubation chambers during early development for observation, in order to compare the early development of the red zebra to that of other cichlids.

**Courtship Behavior Observations**

I examined the courtship behavior of the red zebra in order to compare it to that of the sympatric species (*C. horei*). I wanted to examine not only whether or not there was a
spawning and courtship difference between sympatric and non-sympatric species to the cuckoo catfish, but also whether or not there were varying levels of aggression shown towards the parasitic catfish. I filmed red zebras during breeding to observe courtship behaviors and compared this information to standardized rock-dwelling cichlid courtship specified in Seehausen (1996). In general, after acquiring a territory, the male cichlid displays (with all fins erect) to a female, thereby initiating spawning. When the female and male are ready to spawn, the female stays in the male’s territory. During breeding, the male quivers his anal fin, where eggs spots are located. These eggs spots on the anal fin of the male serve as dummy eggs to distract the female long enough for the male to fertilize her eggs. The female releases the eggs and the male releases sperm, which is then ingested and fertilizes the eggs she picks up into her mouth (Figure 3).

Visual Documentation of Early Development

In this study, I documented the early development of the Red Zebra in order to compare the developmental timing with that of the parasitic catfish, and a sympatric species to the catfish. With many brood parasites in the population, it could be beneficial to the host to develop faster, in order to reduce the amount of host progeny that the catfish young are able to consume. I wanted to test the hypothesis that the red zebra development is not slower than that of the *C. horei* (the species that coevolved with the cuckoo catfish) even with to the lack of pressures caused by brood parasitism. I put the broods that I stripped from the holding females into incubation chambers. These incubation chambers were tubes I placed in the tank that allowed airflow to tumble the developing embryos. I took pictures of the early developmental stages of the broods (of each of the young daily for 23 days) using a Carl Zeiss Stem SV II.
The microscope was connected to a Carl Ziess AxioCam HRc camera and AxioVision Rev. 4.6.3 software. Using the program AxioVision, I viewed images of the developing *Maylandia estherae*.

**Statistical Analysis**

**Red Zebra Length and Brood Size**

In order to analyze whether body size is linearly related to brood size, I used a Pearson’s product-moment correlation test, followed by a regression analysis on brood size and body size.

**Parasitism Rate**

I calculated the percent parasitism of the red zebra broods by looking at how many of the total broods of red zebra were successfully parasitized. For every brood that is parasitized, most to all of the red zebra young are eaten by the parasitic catfish young.

**Cuckoo Catfish Host Preference**

I created a general linear model for whether the cichlid that was parasitized could be predicted by size, and another to see if it could be predicted by brood size. I used ANCOVA after verifying a normal distribution of the data.

To test whether cichlid size distribution differed for parasitized and unparasitized cichlids, I used the two-sample Kolmogorov test. I then calculated the variance of the sizes of cichlids that were parasitized to compare to the variance of the sizes of cichlids that were not parasitized. The hypothesis I tested was, is the variance of parasitized cichlids sizes equal to
the variance of unparasitized cichlids sizes. I began to analyze this using the F-test of equal variances. To test this significance further I used a Fligner-Killeen test of homogeneity of variances.

**Results**

**Red Zebra Length and Brood Size**

In this study, I examined at 101 unparasitized broods of red zebra. Female cichlid body length ranged from 60 mm to 90 mm, and brood size varied from 8 to 60 red zebra eggs. From the regression analysis and the Pearson’s product-moment correlation test on body length and brood size I received a p-value of p<0.01, and r=0.46 (Figure 4). This indicates a significant positive relationship between cichlid body size and brood size.

**Rate of Parasitism**

Of the 136 broods of red zebra, 35 were parasitized by the cuckoo catfish, resulting in a rate of parasitism (parasitized/total broods) of the red zebra by the cuckoo catfish 25.7% (Table 1). The average number of catfish eggs per parasitized brood was 9.36±√59.1, as the number of catfish eggs picked up by the red zebra is highly variable. The mean clutch size for parasitized broods was 49 eggs, much larger than non-parasitized broods because catfish eggs are included.

**Courtship Observations**

The red-zebra courtship study was similar to previous studies done on rock dwelling cichlids (Figure 3). The courtship did not vary from that of the sympatric species (*C. horei*)
nor did it differ from the spawning sequence of the non-sympatric species (P. zebra). The red zebra did however show less aggression towards the cuckoo catfish than the sympatric species during courtship.

Developmental Staging

The early developmental stages of the red zebra were divided into the three stages described by Balon (1985), including the cleavage, embryonic, and eleutheroembryonic phase.

Cleavage Phase (day 0 to day 1) (Figure 5a-5e)

Cells divide until they form a blastula (Figure 5a-5c) and the blastula then collapses into the yolk cell (Figure 5d). The Blastodisc is then formed (Figure 5e) followed by the formation of the germ layer (Figure 5f-5g).

Embryonic Phase (day 2 to day 5) See Figure 6a-6d

This phase is outlined by the formation of the head, germ ring, and tail.

Eleutheroembryonic Phase (day 6 to free swimming) See figure 7a-7h

This phase begins with hatching, where the tail becomes detached from the yolk sac, and outlines development until the red zebra fry is free swimming.

In all three stages, the red zebra development did not differ from that of previous studies of the development of non-sympatric species (albino zebra), nor the sympatric species (C. horei). (A. Cruz and M. Cohen, unpublished data)
Cuckoo Catfish Host Preference

From the general linear model testing of whether or not parasitism could be predicted by brood size or body size, no significant relation was found. The variances of parasitized versus non-parasitized broods were analyzed as dependent on egg number (Figure 8A) versus adult size (Figure 8B). A two-sample Kolmogorov test revealed that these distributions are different in Figure 8B (p <0.05; Table 1). Table 2 shows that the magnitude of the variance of cichlids that were not parasitized is almost double that of the parasitized. There was a significant difference between the variances. This difference was marginally significant (p = 0.05), suggesting that catfish may prefer hosts of an average size (Figure 9).

Discussion

Obligate brood parasitism usually occurs between two specific species that coevolve in the same ecosystem, and not between species in different ecosystems. The cuckoo catfish was thought to only parasitize Lake Tanganyika cichlids (Sato 1986), but the present study, along with unpublished data (A. Cruz and M. Cohen, Cruz lab), demonstrates the cuckoo catfish’s ability to parasitize a cichlid found in Lake Malawi. The naïve host (host and parasite have not come into contact prior to study) in this study is *Maylandia estherae* (native to the rocky shores of Lake Malawi) commonly referred to as the red zebra.

Red zebra length and brood size

The red zebra clutch size ranged from about 8 to 68 eggs with a range in female body size from about 60 mm to 90 mm. The present study found a significant relation between red zebra brood size and female body size. This may be due in part to the fact that the larger the
female, the larger her buccal cavity (which encloses the brood). The significant positive relationship between brood size and cichlid length indicates that older, larger cichlid females are able to carry more eggs and therefore have a higher fecundity. This relation was also found in previous studies (Fawole and Arawomo 2000, Campos-Mendoza et al. 2004, Mannon 2013, Roscow 2012).

**Parasitism Avoidance strategies**

Although red-zebra breeding males showed some aggression towards the cuckoo catfish, most aggression was directed at other red zebra males coming into the breeding territory. Over time, there was no significant decrease in parasitism success of the cuckoo catfish. Over the period of this study (about three years) the red zebra did not develop any successful parasitism avoidance strategies. More studies should be done over a longer period of time to further address the question of whether or not the red zebra can develop adaptations to reduce brood parasitism.

**Red Zebra Rate of Parasitism and comparison with other host species**

I found that the (not naturally co-occurring) red zebra was parasitized significantly more than (the naturally co-occurring) *C. horei* used in previous, unpublished studies in the Cruz lab (A. Cruz and M. Cohen). The *C. horei* is native to Lake Tanganyika, and is naturally parasitized by the cuckoo catfish. It has also presumably coevolved with the cuckoo catfish. The fact that *C. horei* was parasitized significantly less (Table 1) suggests that the sympatric hold species likely developed adaptations against brood parasitism. This is a novel insight in the current ichthyology (fish) literature, while the use by avian brood parasites of naïve and sympatric hosts has been the subject of several studies. These studies include the study of avian brood parasitism of a naïve host the village weaver (*Ploceus cucullatus*) and the shiny cowbird (*Molothrus bonariensis* in
Hispaniola. Cruz et al. (2004) inferred that, although egg rejection likely has a genetic component, differences in rates of egg rejection between different types of parasitic eggs likely had to do with phenotypic plasticity (ability of an organism to change its phenotype in response to changes in the environment). In areas with more brood parasites, more defenses against brood parasitism may be found. When parasitism avoidance behaviors such as egg rejection have minimal costs, they have also been found to be maintained in avian populations as relic behaviors, even when there are no longer parasites in the population (Rothstein 2001). In the present study, both the red zebra and albino zebra showed no apparent new successful adaptations against parasitism, even with parasites present in the population. This is unlike what has been found in avian brood parasitism studies, but should continue to be studied over a longer period of time to see if adaptations are developed. Also unlike avian brood parasitism (Rothstein 1975, Cruz et al. 2004), there is no egg rejection behavior from the host and no egg mimicry adaptations by the cuckoo catfish.

**Parasitism of the Red Zebra vs. Albino Zebra**

The question of how eyesight affects the ability of hosts to avoid brood parasitism is poorly understood. For this reason, the albino zebra, an albino morph of the zebra cichlid, a closely related species to the red zebra, and also a rock-dwelling Lake Malawi mbuna species. One of the obvious morphological differences between the red zebra and the albino zebra is that the albino zebra has red eyes, indicating a lack of pigment. Many authors have noted that the lack of pigment in the eyes of albino morphs causes reduced visual acuity (Lashley 1930, Wilson et al. 1988, Abadi and Pascal 1991, Ren et al. 2002). Because visual aspects, including the ability to see color, have been shown to be important in cichlid mating (Seehausen and van Alphen 1998), having reduced visual capabilities could impact the breeding success of a species. Unpublished Cruz lab data (A.
Cruz and M. Cohen) show that the albino zebra was parasitized 41% of the time, almost double the rate of parasitism of the red zebra (Table 1). This result leads to the conclusion that eyesight plays a major role in parasitism avoidance.

**Early developmental stages of the red zebra**

The timing of the early developmental stages of the red zebra coincided with other cichlid studies, including Balon (1985) (Figures 5-7). The timing of the development is important because for mouthbrooding cichlids, the female does not eat while she carries her young. This creates a fitness tradeoff for female fitness and progeny fitness. For species that are parasitized by the cuckoo catfish, developing faster could alter the reproductive success of the cuckoo catfish, since the cuckoo catfish young hatch earlier than their cichlid host and consume the host embryos. The cuckoo catfish young will only eat the cichlid young while they still carry most of their yolk (used for sustenance before they leave the mothers mouth), so if the cichlids are able to reach a certain point in their development without being consumed by the catfish young, they will survive.

**Courtship Observations**

The courtship of the red zebra falls in line with the standardized cichlid courtship behavior shown in figure 3. Further studies should be done to investigate how different courtship behaviors affect cuckoo catfish parasitism success, as all the cichlids in this study share similar courtship behaviors.
Cuckoo catfish host preference

I found that the cuckoo catfish was successful in parasitizing a naïve host (red zebra) in this study. This posed the question of whether the cuckoo catfish parasitizes hosts at random whenever possible, or whether there are host preferences at play. In this study, I found that the cuckoo catfish parasitized red zebras of average size over the largest and smallest in the population. This may be because the smallest breeding red zebras have a smaller buccal cavity and therefore less space for the developing catfish young, as well as fewer red zebra young for the catfish to feed on. The larger the spawning red zebra, the higher the risk of physical harm to the catfish attempting to interrupt the spawning. Cuckoo catfish may therefore prefer an intermediate to maximize buccal cavity space and red zebra young, and to minimize risk of physical harm to the catfish. This also implies that the negative effects of parasitism on brood size are most relevant to female red zebras of average size. This cuckoo catfish host preference is a novel finding that has not been discussed in the literature. More studies should be done to determine why the cuckoo catfish prefers to parasitize cichlids of average size. This also leads to question of what other (if any) host preferences does the cuckoo catfish have.

Conclusion

By investigating the interaction between the red zebra, a member of a fish group known for its adaptive capabilities, and a brood parasite, this study gives important insight into whether or not these cichlids adapt in order to increase their reproductive success. Over a period of 3 years, the red zebra seemingly did not develop successful parasitism avoidance strategies against the cuckoo catfish. The catfish however was able to successfully parasitize a
host that it had not previously encountered in the wild. The cuckoo catfish was also found in this study to have a size preference for the hosts it parasitized. Although the red zebra was not able to hamper the rate of parasitism among its broods, it was parasitized much less than the albino zebra, suggests that eyesight plays a role in parasitism avoidance. Another factor that could have caused such a high rate of parasitism in the albino zebra is that the albino zebras in the Cruz lab are further removed from the wild than the red zebras, that is, they have been bred in captivity for longer. Lastly, the red zebra was parasitized significantly more than the species in the lab that coevolved coexist naturally with the catfish. This suggests that there are adaptations against brood parasitism that the sympatric species have that the non-sympatric species (red zebra and albino zebra) do not have. This may include increased aggression towards the cuckoo catfish. Future studies should be conducted to identify these adaptations, to gain further knowledge on adaptations and brood parasitism avoidance.

**Acknowledgements**

My study was done in the lab of Dr. Alexander Cruz. Resources of Dr. Pamela Diggle were also used. Funding was provided through URAP and UROP grants provided by the University of Colorado at Boulder. Special thanks to Dr. Alexander Cruz and Ph.D. student Marcus Cohen for their support in developing this honors project. Without their guidance this project would not have been possible. I would also like to thank other members of the Cruz lab including Robert Roscow for helping to train me to conduct research in the lab. Lastly, thank you to my committee members Dr. Alexander Cruz, Dr. Barbara Demmig-Adams, and Dr. Holly Barnard for their time and support.
Reference List


Description of a New Genus and Ten New Species.” *Academy of Natural Sciences*, 148 (1997): 189-230


Figures and Tables

Figure 1: East African Great Lakes: Lake Victoria, Lake Tanganyika, and Lake Malawi (from Nyamweru C.K. 1983)
Figure 2: Lake Malawi (Owen, et. al. 1990)
Figure 3: Cichlid Courtship sequence based on (Seeausen 1996)
Table 1. Frequency of parasitism for three species of African mouthbrooding cichlids. Lakes of origin are denoted in parentheses. Allopatric parasitism frequencies are significantly higher for allopatric species (p < 0.001 for the red zebra and the albino zebra).

<table>
<thead>
<tr>
<th></th>
<th>C. horei (Tanganyika)</th>
<th>Red zebra <em>M. estherae</em> (Malawi)</th>
<th>Albino zebra <em>M. zebra</em> (Malawi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitized</td>
<td>17</td>
<td>35</td>
<td>92</td>
</tr>
<tr>
<td>Unparasitized</td>
<td>86</td>
<td>101</td>
<td>133</td>
</tr>
<tr>
<td>% Parasitism</td>
<td>16.5%</td>
<td>25.7%</td>
<td>40.9%</td>
</tr>
</tbody>
</table>
Figure 4: Relationship between cichlid brood size and body size, $p < 0.01$ and $r = 0.46$. 

Cichlid Size and Brood Size

![Graph showing the relationship between cichlid brood size and body size. The graph includes data points that indicate a positive correlation, with the Pearson correlation coefficient $r = 0.46$.](image-url)
Figure 5a-e: Cleavage Phase Day 0 of *Maylandia estherae* (red zebra cichlid).

Figure 5f-g: Cleavage Phase Day 1 of *Maylandia estherae* (red zebra cichlid).
Figure 6 a-d: Embryonic Phase of *Maylandia estherae* (red zebra).

Figure 7a-h: Eleutheroembryonic Phase (post hatching) of *Maylandia estherae* (red zebra).
Figure 7 Continued
Figure 8A and 8B. The distribution of the sizes of cichlids that were parasitized versus the distribution of the sizes of cichlids that were not parasitized. Figure 5A shows that there is no difference in distribution of number of cichlid eggs in broods that are and are not parasitized. Figure 5B shows that there is a difference in distribution between the lengths of parasitized cichlids compared to unparasitized cichlids.

**Figure 8A**

![Figure 8A](image)

**Figure 8B**

![Figure 8B](image)
Table 2: Significant difference of variances of host size (p < .05).

<table>
<thead>
<tr>
<th>Differing Variances of Parasitized vs. Non-Parasitized Cichlids</th>
<th>Parasitized</th>
<th>Not Parasitized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variance of Cichlid Size</td>
<td>25.86</td>
<td>47.03</td>
</tr>
</tbody>
</table>
Figure 9: Significant difference in distributions between non-parasitized and parasitized cichlids (p<0.05)

Non-parasitized vs. Parasitized Cichlid Length