

HOST-PARASITE INTERACTIONS OF THE AFRICAN CUCKOO CATFISH
(*Synodontis multipunctatus*)

by

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Host-Parasite Interactions of the African Cuckoo Catfish (*Synodontis multipunctatus*)

Dissertation directed by Professor Alexander Cruz

Obligate brood parasitism (completely relying upon a host for successful breeding) has been studied in insects and birds, with a considerable amount of research concentrating on birds such as cuckoos and cowbirds. The Lake Tanganyikan cuckoo catfish (*Synodontis multipunctatus* Siluriformes: Mochokidae) is the only known non-avian obligate brood parasite among vertebrates, and it uses female mouthbrooding cichlids (Perciformes: Cichlidae) as hosts. Although the basic breeding mechanism of the cichlid-cuckoo catfish system has been well documented through a series of observational studies, there have been few manipulative experiments concentrating on host-parasite interactions. The goal of my dissertation work was to examine this parasitic relationship from the perspectives of both the host and the parasite in the context of ecology and coevolution. Specifically, I was interested in how hosts avoid or mitigate parasitism, as well as features of the parasite that allow for successful parasitism. First, I assessed parasitism frequencies among cichlid hosts and discovered that sympatric Lake Tanganyikan hosts were parasitized significantly less than allopatric hosts from other lakes in laboratory conditions, possibly explained by subtle differences in mating ritual and oviposition, or increased aggression by sympatric hosts. Next, I compared unparasitized and parasitized broods to show that the cuckoo catfish indiscriminately parasitize all sizes of host, followed by complete elimination of host progeny and subsequent cannibalism while being brooded in the host mouth. Finally, using live fish and video playback techniques, I found that visual cues play an important role in breeding synchrony between the cuckoo catfish and cichlid hosts. Together, my dissertation provides the groundwork for using this unique cichlid-cuckoo catfish system as a model for studying brood parasitism in controlled laboratory conditions, where questions regarding parasitism dynamics in the context of both ecology and coevolution can be addressed.

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Chapter 1

Introduction

Parasitism and Host-Parasite Interactions

Parasitism is a symbiotic relationship where one organism (the parasite) benefits, while another organism (the host) is harmed. Brood parasitism is a form of social parasitism in which a host broods and raises the young of the parasite, and can be either intraspecific or interspecific. Brood parasitism has been studied extensively in insects and birds, with the majority of research focusing on avian obligate brood parasites (completely relying upon a host for successful breeding), as is the case for cuckoos and cowbirds (e.g., Ortega 1998; Davies 2000). A facultative brood parasite may resort to parasitism, but does not require parasitism to successfully reproduce. Among fishes, most species practice facultative brood parasitism (Sato 1986; Wisenden 1999; Yom-Tov and Geffen 2006). However, the only known non-avian obligate brood parasite among vertebrates is the cuckoo catfish (*Synodontis multipunctatus* Siluriformes: Mochokidae), a species endemic to Lake Tanganyika that uses female mouthbrooding cichlids (Perciformes: Cichlidae) as hosts (Finley 1984; Sato 1986; Cruz *et al.* 2004). There have been anecdotal reports of the cuckoo catfish being persuaded to spawn facultatively in aquariums, but is otherwise assumed to be an obligate brood parasite (Barlow 2000). Additionally, in over 15 years of daily observations in our lab, no facultative spawning has ever been observed (pers. comm. A. Cruz, pers. obs.). Mouthbrooding is a system whereby one, or both, of the parents provide protection for young by carrying the eggs and/or fry in the mouth (Sato 1986; Keenleyside 1991; Barlow 2000). While in nature the cuckoo catfish can only parasitize mouthbrooding cichlids found in Lake Tanganyika, under laboratory conditions, the cuckoo catfish is capable of parasitizing mouthbrooding cichlids from other Rift Valley lakes

and have even been observed parasitizing a South American mouthbrooding cichlid species *Geophagus steindachneri* (Loiselle 1998).

Much of the research on the biology and ecology of the fish fauna of the Rift Valley lakes has concentrated on cichlid species. Although cichlids make up a large part of the fish fauna in the Rift lakes, non-cichlid fishes account for about 30% of the fish diversity, and many species are catfishes (Snoeks 2000; Koblmüller *et al.* 2006). Therefore, it is important to include non-cichlid species in studies of Rift Valley fishes. The basic breeding mechanism of the cichlid-cuckoo catfish system has been well documented through a series of observational studies. Brichard (1979) was the first to observe the cuckoo catfish fry in the mouths of captured Tanganyikan cichlids, and thought they got there accidentally. There were also descriptions offered from aquarists (Ferguson 1983; Finley 1983), until a field study by Sato (1986) confirmed that the cuckoo catfish was an obligate brood parasite of Lake Tanganyikan cichlids. In addition, Wisenden (1999) mentioned unpublished parasitism rates observed in aquariums of cichlid species from Lake Victoria and Lake Malawi, while Cruz *et al.* (2004) described the breeding biology and ontogeny of a host species and the catfish. While these studies provide a good foundation, there have been few manipulative experiments focused specifically on addressing the interactions of host and parasite.

This dissertation aims to provide a deeper understanding of the host-parasite interactions between cichlids and the cuckoo catfish. African cichlids have been proposed as a model group of organisms for studying genetics and adaptive radiation (Seehausen 2006; Santos and Salzburger 2012). Thus, the findings presented in the following chapters lay the groundwork for using cichlids and the cuckoo catfish as a model system to study brood parasitism under controlled laboratory conditions, where questions regarding parasitism dynamics in the context

of both ecology and coevolution can be addressed. For example, brood parasitic birds (e.g. cowbirds or cuckoos) can reduce host fitness by egg removal or competition between host and parasite young, whereas hosts can reduce the parasite's fitness by recognizing and rejecting the eggs of the parasite or by showing increased aggression towards the parasite (Rothstein 1975a; Payne 1977; Rothstein 1990; Fossøy *et al.* 2011). This results in an evolutionary arms race between host and parasite (Dawkins and Krebs 1979; Davies *et al.* 1989; Rothstein 1990). Since selection is both spatially and temporally focused in the breeding area, factors affecting host-parasite interactions are easily discernible (Rothstein 1990). Although research has focused on insects and birds, little is known about the subtleties of the host-parasite relationship in brood parasitic fishes. The interactions between cuckoo catfishes and mouthbrooding cichlids are a unique example among vertebrates of an obligate host-parasite system.

Experimental Questions and Objectives

The overall objective of this dissertation was to study the obligate brood parasitic cuckoo catfish-cichlid system, approaching the parasitic relationship from the perspectives of both the host and the parasite. Specifically, I was interested in how hosts avoid or mitigate parasitism, as well as features of the parasite that allow for successful parasitism.

- Chapter 1 – In this chapter I provide a brief review of the cuckoo catfish-cichlid study system by first describing the East African Rift System (EARS) and its cichlid diversity, followed by a description of the species used in this study. Then I make developmental comparisons between parasite and hosts and between parasite and a congener.
- Chapter 2 –Research Questions: Are host species from Lake Tanganyika that have coevolved with the cuckoo catfish less frequently parasitized than species from Lake Victoria or Lake Malawi? If so, what are hosts doing to avoid or mitigate parasitism?

Here I compare the parasitism rates by the cuckoo catfish between sympatric host species from Lake Tanganyika and allopatric host species from Lake Malawi and Lake Victoria to investigate whether sympatric host species have coevolved means to alleviate parasitism.

- Chapter 3 – Research Question: How costly is parasitism to the cichlid hosts? In this chapter I examine the effect of parasitism on host breeding success, determine which host sizes are parasitized most frequently, and document cannibalism among cuckoo catfish fry.
- Chapter 4 – Research question: What is the role of visual cues in achieving breeding synchrony with the host? Here I assess the role of visual cues used by the cuckoo catfish to synchronize their breeding with cichlid hosts, assuming the breeding synchrony of the cuckoo catfish with the host species is achieved at least in part via visual cues, because the cichlid spawning sequence has repeated behaviors that are easily discernable.

Notes About Formatting of Dissertation

Chapters 2 through 4 were each prepared as individual papers to be submitted to peer-reviewed journals. I have formatted the contents of this dissertation to be as consistent as possible throughout the document, with the intention of arranging each chapter to fit the requirements of individual journals at a later time. I have included more figures in each chapter than will appear in a final submitted manuscript for that chapter. I will use a subset of the figures for publication.

The East African Rift System (EARS)

The East African Rift System (EARS) is over 3000 km long, with its northern boundary at the intersection of Ethiopia, Eritrea, and Djibouti along the Red Sea's southwestern coast. The

rift system meanders south through eastern Africa to Mozambique. The EARS makes up a large portion of the Great Rift Valley (Gregory 1894) that begins farther north in Lebanon. The EARS began forming around 35-40 mya and currently contains over 35 lakes of various sizes (Watchorn *et al.* 1998; Tiercelin and Lezzar 2002), including the three largest lakes in Africa (Fig. 1.1). These three lakes (Victoria, Tanganyika, and Malawi) are often collectively referred to as, “The Great Lakes of Africa.” Lake Tanganyika is the second deepest lake in the world with a maximum depth of 1470m, Lake Victoria is the second largest freshwater lake by surface area at 68,870 km², and Lake Malawi is the fourth deepest (706m) and eighth largest by surface area (29,500km²). There are two main branches of the EARS, the Eastern Branch and the Western Branch (Fig. 1.2). Lake Malawi and Lake Tanganyika are graben lakes located within the rift valleys of the Western Branch, while Lake Victoria is unique in that it lies between the two rift valley branches and not within any of the major rifts themselves (Tiercelin and Lezzar 2002; Chorowicz 2005). Many volcanic and tectonic activities have been involved in creating and shaping the EARS, however the precise processes and their extent are still debated. The tectonic activity in this region has resulted in the Arabian Peninsula slowly pulling away from Northeastern Africa and the separation of the two East African Plates (Nubian and Somalian) from each other (Fig. 1.3). The unique features of this region have provided for an incredible natural experiment in speciation and biodiversity, and African cichlids in the EARS are a great example of diversity and adaptive radiation.

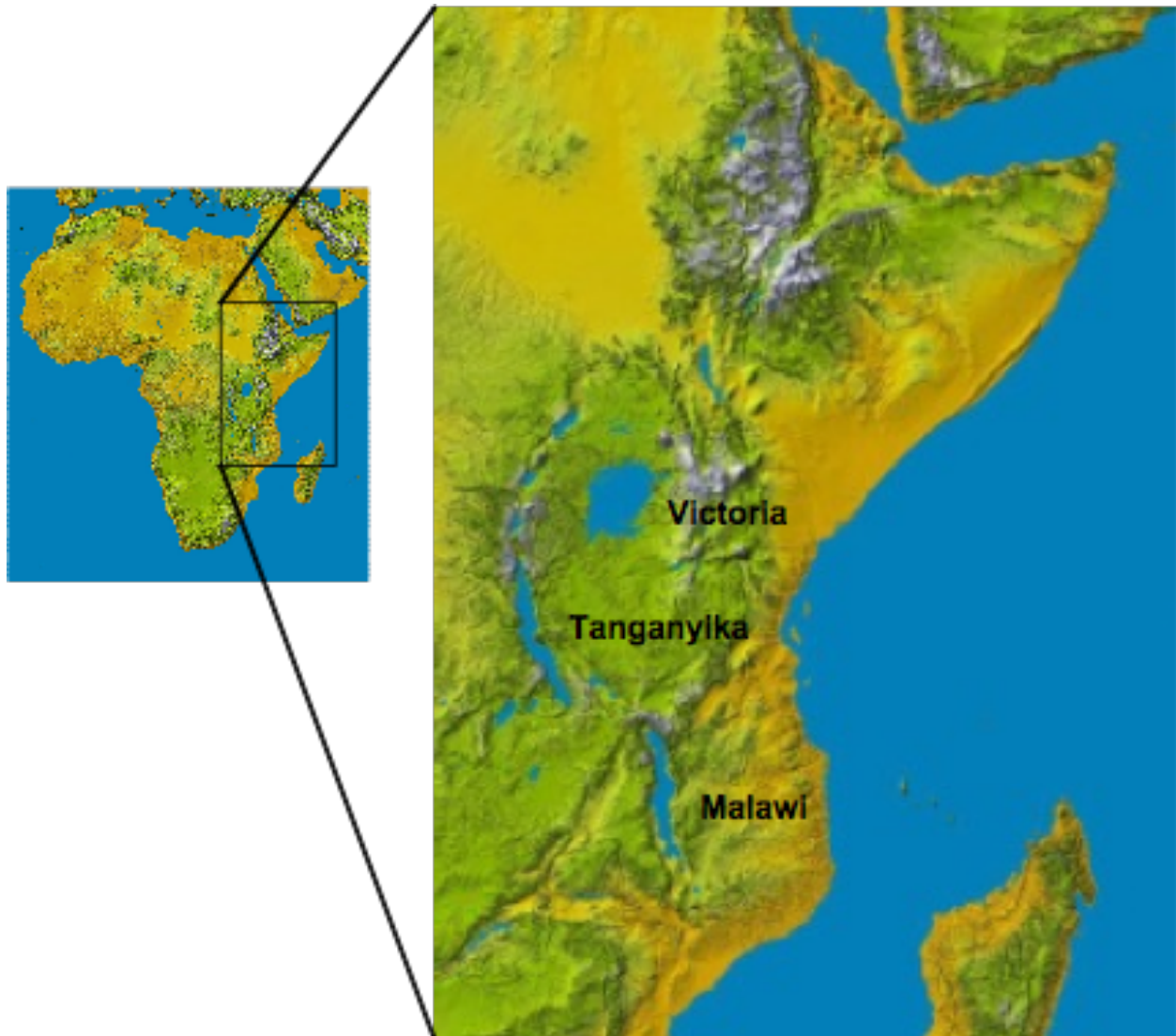


Fig. 1.1 SRTM color shaded relief image of Africa expanded to show the extent of East African Rift System and three largest lakes. Topographic height is indicated by color; with brown and yellow at lowest elevations, rising through green to gray and then white at highest elevations (background image source: NASA/JPL/NIMA).

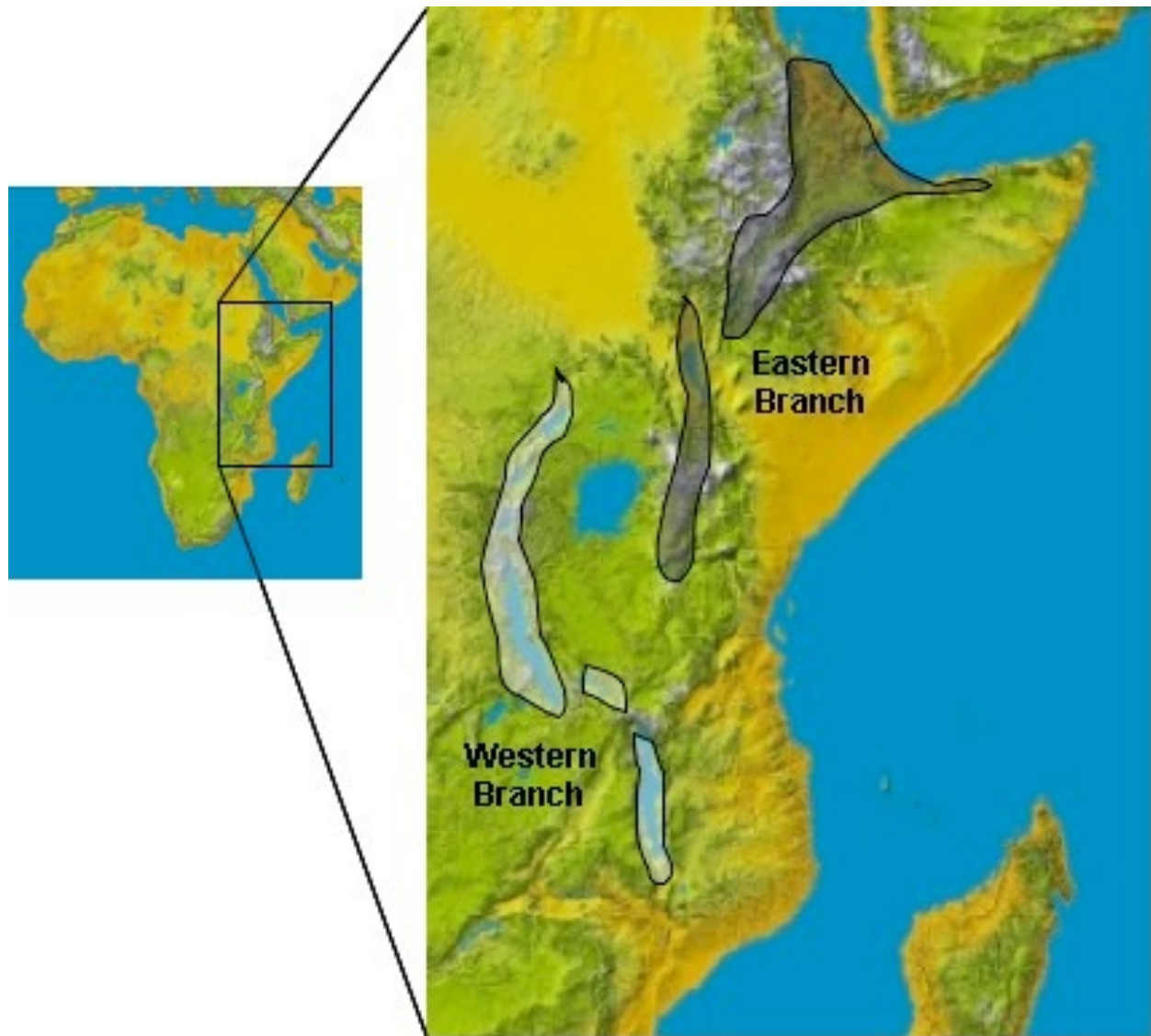


Fig. 1.2. SRTM color shaded relief image illustrating the two main branches of the East African Rift System, the Western Branch (light gray shaded areas) and the Eastern Branch (dark gray shaded areas) (background image source: NASA/JPL/NIMA).

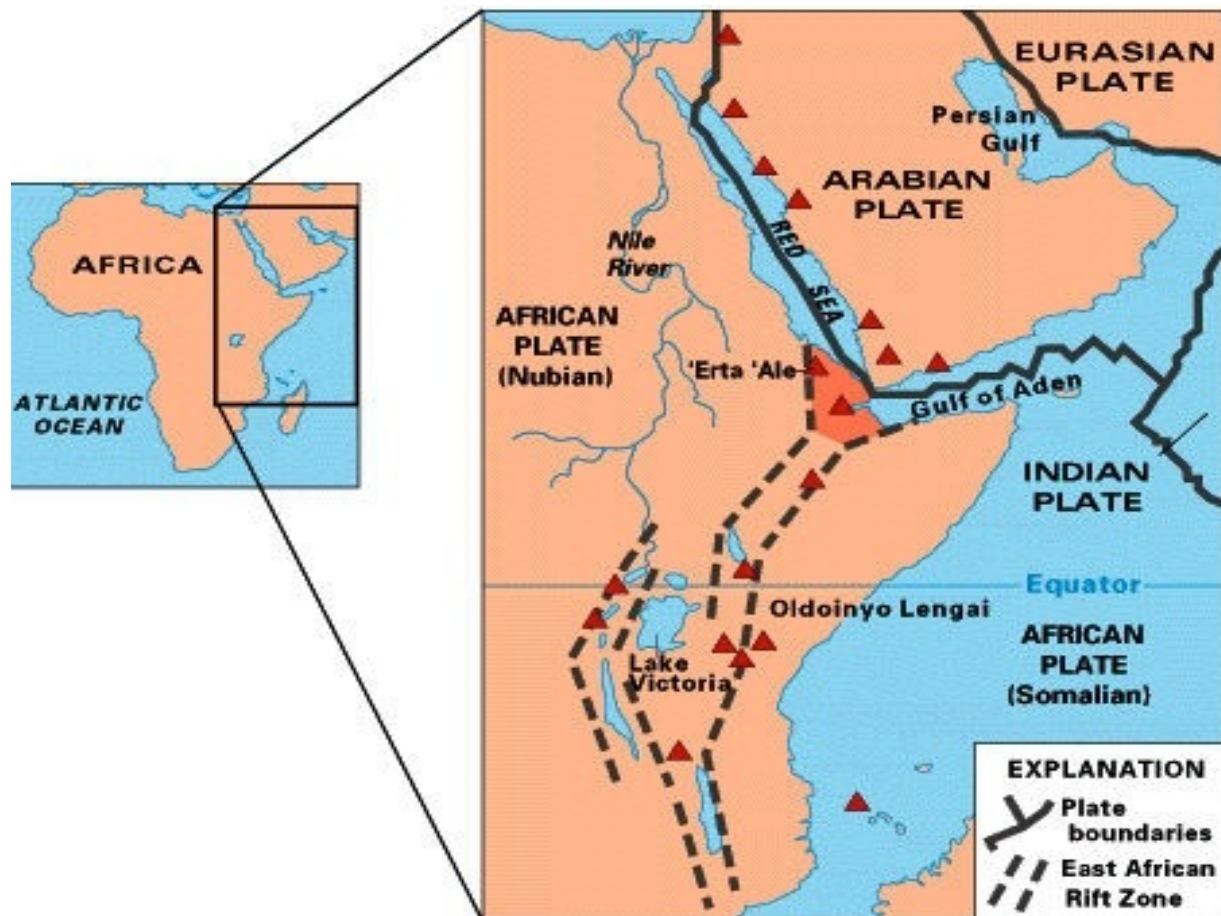


Fig. 1.3. Map of East Africa showing the plate boundaries (solid gray lines), historically active volcanoes (red triangles), and the Afar Triangle (shaded dark orange). The Afar Triangle is the location where the Arabian Plate and the two parts of the African Plate (Nubian and Somalian) are splitting along the East African Rift Zone (dashed lines) (image source: USGS).

Cichlid Diversity

Cichlids are perch-like fishes (Perciformes) of the family Cichlidae. They can be distinguished from other closely related species by the presence of a single nostril on each side of the head and a discontinuous lateral line. Cichlids are extraordinarily diverse in color, morphology, and feeding modality, and this diversity is most pronounced in cichlids from the three major Rift Valley lakes (Tanganyika, Malawi, and Victoria) of East Africa, where as many as 2000 species have evolved within the last 10 million years, resulting in one of the most dramatic cases of vertebrate speciation (Barlow 2000; Danley and Kocher 2001; Lande *et al.* 2001; Kocher 2004; Sturmbauer *et al.* 2011). Lake Tanganyika has up to 250 species and

subspecies of cichlids, while estimates for Lake Malawi are 500-700 species, and Lake Victoria 700-800 species, with new species still being discovered (Coulter 1991; Snoeks 2000; Turner *et al.* 2001; Koblmuller *et al.* 2008; Takahashi and Koblmuller 2011). Endemism is widespread among cichlids in these lakes, with virtually all (>95%) of the cichlid species found only in a single lake, and species are not shared between lakes (Fryer and Iles 1972; Kocher *et al.* 1993; Meyer 1993; Snoeks 2000; Salzburger and Meyer 2004; Seehausen 2006; Takahashi and Koblmuller 2011). Molecular evidence suggests that the cichlids in lakes Victoria and Malawi are monophyletic, and derived from one or two ancestral lineages (Meyer *et al.* 1990; Meyer 1993; Nagl *et al.* 2000). This is contrasted by polyphyletic origins of the 12-16 tribes of cichlids in Lake Tanganyika, which is at least partly due to the lake being much older (Fryer and Iles 1972; Poll 1986; Danley and Kocher 2001; Salzburger *et al.* 2002; Takahashi 2003; Salzburger and Meyer 2004).

Evolution of 2000 distinct species in 10 million years would be considered rapid, in and of itself, but it is thought that Lake Victoria completely dried up at roughly 12,400-15,000 years ago, and therefore speciation of the current collection of cichlid species would likely have started at that point if no appropriate habitat remained (Johnson *et al.* 1996; Kornfield and Smith 2000; Nagl *et al.* 2000; Danley and Kocher 2001). Lake Malawi and Lake Tanganyika would have been exposed to similar drought conditions, although there is no evidence that they dried completely. However, there are indications that Lake Tanganyika, thought to be the oldest of the three great lakes, has had many drastic lake level fluctuations over the last 1 million years (Lezzar *et al.* 1996). The exact age of Lake Tanganyika has been debated for more than a century, with most estimates ranging from 5-25 million years; however, more contemporary analyses tend to fall within the 9-12 million year range (Cohen *et al.* 1993; Sturmbauer *et al.*

2001; Tiercelin and Lezzar 2002). Lake Tanganyika can be broken up into the northern, central, and southern basins. The central basin is thought to be the oldest with sediment analyses generating a maximum age of 9-12 myo, while the northern and southern basins formed later (Cohen *et al.* 1993). If lake levels dropped low enough during extended periods of extreme drought, or experienced prolonged times where high heat and evaporation occurred, Lake Tanganyika may well have repeatedly become three separate lakes. This would reinforce barriers to mating and help to facilitate allopatric speciation. There is evidence that a long dry period roughly 25,000 years ago caused Lake Malawi's water level to be 250-500 meters lower than its current level, while Tanganyika's water level decreased more than 600 meters, supporting evidence that indeed three separate basins were present during this timeframe (Scholz and Rosendahl 1988) .

While vicariance is important to species interactions and evolution in Rift Valley cichlids, other modes of selection like assortative mating or sexual selection are also likely involved. For example, research suggests that the radiation of the cichlids of Lake Malawi occurred in three stages. First, cichlids adapted to either rocky or sandy habitats, which led to the evolution of different jaw morphologies based on differential feeding habits, and finally males diversified color patterning within each lineage through means of sexual selection (Danley and Kocher 2001). Cichlids' ability to adapt to a wide variety of habitats and feeding modalities has permitted rapid and widespread speciation. Specifically, cichlids have pharyngeal jaws at the back of their throat that are involved in most of the food processing and allows their oral jaws to adapt to different specialized feeding modalities. Having such plasticity in oral jaw functionality

has likely led to the swift evolution of the diverse feeding strategies seen among these fishes (Liem 1973; Meyer 1993; Salzburger and Meyer 2004; Kocher 2005).

Another feature of African cichlids that allows them to be so successful is extensive parental care. Although the underlying forces driving parental care can be debated, the high levels of cichlid predation in the Rift Valley lakes are indeed a contributing factor. Humans are an important predator of cichlids, as these fish are a primary source of protein in the region, but there are also many mammals, birds, reptiles, and other fish that rely on cichlids as a significant part of their diet (Fryer and Iles 1972). Cichlids in Lake Tanganyika have evolved many parental care systems including: mouthbrooding, substrate guarding, care by one parent, and care by both parents. In addition, the cichlids employ different mating systems including monogamy and various types of polygyny (Kuwamura 1997). A phylogeny of the family Cichlidae shows that substrate guarding is the ancestral state, while mouthbrooding is the most common form of care, and has evolved more than once (Goodwin *et al.* 1998).

Mouthbrooding as a reproductive strategy is well represented in Rift Valley cichlids. All but one of the endemic cichlid species in both Lake Malawi and Lake Victoria (Kuwamura 1986; Duponchelle *et al.* 2008), and many in Lake Tanganyika are maternal mouthbrooding species (Fryer and Iles 1972; Rossiter 1995; Salzburger *et al.* 2002). In maternal mouthbrooding species, the offspring will develop using their yolk sac as a nutrition source and will use the mouth of their mother for protection until becoming independent. This form of oral incubation is one of the most advanced parental care systems among fishes (Keenleyside 1979; Ochi *et al.* 2001).

Study Species Used in Experiments (Fig. 1.4)

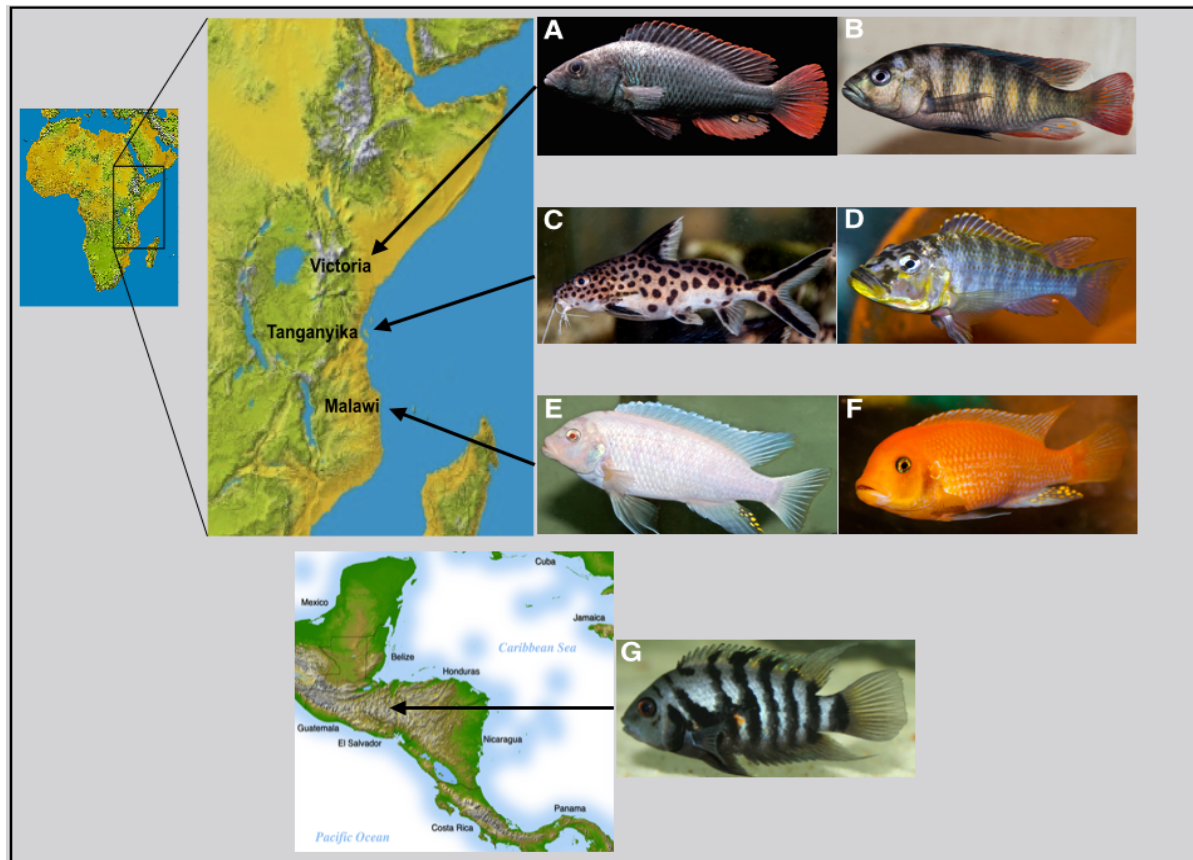


Fig. 1.4. Five African cichlid host species: (A) *H. nubilus*, (B) *H. latifasciatus*, (D) *C. horei*, (E) *M. zebra*, and (F) *M. estherae*. (C) The brood parasitic cuckoo catfish (*S. multipunctatus*). (G) *A. nigrofasciata* is native to Central America. Black arrows indicate the locations where the natural populations of these species are found, and fish from the same area are organized by row (background map images source: NASA/JPL/NIMA).

Sympatric Study Species

Synodontis multipunctatus (Boulenger 1898), the cuckoo catfish, is a brood parasitic catfish in the Mochokidae family endemic to Lake Tanganyika in Africa. This catfish is a generalist brood parasite on different species of mouthbrooding cichlids. They have brownish gray coloration dorsally, are uniformly white ventrally with conspicuous dark spots across the entire body, reach a maximum reported standard length (SL) of 27 cm, and can be distinguished from similar looking congeners by a humeral process that is narrow posteriorly (Eccles 1992)

(Fig. 1.5). It should be noted that the cuckoo catfish rarely exceed a SL greater than 13 cm. when kept in laboratory aquaria (pers. obs.). Males can be distinguished from females by the presence of a triangular shaped genital papilla that is well defined and located ventrally between the pelvic and anal fins, while females have only a bulging around the urogenital opening (Fig. 1.6). These catfish travel in large shoals in Lake Tanganyika (Finley 1984), and inhabit the muddy bottoms down to at least 100 m in the lake. They feed on small invertebrate and are specialized to feed on an abundant gastropod species (*Neothauma tanganyicense*) (Poll 1953; Coulter 1991; Eccles 1992).

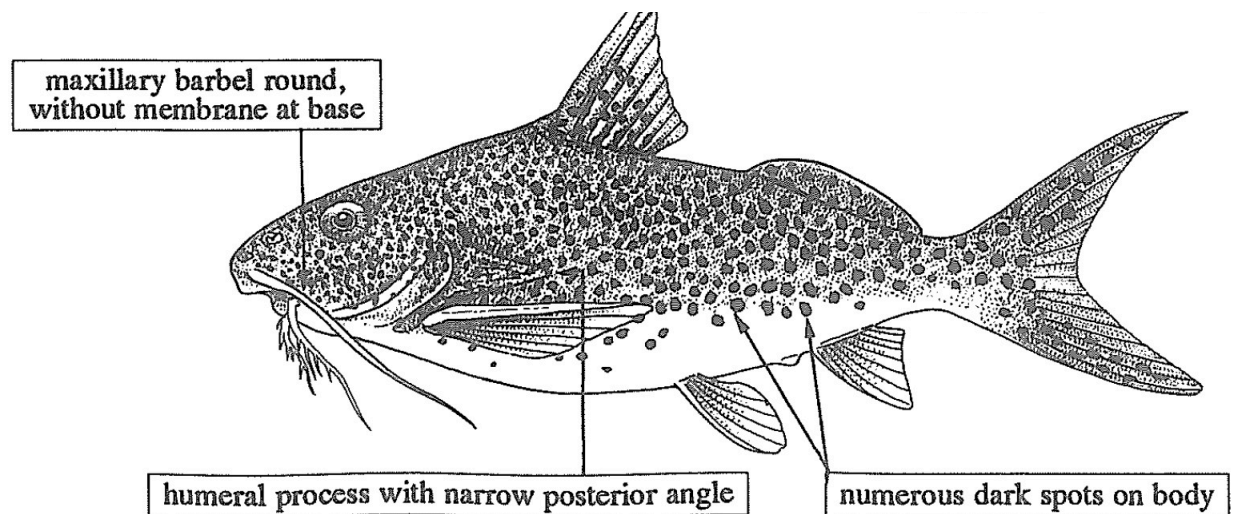


Fig. 1.5. Modified drawing from (Eccles 1992) showing some distinguishing features of the cuckoo catfish (*S. multipunctatus*).



Fig. 1.6. Male cuckoo catfish (*S. multipunctatus*). Males can be distinguished from females by the presence of a triangular genital papilla located ventrally between the pelvic and anal fins.

Ctenochromis horei (Günther 1894) is a mouthbrooding cichlid endemic to Lake Tanganyika and surrounding river tributaries, and is a naturally occurring host of the cuckoo catfish (Sato 1986). They are tan to yellowish brown in color, and have variable dark markings on the head as well as vertical black bars and irregular reddish circles on their sides. The males have more prominent black markings on the head and brighter coloration overall than females do. Ocelli are present in the anal fin. The maximum reported SL is 20 cm., and they live in the benthopelagic inshore regions of the lake and lower reaches of the river tributaries (Eccles 1992).

Allopatric Study Species

Metriaclima zebra (Boulenger 1899), the zebra cichlid, is a mouthbrooding cichlid endemic to Lake Malawi. The albino morphs were used in this study, as aquarists have reported using them successfully to rear cuckoo catfish. They are white to slightly yellowish in background color with pink vertical bars along their sides. They have red pupils and no pigment

in the iris. Ocelli are present in the anal fin. The maximum reported SL is 11 cm. (Maréchal 1991). They are found in rocky areas and feed at ninety degrees to the substrate using their teeth to comb loose material away, and also feed on plankton in the open water (Konings 1990).

Metriaclima estherae (Konings 1995), the red zebra cichlid, is a mouthbrooding cichlid endemic to Lake Malawi. They are beige to orange-red in color; lacking broad vertical bars on body. Ocelli are present in the anal fin. The maximum reported size is 8 cm. SL (Konings 1995), however they have been observed to reach 9 cm SL in the aquariums (pers. obs.). They are found in rocky areas and feed the same way as *M. zebra* described above.

Haplochromis nubilus (Boulenger 1906) is a mouthbrooding cichlid endemic to not only Lake Victoria, but also surrounding smaller lakes and associated river tributaries in the greater Victoria system (Van Oijen *et al.* 1991). Females are beige colored throughout. Males range from beige with dark markings to bluish-black with pinkish to red tips on all fins. Dark vertical bars may or may not be visible. Ocelli are present in the anal fin. The maximum reported size is 9 cm. SL (Lippitsch 2003). This species inhabits shallow inshore areas, and are omnivorous non-specialized feeders with plants and invertebrates making up part of their diet (Greenwood 1981; Seehausen 1996).

Haplochromis latifasciatus (Regan 1929) is a mouthbrooding cichlid endemic to Lake Kyoga and Lake Nawampasa (belonging to the greater Victoria system), and little is known about the habitat and ecology of the species as it is critically endangered in the wild (Van Oijen *et al.* 1991; Kaufman 1996). They range from beige to yellowish green with dark vertical bars that extend onto dorsal fins. Males are more colorful than females and can have regions of orange to bright red along sides, breast, and abdomen. Ocelli are present in the anal fin. The

maximum reported size is 11 cm. total length (TL). Although endangered in the wild, tank-raised specimens of this species are readily available for purchase through the aquarium trade.

Amatitlania nigrofasciata (Günther 1867), the convict cichlid, is a substrate spawning cichlid native to El Salvador, Guatemala, and Honduras (Schmitter-Soto 2007). They range in color from gray to blue with 8 or 9 dark vertical bars, many of which extend onto dorsal fins. Mature females are more colorful than males and have regions of pink to orange coloration in the ventral area and on the dorsal fin. The maximum reported size is 10 cm. SL (Kullander 2003). They inhabit the fast moving water areas of creeks, streams, and rivers, and prefer small cracks and crevices amongst the rocks (Conkel 1993).

Developmental Comparison of Parasite to Hosts

The cichlid and cuckoo catfish host-parasite system most resembles avian brood parasitism, and although they share many similarities there are also some unique differences. The cuckoo catfish travel in large shoals in Lake Tanganyika, and when cichlid breeding is detected, a female catfish will lead one to several males into the cichlid spawning area. The catfish will then interrupt the cichlid breeding sequence and breed simultaneously with the cichlids (Fig. 1.7). Unlike birds that can be parasitized for days to as long as a week (Ortega 1998), the cichlids only spawn for a couple of hours or less (Cruz *et al.* 2004). Therefore, it is crucial that the catfish are capable of finding cichlids during this timeframe, and that they have the ability to lay their eggs in synchrony with the cichlids. The cuckoo catfish appears to be a fractional egg spawner capable of releasing small quantities of eggs over periods of days to weeks, which would explain how they are able to accomplish coordinated spawning with cichlids (pers. obs, and unpublished data in Wisenden 1999). The catfish eat some of the cichlid eggs and lay their own eggs, which will in turn be inadvertently picked up by the female cichlid mouthbrooder. In the case of the

cuckoo catfish, the female cichlid picks up the catfish eggs, despite them being smaller in size and different in appearance (Fig. 1.8a). Cichlid hosts are egg acceptors and will pick up anything that looks like an egg (e.g., small rocks). Conversely, host birds of brood parasites can be either egg acceptor species or egg rejector species when exposed to brood parasitism (Ortega 1998). The host cichlid will then brood the catfish embryos in their buccal cavity together with the host embryos, until 5 dpf (days post fertilization) at 26°C, when the cichlids begin to hatch and the catfish exhaust their yolk sacs as a source of nutrition (Figs. 1.8b and 1.9). By 6dpf, the cichlids are all hatched and the catfish will begin to devour the host fry while still in the host's mouth (Figs. 1.8c and 1.9). Therefore, the cichlid host is providing both protection and a food resource for the young catfish (Cruz *et al.* 2004). The timing of cichlid hatching is important because cuckoo catfish fry appear to be unable to grasp and eat the cichlid larvae until they have hatched (pers. obs.). As is the case with cuckoo bird nestlings (Davies 2000), the young cuckoo catfish usually eliminate the entire host progeny (pers. obs.).

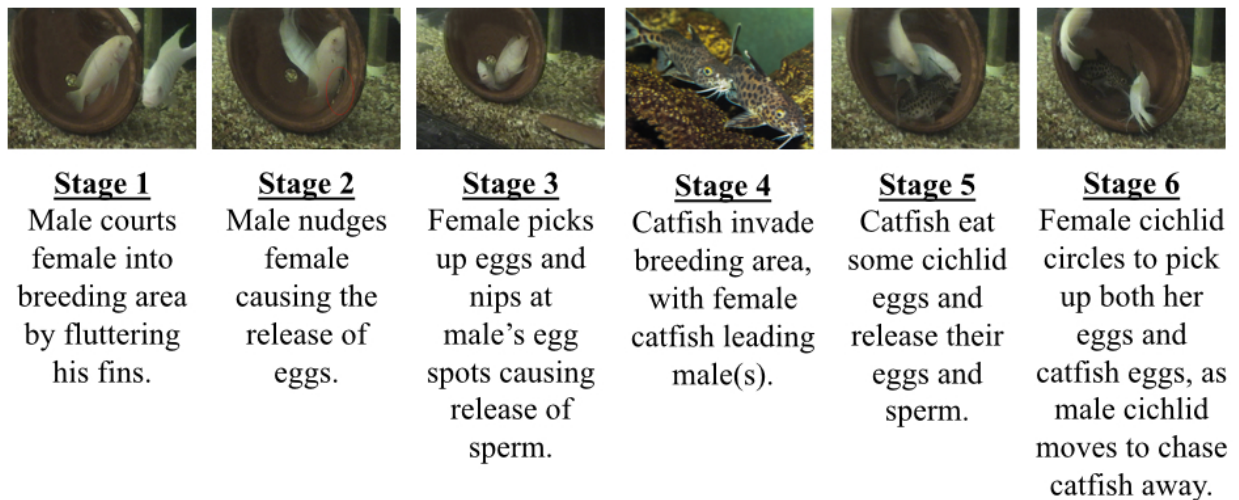


Fig. 1.7. Spawning sequence and parasitism of *M. zebra* cichlid by the cuckoo catfish (*S. multipunctatus*). Stages 1-3 illustrate the typical spawning sequence of *M. zebra*, while stages 4-6 show the interruption of this normal cichlid spawning sequence and subsequent parasitism by the cuckoo catfish (*S. multipunctatus*).

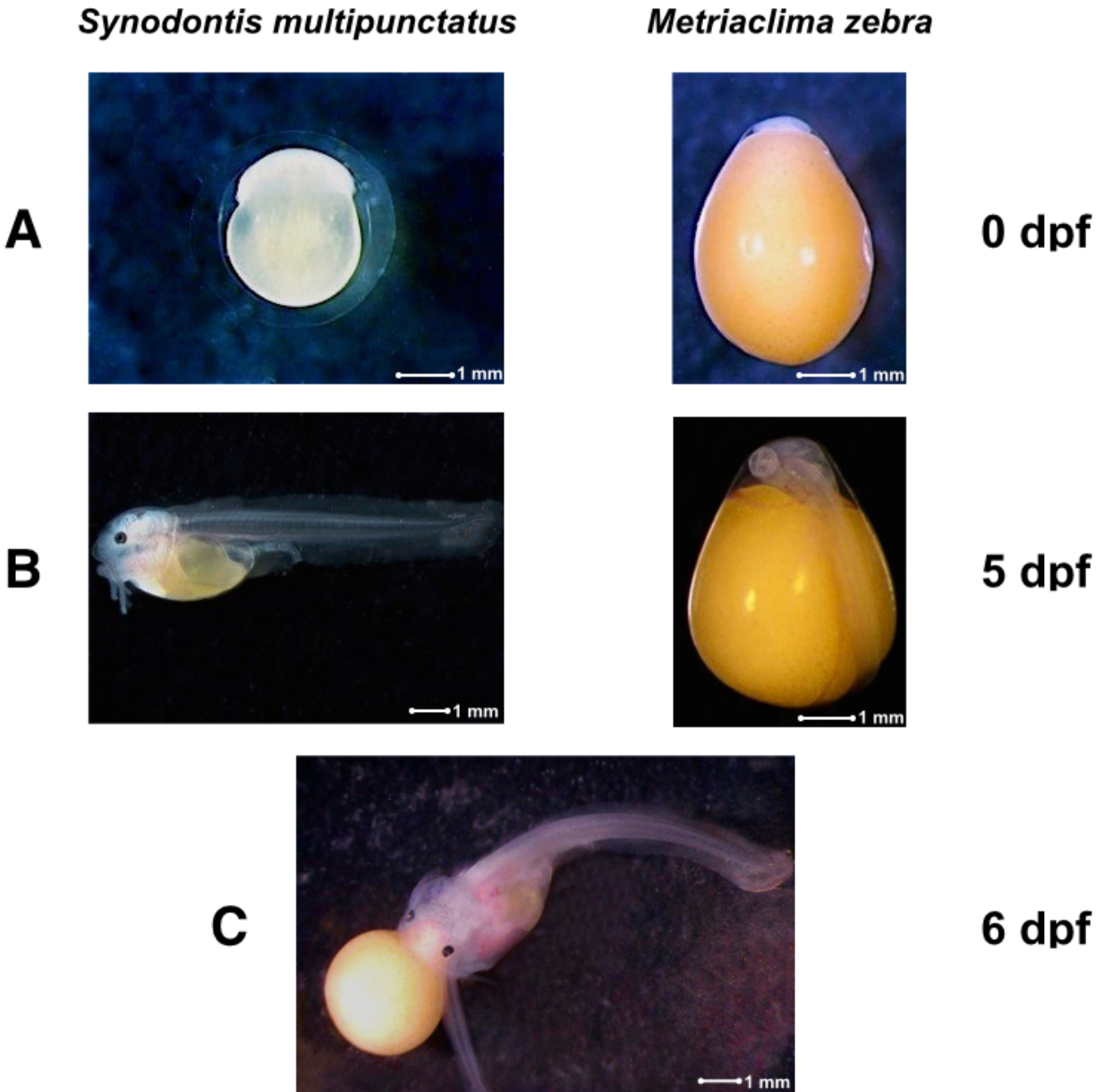


Fig. 1.8. The top two images (A) are of embryos taken shortly after fertilization, and illustrate the differences in appearance of eggs from the cuckoo catfish (*S. multipunctatus*) and *M. zebra* cichlid. The middle two images (B) represent 5-6 dpf, at which point the cichlid will hatch and the catfish will exhaust their yolk as a nutrition source and begin to feed exogenously. The bottom image (C) is a 6dpf cuckoo catfish (*S. multipunctatus*) consuming a newly hatched *M. zebra* cichlid larva. All fish were maintained at 26°C.

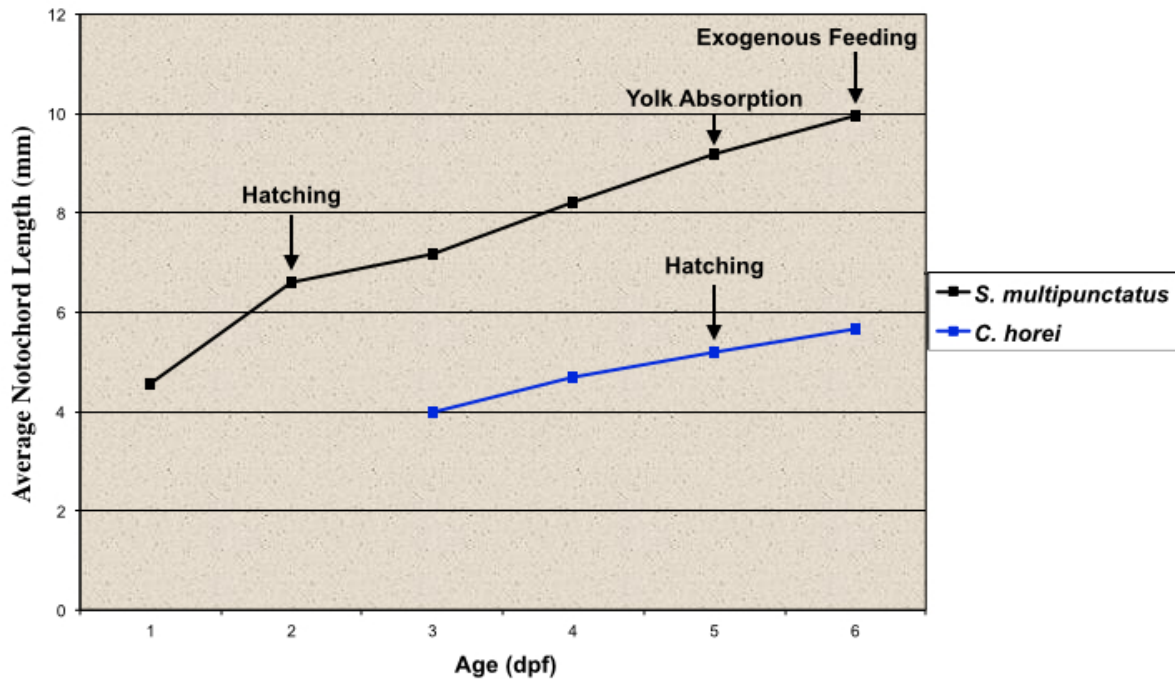


Fig. 1.9. Growth curves of the cuckoo catfish (*S. multipunctatus*) (black, $n = 19$) and *C. horei* cichlid (blue, $n = 52$) from 1 dpf to 6 dpf. Arrows indicate the timing of key life history events. Note how cichlid hatching occurs at the same time as yolk absorption by the cuckoo catfish (*S. multipunctatus*). Cichlid yolk absorption and exogenous feeding occur by about 21 dpf. All fish were maintained at 26°C.

Developmental Comparison of Parasite to a Congener

Methods and more detailed results for this section can be found in an honors thesis by Hawkins (2008). There are approximately 120 described species of *Synodontis*, with as many as ten species endemic to Lake Tanganyika (Poll 1971; Day and Wilkinson 2006; Friel and Vigliotta 2006; Koblmüller *et al.* 2006; Wright and Page 2006). While the non-parasitic congeners are all egg scatterers, the cuckoo catfish is the only species exhibiting obligate brood parasitic behavior. The cuckoo catfish may possess adaptations that their non-parasitic congeners lack, which allows for successful brood parasitism of cichlids.

Molecular studies of endemic *Synodontis* in Lake Tanganyika have placed the cuckoo catfish and their closest sister species (*S. granulatus*) in one clade, and the remaining species in a second clade (Day and Wilkinson 2006; Koblmüller *et al.* 2006). One species in the second clade

is *S. lucipinnis*. They are a good candidate species to use for comparison to the cuckoo catfish since they are sympatric, have very similar adult feeding habits, and they readily spawn in aquariums.

A comparison of juvenile *S. lucipinnis* and the cuckoo catfish reveals that there is a difference between the shape of their head skeletons, whereby the cuckoo catfish has a much more robust oral morphology than *S. lucipinnis* (Figs. 1.10 and 1.11). These differences in craniofacial morphology may be related to diet. Food resources acting as the selective agents on feeding morphology have been shown in many vertebrates, from Darwin's finches (Grant and Grant 1997) to East African cichlids (Albertson *et al.* 2003a; Albertson *et al.* 2003b; Streelman and Danley 2003). *S. lucipinnis* have variable diets consisting of algae and small invertebrates from 6 dpf on, while at about two to three weeks of age the cuckoo catfish leaves the cichlid's mouth and switch their feeding from cichlid fry to small invertebrates and specialized feeding on gastropods (*Neothauma tanganyicense*) for the remainder of their lives (Poll 1953; Coulter 1991). Also, when examining the appearance of mineralized structures (from 5dpf to 12 dpf) it should be noted that the cuckoo catfish has more teeth at all ages, a wider range of teeth on the dentary, and has a greater number of teeth on the dentary compared to the premaxilla than *S. lucipinnis* does (Table 1.1 and Fig. 1.12). These findings suggest that differences in oral morphology may help enable the cuckoo catfish to consume cichlid young and what prevents non-parasitic congeners from doing so.

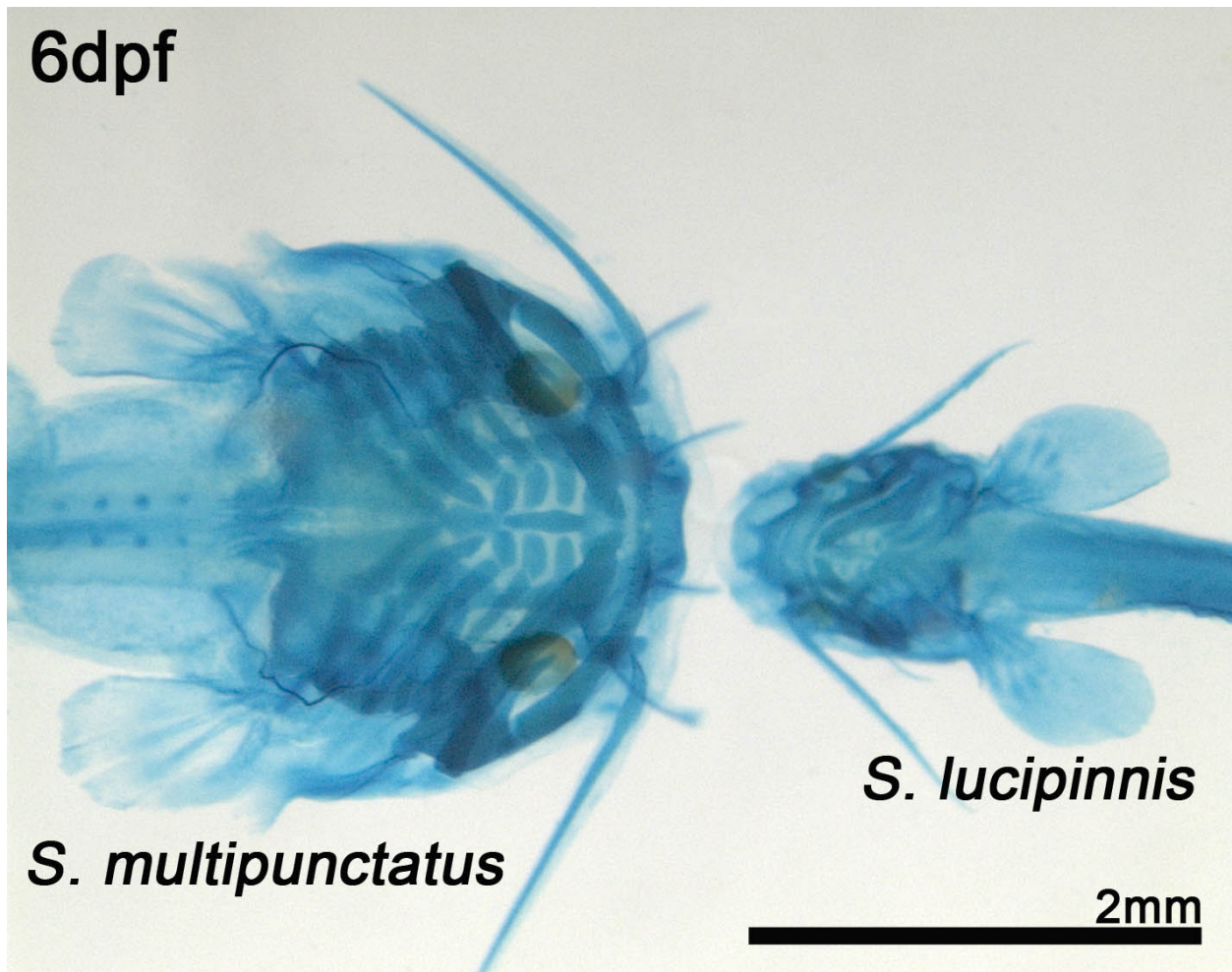


Fig. 1.10. The cuckoo catfish (*S. multipunctatus*) and the congeneric *S. lucipinnis* at 6 dpf cleared and stained with Alcian Green.

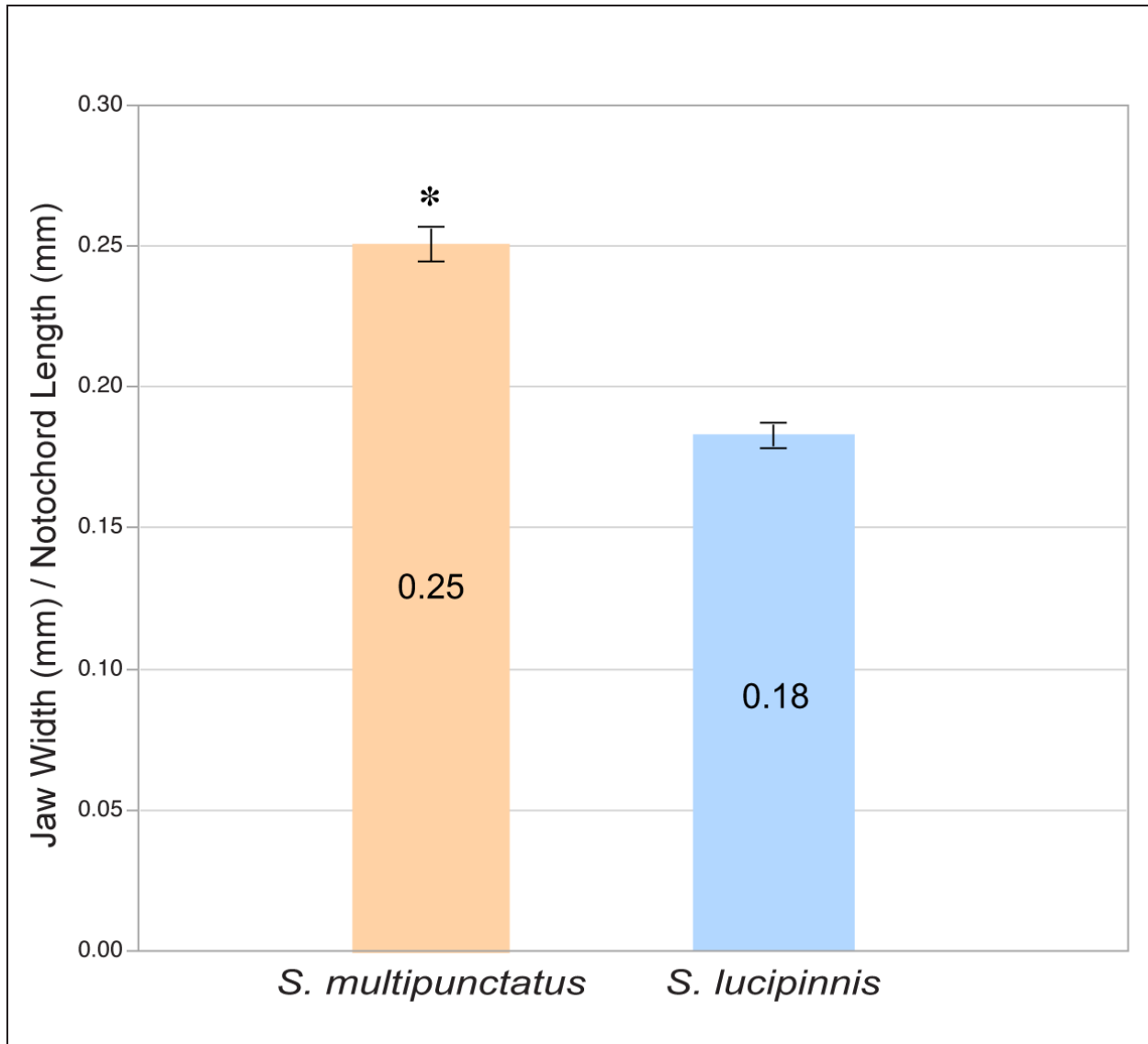


Fig. 1.11. Jaw width (mm) to notochord length (mm) ratio comparison between the cuckoo catfish (*S. multipunctatus*) and congeneric *S. lucipinnis* at 6 dpf. The cuckoo catfish (*S. multipunctatus*) has a significantly larger relative jaw width than the congeneric *S. lucipinnis* (indicated by *, *t*-test assuming equal variances, $t(11) = 11.13$, $p < 0.0001$). Error bars indicate one standard error of the mean.

Table 1.1. Summary of the pattern of mineralized teeth in larval cuckoo catfish (*S. multipunctatus*) and the congeneric *S. lucipinnis* from 5 dpf to 12 dpf. PMAX = premaxillary teeth; DENT = dentary teeth.

days post fertilization (dpf)	<i>S. multipunctatus</i>	<i>S. lucipinnis</i>
5	24 oral teeth (10 PMAX, 14 DENT)	8 oral teeth (8 PMAX, 8 DENT)
6	40 oral teeth (16 PMAX, 24 DENT)	14 oral teeth (8 PMAX, 6 DENT)
7	44 oral teeth (16 PMAX, 28 DENT)	24 oral teeth (12 PMAX, 12 DENT)
8	64 oral teeth (24 PMAX, 40 DENT)	24 oral teeth (12 PMAX, 12 DENT)
9	68 oral teeth (28 PMAX, 40 DENT)	28 oral teeth (14 PMAX, 14 DENT)
10	72 oral teeth (32 PMAX, 40 DENT)	32 oral teeth (18 PMAX, 14 DENT)
11	90 oral teeth (40 PMAX, 50 DENT)	No Data
12	90 oral teeth (40 PMAX, 50 DENT)	40 oral teeth (20 PMAX, 20 DENT)

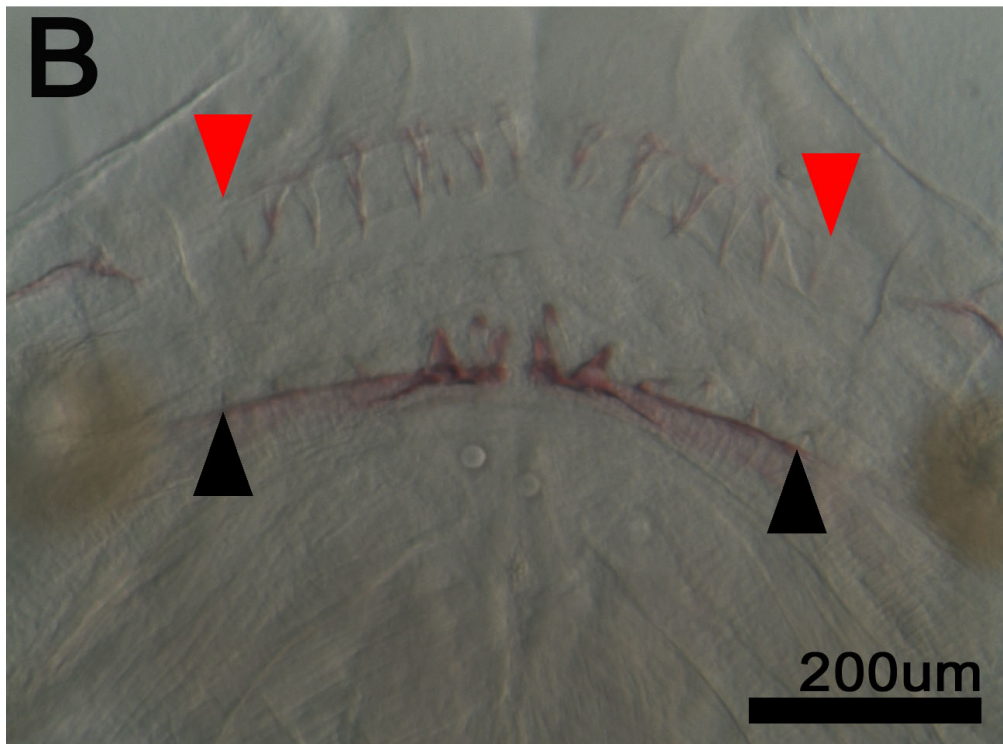
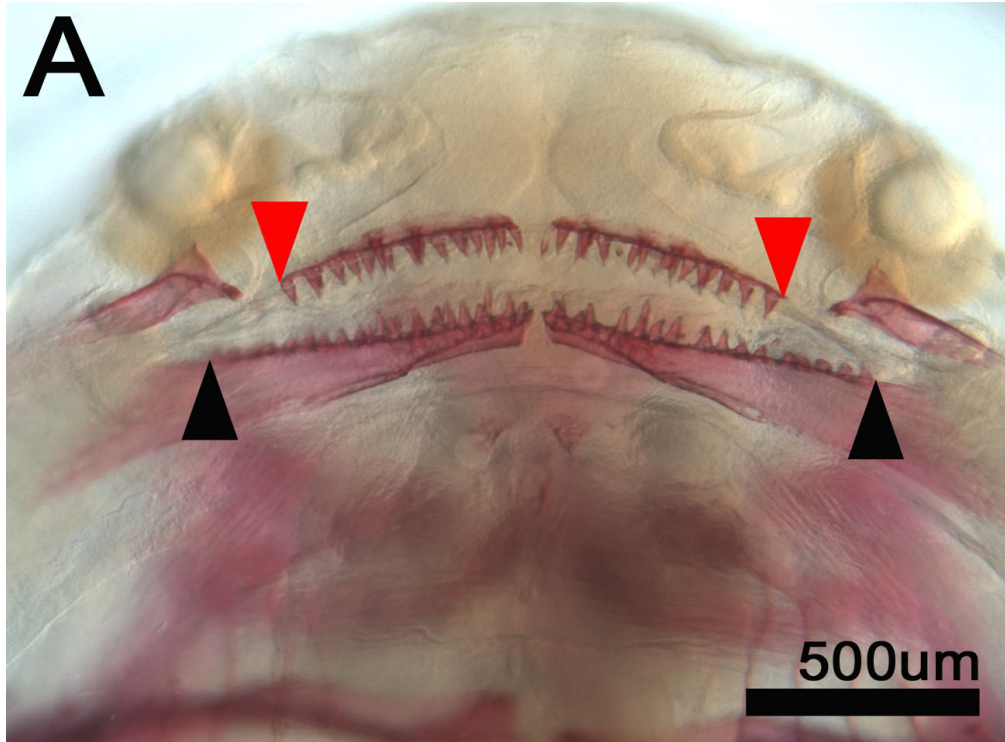


Fig. 1.12. The oral teeth of 12 dpf (A) cuckoo catfish (*S. multipunctatus*) and (B) *S. lucipinnis*. In both images red arrowheads indicate the lateral limit of teeth on the upper jaw (premaxilla) while black arrowheads indicate the lateral limit of teeth on the lower jaw (dentary).

Another major difference between the two congeneric catfish species is that the cuckoo catfish is 50% longer at 6 dpf than *S. lucipinnis*, and *S. lucipinnis* may simply not be large enough to grasp the cichlid larvae and consume them (Fig. 1.13). Beyond this size disparity, we found no major early ontogenic differences in the timing of crucial life history stages that would help explain why non-parasitic *Synodontis* do not parasitize cichlids. Although *S. lucipinnis* hatch a day earlier than the cuckoo catfish, the timing of yolk absorption and exogenous feeding are the same in both species. So, *S. lucipinnis* would be exogenously feeding at the correct time to coincide with cichlid hatching. Understanding any differences in ontogeny and odontogenesis present between these two species may help to better understand how obligate brood parasitism evolved in the cuckoo catfish.

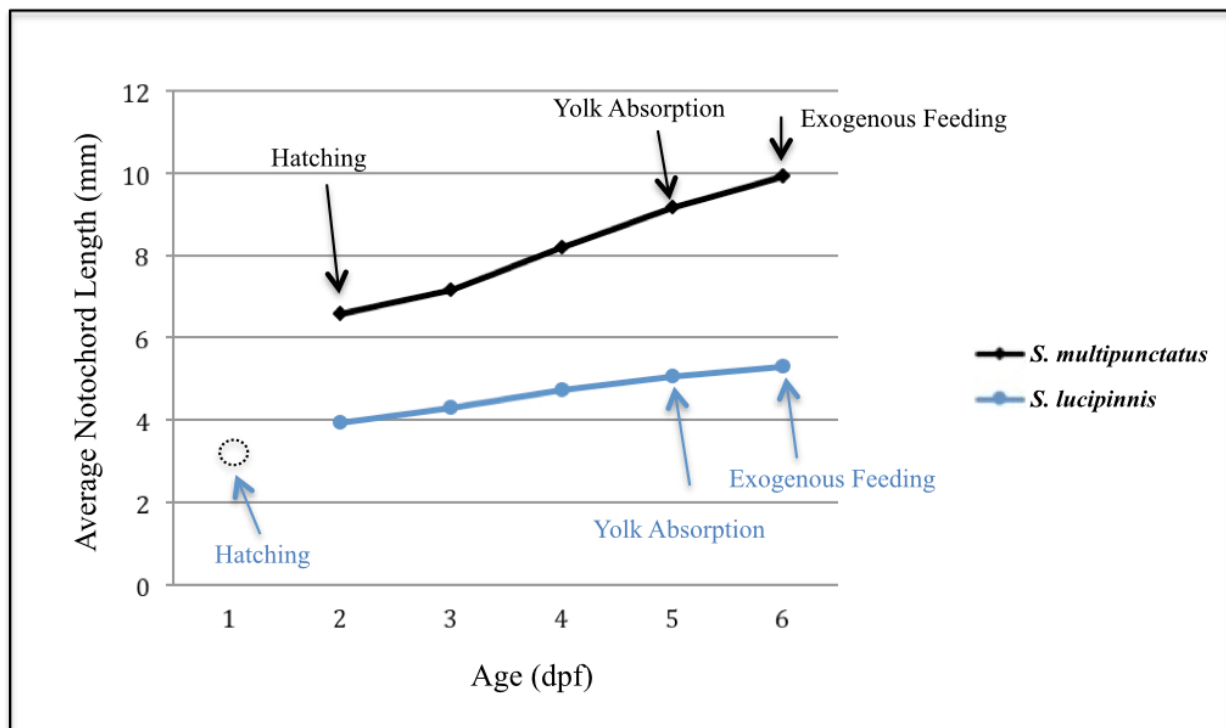


Fig. 1.13. Growth curves of the cuckoo catfish (*S. multipunctatus*) (black, n = 19) and congeneric *S. lucipinnis* (blue, n = 50) 2 dpf-6 dpf. Hatching, yolk absorption, and the onset of exogenous feeding are noted with black arrows on the graph. Hatching of *S. lucipinnis* occurs around 1 dpf, but no NL measurements at this stage were obtained. Therefore, placement of the dashed circle for *S. lucipinnis* hatching is for illustration purposes only. All fish were maintained at 26°C.

Chapter 2

Sympatric cichlid hosts of the Lake Tanganyikan *Synodontis multipunctatus* (cuckoo catfish) are parasitized less than allopatric hosts from Lake Malawi and the greater Lake Victoria system.

Abstract

The only known non-avian vertebrate obligate brood parasite is the cuckoo catfish (*Synodontis multipunctatus*), a Tanganyikan endemic. The cuckoo catfish parasitizes mouthbrooding cichlids, and in nature can only parasitize sympatric cichlids from Lake Tanganyika; however, under laboratory conditions, cuckoo catfish will parasitize mouthbrooding cichlids from other Rift Valley lakes. Little is known about the cuckoo catfish beyond the basics about their breeding biology. In the present study I examine the frequency of parasitism by the cuckoo catfish in both sympatric (from Lake Tanganyika) and allopatric (from Lake Malawi and the greater Lake Victoria system) host cichlid species in a laboratory setting. *Ctenochromis horei*, a natural sympatric host species of the cuckoo catfish, was parasitized significantly less than the allopatric species were. The lower rates of parasitism may be due to subtle differences in the mating ritual and oviposition, or behavioral adaptations (e.g., increased aggression towards the cuckoo catfish) on the part of sympatric hosts. *Metriaclima zebra* parasitism rates and number of catfish per brood were higher than all other species, and may be due to the use of an albino morphotype of this species for the study. The number of catfish per parasitized brood was similar for all other host species. My results are comparable to findings from the field for *C. horei* parasitism frequency and number of cuckoo catfish per brood. Cuckoo catfish and mouthbrooding cichlids provide a model system for rigorously testing brood parasitism in a laboratory setting.

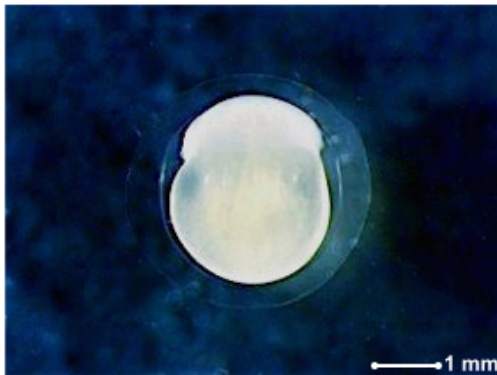
Introduction

The lakes of the East African Rift Valley contain an incredibly rich fish fauna. Cichlids (Cichlidae) in particular exhibit a diverse range of colors, morphology, and feeding methods. As many as 2000 species of cichlids have evolved within the last 10 million years, resulting in one of the most dramatic known cases of vertebrate speciation (Barlow 2000; Danley and Kocher 2001; Lande *et al.* 2001; Kocher 2004; Sturmbauer *et al.* 2011), and over 95% of the cichlids in the three major Rift Valley lakes (Tanganyika, Malawi, and Victoria) endemic to an individual lake (Snoeks 2000). In Rift Valley cichlids, maternal mouthbrooding as a reproductive strategy is well represented. Mouthbrooding involves the female cichlid picking up recently oviposited eggs in her mouth, where they are brooded in the buccal (oral) cavity until free swimming (Keenleyside 1991; Barlow 2000). An obligate brood parasitic catfish (*Synodontis multipunctatus*, the cuckoo catfish, Siluriformes: Mochokidae) has been documented in Lake Tanganyika to use female mouthbrooding cichlids (Perciformes: Cichlidae) as hosts (Finley 1984; Sato 1986; Cruz *et al.* 2004).

Brood parasitism has been studied extensively in insects and birds, with the majority of research focusing on avian obligate brood parasites (completely relying upon a host for successful breeding), as is the case for cuckoos and cowbirds (e.g., Ortega 1998; Davies 2000). Unlike the cuckoo catfish, other fish species are facultative brood parasites where they may resort to parasitic behavior, but do not rely on the host for successful breeding (Sato 1986; Wisenden 1999; Yom-Tov and Geffen 2006). The cuckoo catfish enters the cichlid spawning area and they breed in synchrony with the cichlids, laying and fertilizing their eggs for the female mouthbrooder to pick up. The female cichlid picks up the catfish eggs, even though they are smaller in size and are different in appearance (Fig. 2.1). The catfish embryos are then

brooded in the buccal cavity of the host together with the host embryos, but hatch earlier. The catfish grow faster, and after they have exhausted their yolk sacs as a nutrition source, they begin to devour the host fry while still in the host's mouth (Fig. 2.2). Thus, the catfish require their host for protection and to provide its first exogenous food source during the earliest stages of development (Sato 1986; Cruz *et al.* 2004).

Synodontis multipunctatus



Metriaclima zebra



0 dpf

Fig. 2.1. The differences in appearance between the cuckoo catfish (*S. multipunctatus*) and *M. zebra* cichlid eggs. These images are of embryos taken shortly after fertilization.



Fig. 2.2. 6dpf cuckoo catfish (*S. multipunctatus*) consuming a newly hatched *M. zebra* cichlid larva.

There are approximately 120 species of *Synodontis* catfishes recognized (Poll 1971; Friel and Vigliotta 2006; Koblmüller *et al.* 2006; Wright and Page 2006), and while the non-parasitic congeners are all egg scatterers, the cuckoo catfish is the only species known to exhibit brood parasitic behavior. Furthermore, the cuckoo catfish is the only known non-avian obligate brood parasite among vertebrates (Sato 1986; Cruz *et al.* 2004). This is an important finding as it provides a model system to study brood parasitism under controlled laboratory conditions, where questions about parasitism dynamics can be addressed. Because selection is both spatially and temporally focused in the breeding area, factors affecting host-parasite interactions are easily discernible (Rothstein 1990). The cuckoo catfish is endemic to Lake Tanganyika, and in nature can only parasitize mouthbrooding cichlids found in that lake. However, under laboratory conditions, cuckoo catfish will parasitize mouthbrooding cichlids from other Rift Valley lakes and have even been observed parasitizing *Geophagus steindachneri*, a South American

mouthbrooding cichlid species (Loiselle 1998). Because of these close parasite-host interactions, the catfish-cichlid system of brood parasitism can help address pressing ecological and coevolutionary questions, particularly regarding how selection pressures and feedbacks influence reproductive behaviors. For example, brood parasitic birds (e.g., cowbirds or cuckoos) can reduce host fitness by egg removal or competition between host and parasite young, whereas hosts can reduce the parasite's fitness by recognizing and rejecting the eggs of the parasite or increased aggression towards the parasite (Rothstein 1975a; Payne 1977; Rothstein 1990; Duckworth 1991; Fossøy *et al.* 2011). This results in an evolutionary arms race between host and parasite (Dawkins and Krebs 1979; Davies *et al.* 1989; Rothstein 1990). Although studied extensively in insects and birds, little is known about the subtleties of the host-parasite relationship in brood parasitic fishes. As is the case with cuckoo bird host nestlings (Davies 2000), the cuckoo catfish usually eliminates the entire host progeny (pers. obs), making it extremely costly for parasitized cichlids. The selection pressures exerted by parasitism from cuckoo catfish should favor counter adaptations in host species. Mouthbrooding cichlids have very complex and ritualistic breeding sequences, and they will pick up their eggs so quickly that the male will fertilize the eggs after they are in the mouth of the female (Fryer and Iles 1972). Because the cichlids pick up the eggs quickly, adaptations against brood parasitism would likely not involve behaviors like egg rejecting as seen in birds. Therefore, cichlids may have behavioral adaptations to combat parasitism (e.g., behaviors related to courtship and oviposition, or aggression towards the parasite). Sympatric host species have coevolved with the cuckoo catfish, and should have developed adaptations against brood parasitism, whereas host species that have never been exposed to the cuckoo catfish should not have adaptations to combat parasitism.

The basic breeding mechanism of this host-parasite system has been well documented through a series of observational studies. Brichard (1979) was the first to observe cuckoo catfish fry in the mouths of captured Tanganyikan cichlids, and thought they got there accidentally. There were also descriptions offered from aquarists (Ferguson 1983; Finley 1983), until a field study by Sato (1986) confirmed that the cuckoo catfish was an obligate brood parasite of Lake Tanganyikan cichlids. Sato examined differences between host species with regard to percentage of cichlid broods parasitized and number of catfish per brood. In addition, Wisenden (1999) mentioned unpublished parasitism rates observed in aquariums of cichlid species from Lake Victoria and Lake Malawi, while Cruz et al. (2004) described the breeding biology and ontogeny of the host species and the catfish. However, there have been few manipulative experiments focused on addressing host-parasite interactions in this system. Here I examine the frequency of parasitism by the cuckoo catfish in both sympatric (from Lake Tanganyika) and allopatric (from Lake Malawi and the greater Lake Victoria system) host cichlid species in a laboratory setting. Because sympatric hosts have likely coevolved adaptations to mitigate parasitism by the cuckoo catfish (e.g., increased aggression towards catfish), I predicted that sympatric host species would be parasitized less frequently than allopatric host species were.

Materials and Methods

Sympatric Study Species (Fig. 2.3.)

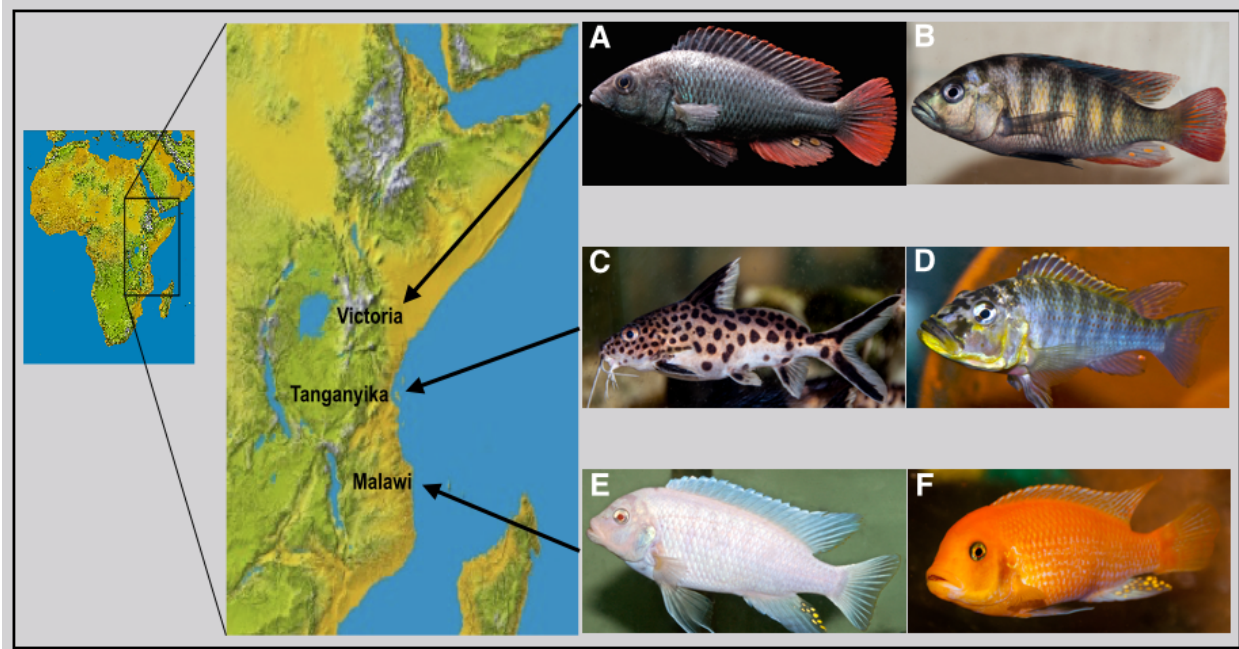


Fig. 2.3. Five African cichlid host species: (A) *H. nubilus*, (B) *H. latifasciatus*, (D) *C. horei*, (E) *M. zebra*, and (F) *M. estherae*. (C) The brood parasitic cuckoo catfish (*S. multipunctatus*). Black arrows indicate the Rift Valley lakes where the natural populations of these species are found and fish from the same area are organized by row (background map images source: NASA/JPL/NIMA).

Synodontis multipunctatus (Boulenger 1898), the cuckoo catfish, is a brood parasitic catfish in the Mochokidae family endemic to Lake Tanganyika in Africa. This catfish is a generalist brood parasite on different species of mouthbrooding cichlids, and can be distinguished from similar looking congeners by a humeral process that is narrow posteriorly (Eccles 1992). Wild-caught specimens were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for experimentation along with their F1 offspring that were bred in the lab.

Ctenochromis horei (Günther 1894) is a mouthbrooding cichlid endemic to Lake Tanganyika and surrounding river tributaries, and it is a naturally occurring host of the cuckoo catfish (Sato 1986). Wild-caught specimens were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for experimentation along with their F1 and F2

offspring that I bred in the lab. Data were collected during 2006 and 2007.

Allopatric Study Species (Fig. 2.3.)

Metriaclima zebra (Boulenger 1899), the zebra cichlid, is a mouthbrooding cichlid endemic to Lake Malawi. The albino morphs were used in this study, as aquarists have reported using them successfully to rear cuckoo catfish. Tank-raised specimens were purchased locally from PetSmart, and used for experimentation along with their offspring that I bred in the lab. Data were collected during 2006.

Metriaclima estherae (Konings 1995), the red zebra cichlid, is a mouthbrooding cichlid endemic to Lake Malawi. Tank-raised specimens were purchased from Atlantis Tropical Fish Hatchery (<http://www.cichlids.net>), and used for experimentation along with their offspring that I bred in the lab. Data were collected from 2011 to 2013.

Haplochromis nubilus (Boulenger 1906) is a mouthbrooding cichlid endemic to not only Lake Victoria, but also surrounding smaller lakes and associated river tributaries in the greater Victoria system (Van Oijen *et al.* 1991). Tank-raised specimens were purchased locally from Golden Fish Aquarium (<http://www.goldenfishaquarium.com>), and used for experimentation along with their offspring that were bred in the lab. Data were collected from 2000 to 2002, and during 2006.

Haplochromis latifasciatus (Regan 1929) is a mouthbrooding cichlid endemic to Lake Kyoga and Lake Nawampasa (belonging to the greater Victoria system), and little is known about the habitat and ecology of the species as it is critically endangered in the wild (Kaufman 1996). Although endangered in the wild, tank-raised specimens of this species are readily available for purchase through the aquarium trade. The tank-raised specimens for this study were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for

experimentation along with their offspring that I bred in the lab. Data were collected from 2011 to 2012.

Fish Husbandry and Experimental Design

Male and female cuckoo catfish were maintained with breeding populations of adult African cichlid species from Lake Malawi (*M. zebra* and *M. estherae*), Lake Tanganyika (*C. horei*), and the greater Lake Victoria system (*H. nubilus* and *H. latifasciatus*). These populations were housed in 110 liter (77 x 32 x 47 cm), 208 liter (122 x 32 x 53 cm), 284 liter (122 x 47 x 53 cm), and 473 liter (184 x 47 x 59 cm) aquariums. The fish were maintained at 24-26°C in a 14:10 light:dark cycle and were fed body size proportional amounts of food daily in the mid-afternoon. Catfish were fed a combination of frozen chironomid larvae and *Artemia* shrimp pellets daily, while cichlids were fed cichlid pellets or cichlid flake foods. Fifty percent water changes were conducted every other week. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was added to the tap water to remove chlorine after the tap water was added to tanks. A pH of 8.0-9.0 was maintained in the tanks by using crushed coral as the tank substrate and then buffering the pH and hardness up with a buffer mixture made from 1:1:1 sodium bicarbonate (NaHCO_3) to Epsom salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) to Instant Ocean[®] sea salt after water changes. The buffer was added in small dosages to aquariums until a dKH of 10-14 was reached, and dKH levels were monitored using a KH carbonate hardness test kit from API[®]. Terracotta pots were placed in the aquariums and served as territorial display and spawning areas for cichlids.

Breeding colonies of the cuckoo catfish were established with either sympatric hosts or allopatric hosts described above. The total number of fish in each tank varied based on the size of the aquarium used. Because the host species are polygynous, the cichlids were maintained at 3:1

(female to male), while the catfish were 1:1 (female to male). However the ratio of host to parasite was 2:1 (cichlids to catfish), as I found these ratios produce good breeding results.

Aquariums were monitored daily for female cichlids carrying eggs, which can be identified by their distended buccal (oral) cavity. Following identification, I carefully removed the carrying females from the tank and recorded their standard length. I obtained eggs by gently holding the female's mouth open in a small volume of tank water and irrigating the oral cavity with a transfer pipette to dislodge the eggs from the mouth. The number of cichlid eggs and catfish eggs, if any, were then recorded. I obtained 100 broods from one cichlid species from Lake Tanganyika (*C. horei*), and 100 broods each from two cichlid species from the greater Lake Victoria system (*H. nubilus* and *H. latifasciatus*) and Lake Malawi (*M. estherae* and *M. zebra*).

Statistical Analyses

I recorded the number of parasitized broods and unparasitized broods and calculated the mean number of catfish eggs per parasitized cichlid brood for the five host species. All statistical test were done using JMP[®] Pro 11 (64-bit). To examine parasitism frequency differences between sympatric and allopatric hosts, I performed an exact one-sided binomial test using the hypothesized test probability set to the parasitism percentage of the sympatric host. I compared the mean number of catfish per parasitized brood between host species using a Wilcoxon/Kruskal-Wallis one-way analysis of variance followed by post-hoc nonparametric comparisons for all pairs using the Steel-Dwass method. I used nonparametric statistics because my data did not meet the assumptions of parametric tests.

Results

Parasitism Rates

I examined 100 broods from each of the five host species and found 17 *C. horei* broods were parasitized by the cuckoo catfish, as compared to 26 *H. nubilus*, 33 *H. latifasciatus*, 25 *M. estherae*, and 46 *M. zebra* broods (Table 2.1). *C. horei* was parasitized significantly less (one-sided binomial exact test, $p < 0.05$) than all species except *H. nubilus*, which was marginally significant ($p = 0.06$), and *M. zebra* was parasitized at a significantly higher percentage than the other host species (one-sided binomial exact test, $p < 0.01$) (Fig. 2.4). When breaking down the parasitism rate by lake of origin, *C. horei* from Lake Tanganyika were parasitized significantly less than the two species from either Lake Malawi or greater Lake Victoria system (one-sided binomial exact test, $p < 0.01$) (Fig. 2.5). Furthermore, when compared to an average rate of parasitism for all of the allopatric species combined, *C. horei* was parasitized significantly less (one-sided binomial exact test, $p < 0.001$) (Fig. 2.6).

Table 2.1. Occurrence of the cuckoo catfish (*S. multipunctatus*) in 100 broods collected from five species of African mouthbrooding cichlids. Lakes of origin are denoted in parentheses.

Species	Parasitized Broods	Unparasitized Broods
<i>Ctenochromis horei</i> (Tanganyika)	17	83
<i>Haplochromis nubilus</i> (Victoria)	24	76
<i>Haplochromis latifasciatus</i> (Victoria)	33	67
<i>Metriaclima estherae</i> (Malawi)	25	75
<i>Metriaclima zebra</i> (Malawi)	46	54

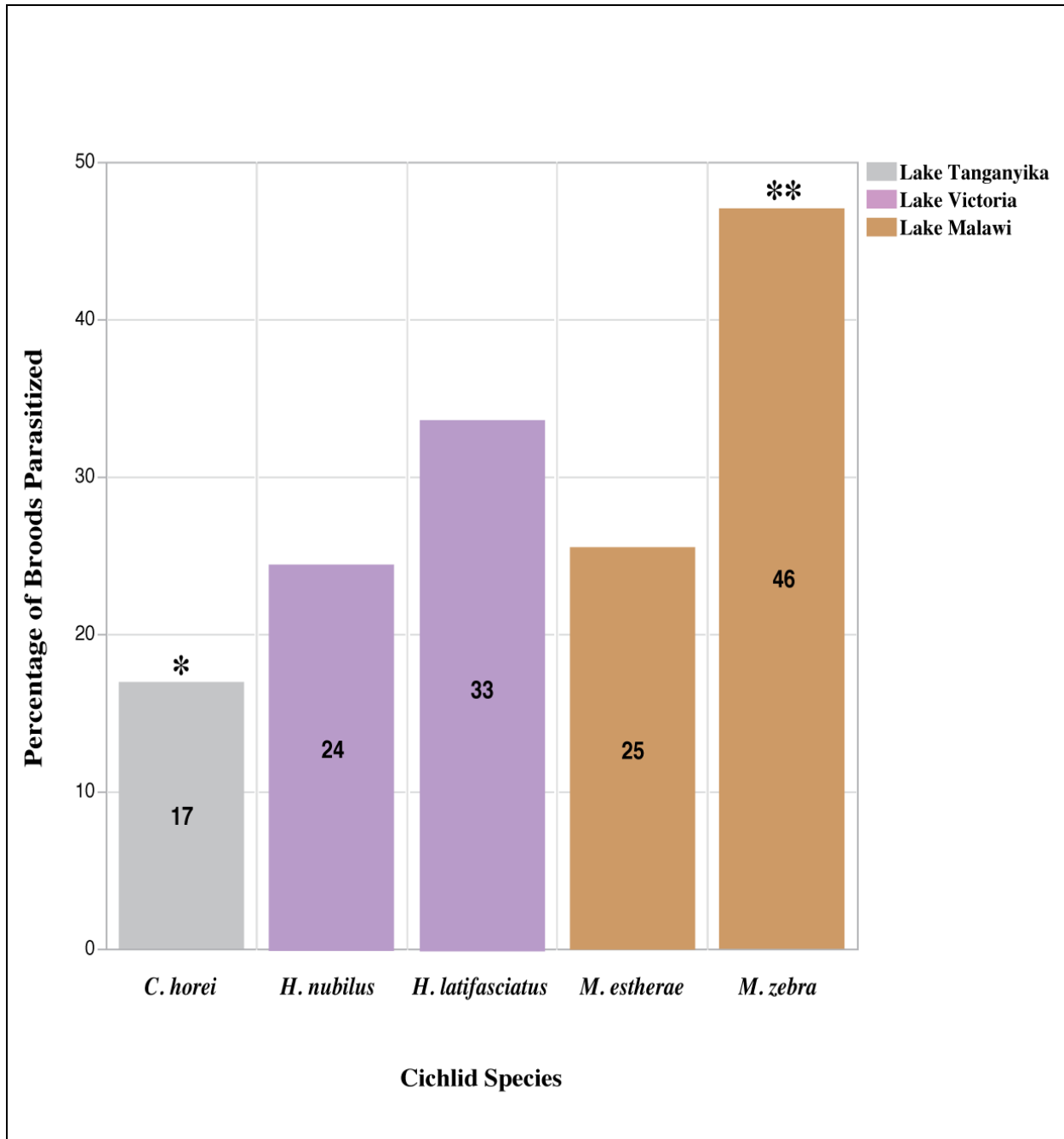


Fig. 2.4. Percentage of broods parasitized by the cuckoo catfish (*S. multipunctatus*) for five cichlid host species. *C. horei* was parasitized significantly less (indicated by *, one-sided binomial exact test, $p < 0.05$) than all species except *H. nubilus*, which was marginally significant ($p = 0.06$). *M. zebra* was parasitized significantly more (indicated by **, one-sided binomial exact test, $p < 0.01$) than all other species.

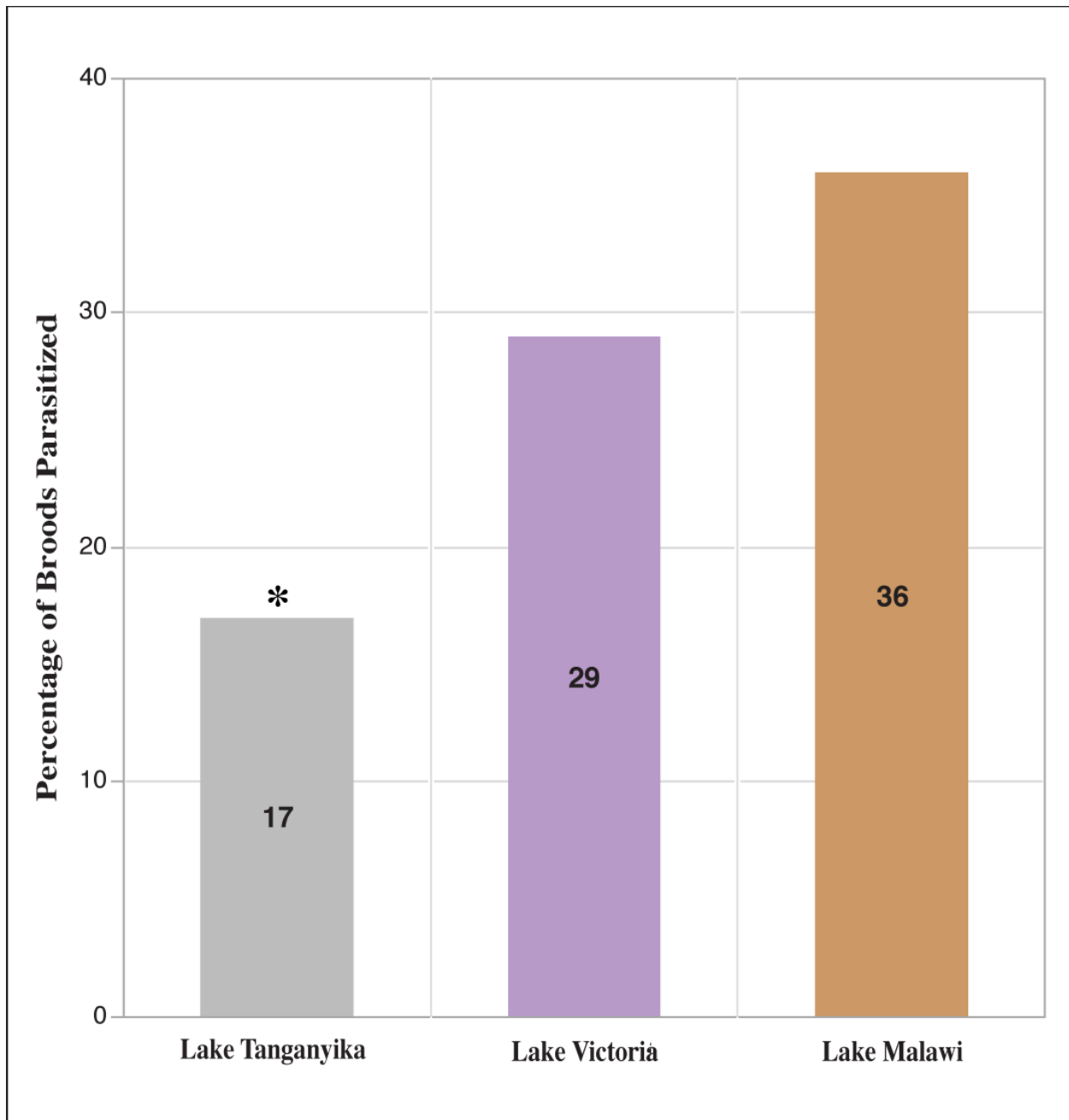


Fig. 2.5. Percentage of broods parasitized by the cuckoo catfish (*S. multipunctatus*) for cichlid host species from three Rift Valley lakes. *C. horei* from Lake Tanganyika was parasitized significantly less (indicated by *, one-sided binomial exact test, $p < 0.01$) than species from Lake Victoria and Lake Malawi.

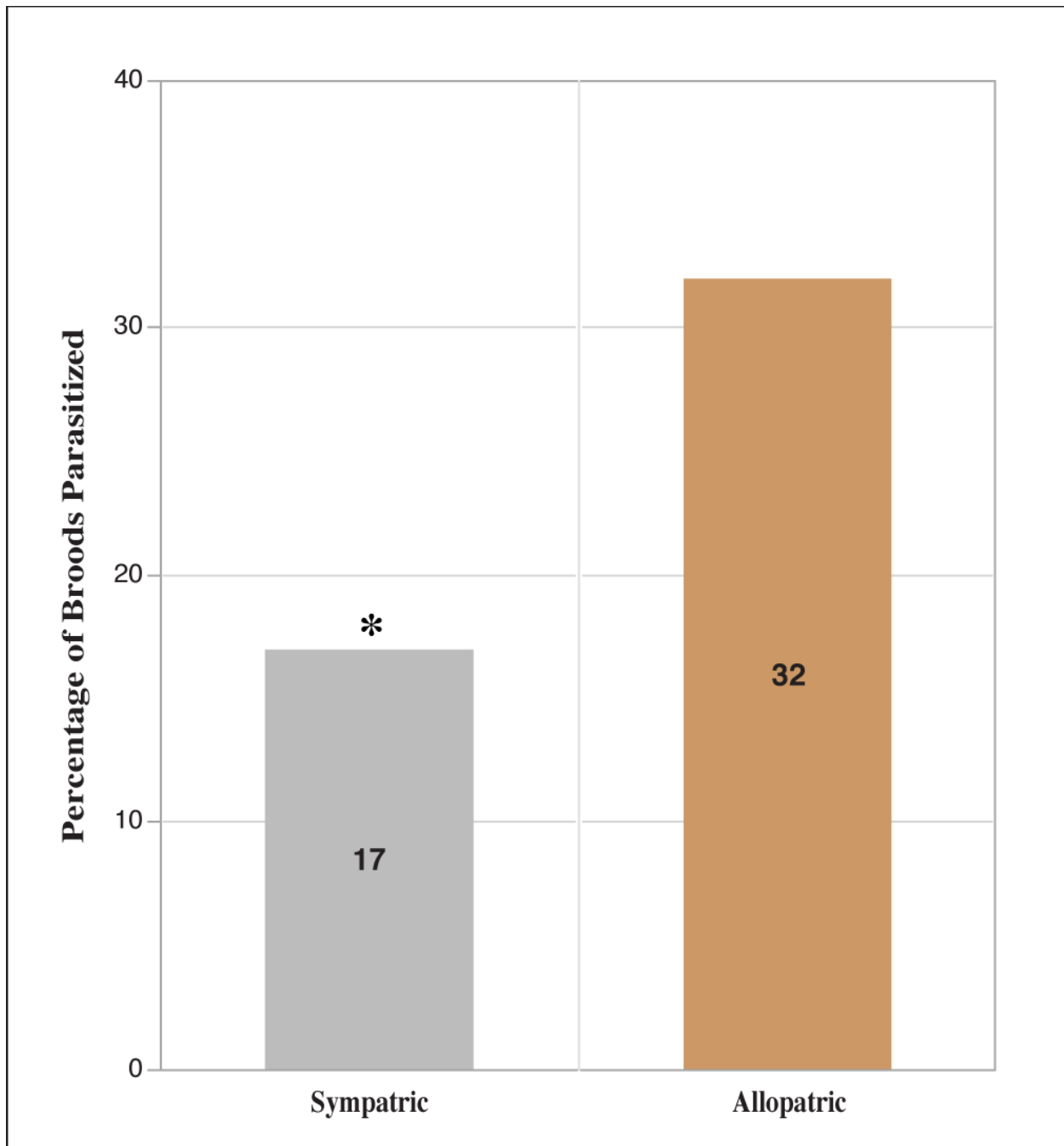


Fig. 2.6. Percentage of broods parasitized by the cuckoo catfish (*S. multipunctatus*) for sympatric and allopatric cichlid host species. *C. horei*, the sympatric host species, was parasitized significantly less (indicated by *, one-sided binomial exact test, $p < 0.001$) than allopatric host species.

Number of Catfish

I found 7.5 catfish per parasitized *C. horei* brood, 6.9 catfish per parasitized *H. nubilus* brood, 5.9 catfish per parasitized *H. latifasciatus* brood, 7.9 catfish per parasitized *M. estherae* brood, and 11.7 catfish per parasitized *M. zebra* brood (Table 2.2). A Wilcoxon/Kruskal-Wallis test showed that there was a significant difference in the number of catfish eggs per parasitized brood between the host species [$\chi^2(4) = 9.82, p < 0.05$] with a mean rank score of 87.0 for *M. zebra*, 74.5 for *M. estherae*, 71.5 for *C. horei*, 64.5 for *H. nubilus*, and 59.2 for *H. latifasciatus*. Post-hoc pairwise comparisons using the Steel-Dwass method indicated that there was a significant difference in the number of catfish eggs between *M. zebra* and *H. latifasciatus* ($p < 0.05, r = 0.31$) with a medium effect size, and that there were no significant differences between any other pairs.

Table 2.2. Average number of cuckoo catfish (*S. multipunctatus*) in 100 parasitized broods collected from five species of African mouthbrooding cichlids. Lakes of origin are denoted in parentheses.

Species	Mean # Catfish per Parasitized Brood
<i>Ctenochromis horei</i> (Tanganyika)	7.5
<i>Haplochromis nubilus</i> (Victoria)	6.9
<i>Haplochromis latifasciatus</i> (Victoria)	5.9
<i>Metriaclima estherae</i> (Malawi)	7.9
<i>Metriaclima zebra</i> (Malawi)	11.7

Discussion

My results show that the sympatric host species *C. horei* was parasitized significantly less (17% of broods) than allopatric host species, with an average of 7.5 catfish per parasitized *C. horei* brood. Sato (1986) found comparable values of a 15% parasitism rate and average number of catfish per parasitized brood of 6.3 for *C. horei* in field experiments. Subtle differences in the mating ritual and oviposition between species may help explain the disparity in parasitism frequencies I recorded between sympatric and allopatric hosts. For example, Ochi (1993) described some of the breeding behaviors of *C. horei* that are unique among mouthbrooders, noting that they spend up to 24 minutes before and after actual spawning engaging in displaying/mating behaviors without oviposition occurring. Furthermore, Ochi (1993) found that *C. horei* males defend females until leading them to a remote spawning site rather than waiting for females to enter a defended territory. These distinctive spawning activities of *C. horei* could present a moving target for the cuckoo catfish to locate, and subsequently make it difficult for the catfish to then synchronize their parasitism with the timing of cichlid spawning. Interestingly, the parasitism rate and number of catfish per brood that Sato found for *C. horei* were the highest among the six Tanganyikan host species he surveyed. Thus, it is possible that other sympatric host species would also have a much lower frequency of parasitism as compared to allopatric species, and may in fact be parasitized even less than *C. horei* were under laboratory conditions, further widening the gap I detected between sympatric and allopatric species.

In the laboratory I compared videos of the mating behaviors of *C. horei* to another mouthbrooding Tanganyikan species, *Simochromis diagramma*, and found that there are also differences among sympatric species in the behaviors associated with spawning and oviposition (unpub.). Once spawning activities started, *C. horei* rarely left the area, while *S. diagramma*

interrupted spawning and left the area multiple times. Interrupted spawning may be yet another way to lower the incidence of parasitism by hindering the ability of the cuckoo catfish to hone in on the exact timing of oviposition. Furthermore, I noticed that *S. diagramma* seem to keep the breeding area much cleaner from small pebbles and other debris than *C. horei*, and that they release less eggs during oviposition as well. Nelissen (1975) specified that *S. diagramma* will begin a bout of spawning by laying three eggs at a time and finish by laying one or two eggs at a time, and these data are consistent with my video recordings. Keeping the site free of debris and releasing a smaller number of eggs per oviposition should make *S. diagramma* more efficient at quickly finding and successfully retrieving their eggs, and likely make it more difficult for the cuckoo catfish to interrupt the spawning of *S. diagramma* to eat some of their eggs and then parasitize them.

Other behavioral differences between host species may also provide an explanation for the lower frequency of parasitism I detected in *C. horei*. Many avian studies have found responses to parasitism (e.g., rates of egg rejection or aggression towards parasite) to be different for sympatric and allopatric host populations, and that the selection pressure of brood parasitism can facilitate the evolution of antiparasite defenses in some hosts (e.g., Payne 1977; Cruz and Wiley 1989; Rothstein 1990; Briskie *et al.* 1992; Røskaft *et al.* 2002; Kuehn *et al.* 2014). Bird species that are hosts of brood parasites tend to fall into two groups, egg acceptors or egg rejecters (Rothstein 1975d). In contrast, African mouthbrooding cichlids will pick up anything that looks like an egg (e.g. small rocks) (pers. obs.). Therefore, cichlids are more similar to egg accepting species of birds, and egg rejection does not appear to be a defense that cichlid hosts use. However, research in our laboratory has found that *M. zebra* previously unexposed to parasitism do show an increase in aggression towards the catfish parasites after an extended

period of exposure (unpub.). Thus, cichlids may use aggression as a means to mitigate parasitism. Studies of cuckoos and cowbirds suggest that host aggression can be an effective behavior to discourage brood parasites, especially if the hosts are of similar size or larger than the parasite, and egg acceptor species appear more aggressive than their egg rejecting counterparts (Robertson and Norman 1976; 1977; Davies and Brooke 1989a; Rothstein 1990). It is unclear whether Tanganyikan host species, like *C. horei*, have coevolved aggression as a specific adaptation against brood parasitism from the cuckoo catfish, or if they are simply aggressive towards any perceived threat (e.g. predators). Barlow (2000) suggests that female cichlids evolving defenses specifically in response to brood parasitism might not be feasible, considering females cannot be selective in what they are picking up and have to gather the eggs into their mouths so quickly (often before they are even fertilized) in order to prevent predation. Furthermore, aggression in birds is often a more universal response towards any nest invader, rather than a coevolved response to brood parasites, and being aggressive can at times be maladaptive for the host (Rothstein 1990). *Ctenochromis horei* males seem to be noticeably more aggressive than the other cichlid species towards both the catfish and the females in their harem (pers. obs.). So, while useful for limiting parasitism, they may just be a more aggressive cichlid species. Additionally, I found no indication that parasitism rates change for any of the host species over time and repeated exposure to the parasite (unpub.), suggesting that aggression alone may not be enough of a deterrent to the parasite.

In addition to the significantly lower rates of parasitism observed for *C. horei*, *M. zebra* broods contained more catfish per brood (11.7 per brood) than all other species sampled, and were parasitized significantly more than all other host species (46% of broods). While higher than all other cichlid species examined, the number of catfish per brood is only significantly

higher for *H. latifasciatus* (5.9 per brood). However, the parasitism rate of *M. zebra* was nearly double the average rate of the other allopatric host species examined (27% of broods). Interestingly, I have collected data on parasitism of two other species of cichlid from Lake Malawi using the same methods, and found that their rates of parasitism are similar to the data presented here at roughly 30% of broods (unpub.). Wisenden (1999) found a parasitism rate of 49% for 65 broods examined from multiple allopatric host species, and surmised that the confined space of the aquarium environment may favor parasitism by the cuckoo catfish and could justify the high levels of parasitism he observed. However, my data shows variability in parasitism rates and number of catfish per brood between cichlid species, so there appears to be more involved than just simply having high parasitism levels due to an aquarium setting. It is important to note that the *M. zebra* I used in this study are an albino morphotype that has been selectively bred by aquarists for many generations, and it is likely highly modified compared to wild caught specimens of the normal morphotype. Thus, as an albino, these fish lack normal eye pigmentation. The question of how eyesight affects the ability of hosts to avoid brood parasitism is poorly understood, but albinism and the absence of eye pigmentation can be associated with poor vision in many species (Lashley 1930; Wahlsten 1972; Wilson *et al.* 1988; Abadi and Pascal 1991; Ren *et al.* 2002). If the albino *M. zebra* suffer from reduced visual acuity, then it is possible they cannot see the catfish as well as the other cichlid species do, and thus experience the much higher level of parasitism I observed.

Conclusions and Future Directions

The sampling done by Sato (1986) in Lake Tanganyika and the results presented here represent only a glimpse of the possible variation in parasitism response by cichlid hosts. The distributions of the cuckoo catfish and mouthbrooding cichlid species in Lake Tanganyika are

largely unknown, and could be important in determining what effects parasitism is having on localized cichlid populations. While endemic cichlid species represent the majority of the fish fauna, there are few species that are considered ubiquitous throughout Lake Tanganyika, and is primarily due to complex species interactions as well as the topography of the littoral zones where the cichlids are predominantly found (Fryer and Iles 1972; Sturmbauer *et al.* 2008). These cichlid distribution patterns suggest that the data provided by Sato (1986) may have been obtained in areas rich in the few species he sampled, and that the extent of the effect of cuckoo catfish parasitism could be very different in other regions of the lake. While many cichlid species in the Rift Valley lakes are stenotopic and philopatric (Ribbink 1990), the cuckoo catfish have been filmed traveling in large shoals among the rocky shores looking for cichlids to parasitize, and have been collected for the aquarium trade from multiple locations around the lake. This suggests that these catfish are widespread in the lake and necessitates the need for further study in other locations to appreciate the extent of their influence on the host species. Also, it would be interesting to compare the parasitism rates of *C. horei* that have colonized the rivers near Lake Tanganyika with my results from individuals that were collected within the lake to see if there are any differences in parasitism frequency. I would suspect that the river populations, in the absence of parasitism, would show higher rates of parasitism than what we found for lake dwelling individuals.

Although it remains unclear what the exact underlying mechanisms driving differential parasitism frequencies are, it is clear that individual host species respond differently to the cuckoo catfish. Bird studies often focus on host species that have either colonized a new area and are no longer sympatric with the brood parasite, have been recently re-exposed to parasitism after some time without that pressure, or have looked at split populations of individuals from one

species in sympatry with the parasite compared to those that are not. The allopatric host species used in this study are all evolutionarily naïve to parasitism because they are confined to different lakes, whereas the sympatric individuals used co-occur with the parasite within the confines of Lake Tanganyika. The cuckoo catfish-cichlid system provides a model for rigorously testing brood parasitism in a laboratory setting, and my comparison of sympatric vs. allopatric hosts offer a novel perspective to the ever-expanding area of host-parasite interactions and brood parasitism.

Chapter 3

The Lake Tanganyikan *Synodontis multipunctatus* (cuckoo catfish) do not discriminate among host sizes, and their fry completely eliminate host offspring then cannibalize siblings.

Abstract

The purpose of this study was to find out in the laboratory if the Tanganyikan obligate brood parasite (*Synodontis multipunctatus*, cuckoo catfish) prefers to parasitize certain sizes of mouthbrooding cichlid host, and to assess the effects of parasitism on hosts. A positive correlation between the size of the female cichlid and the number of eggs carried in the buccal cavity of unparasitized broods was found, agreeing with other research showing larger cichlids have larger broods. There was no difference in the frequency distributions of the sizes of female cichlids that were carrying eggs between unparasitized and parasitized broods, indicating that cuckoo catfish do not favor any particular size of host. The highest frequency of broods sampled occurred in intermediate host sizes in both unparasitized and parasitized broods, so if the cuckoo catfish prefers an intermediate size of host we simply may not be detecting it in the laboratory. A significant reduction in the mean number of cichlid eggs carried by female cichlids in parasitized broods was found, demonstrating that just the act of parasitism reduces reproductive success. To find the effect of parasitism on a natural host of the cuckoo catfish (*C. horei*) after mouthbrooding of young was complete, an egg tumbler was used to simulate the cichlid buccal cavity in a brood success experiment. Progeny of *C. horei* were completely eliminated in parasitized broods compared to a 69.9% survival rate in unparasitized broods, representing a very high cost of parasitism to the host. Once the host fry were exhausted, the cuckoo catfish cannibalized their siblings that resulted in a 35.2% survival rate for the catfish.

Introduction

Larger fish have been shown to have more reproductive success than smaller ones in salmonids (Thorpe *et al.* 1984), sticklebacks (Wootton 1973), cyprinids (Uusi-Heikkilä *et al.* 2010), and other groups. For cichlids (Perciformes: Cichlidae), research has also shown that larger females have larger broods (Fryer and Iles 1972; Nelissen 1975; Kuwamura 1986; Duponchelle *et al.* 2000). Larger females not only have larger broods but also are presumably better able to protect their offspring. Parental care is one strategy to help ensure increased offspring survival. Estimates are that roughly one quarter of teleost families have species providing some form of early offspring care (Breder Jr and Rosen 1966; Blumer 1982; Crawford and Balon 1996; Wootton and Smith 2014). Cichlids exhibit extensive levels of parental care, and their diversity is most pronounced in cichlids of the three major Rift Valley lakes (Tanganyika, Malawi, and Victoria) of East Africa. With numbers approaching 2000 species, the cichlids in these lakes have provided a model system to study adaptive radiation and spectacular rates of vertebrate speciation (Barlow 2000; Danley and Kocher 2001; Lande *et al.* 2001; Kocher 2004; Sturmbauer *et al.* 2011). One important feature that has allowed African cichlids to be so successful is undoubtedly their well-developed care of their offspring. Although the underlying forces driving the prevalence of parental care in the Rift Valley lakes can be debated, the high levels of predation are certainly a contributing factor. In Lake Tanganyika, cichlids have evolved many parental care systems including: mouthbrooding, substrate guarding, care by one parent, and biparental care. The cichlids also engage in different types of mating systems, such as monogamy and various types of polygyny (Kuwamura 1997). A phylogeny of the family Cichlidae shows that substrate guarding of eggs and young is the ancestral state, while

mouthbrooding is the most common form of care, and it has evolved more than once (Goodwin *et al.* 1998).

Mouthbrooding is widespread among Rift Valley cichlids, and all but one of the endemic cichlid species in both Lake Malawi and Lake Victoria (Kuwamura 1986; Duponchelle *et al.* 2008), and many in Lake Tanganyika are maternal mouthbrooding species (Fryer and Iles 1972; Rossiter 1995; Salzburger *et al.* 2002). In maternal mouthbrooding species, the altricial offspring will develop using their yolk sac as a nutrition source, and will use the mouth of their mother for protection until becoming independent. This period of incubation in the buccal (oral) cavity can last for many weeks, and is considered one of the most advanced parental care systems among fishes (Keenleyside 1979; Ochi *et al.* 2001).

Providing such a high-level of parental care is generally considered costly to the caregiver. In instances where there is no fitness benefit, as in raising unrelated young, selection should favor adaptations that would reduce these costs. For example, research has demonstrated that avian brood parasitism will reduce the reproductive success of hosts to varying degrees, and is a strong selective force on the host (e.g., Davies and Brooke 1988; Rothstein 1990; Ortega 1998; Davies 2000). This in turn can result in strong reciprocal selection pressures for the parasite to overcome these defenses, followed by counter-adaptations in the host, and often leads to an evolutionary arms race between host and parasite (Dawkins and Krebs 1979; Davies *et al.* 1989; Rothstein 1990).

In Lake Tanganyika, the endemic and obligate brood parasitic cuckoo catfish (*Synodontis multipunctatus*, Siluriformes: Mochokidae) uses female mouthbrooding cichlids as hosts (Finley 1984; Sato 1986; Cruz *et al.* 2004). The catfish interrupt the spawning of the mouthbrooders and will eat some of their eggs, followed by deposition of their own eggs. These catfish eggs will be

picked up by the female cichlid to be brooded with her own. The host cichlid will then brood the catfish embryos in their buccal cavity together with the host embryos. By 6dpf (days post fertilization) the cichlids are all hatched and the catfish have exhausted their yolk sac as a food source, so the catfish will begin eating host fry while inside the host's mouth. The timing of cichlid hatching is important because cuckoo catfish fry appear to be unable to grasp and eat the cichlid larvae until they have hatched (pers. obs.). As is the case with cuckoo bird nestlings (Davies 2000), the young cuckoo catfish usually eliminate the entire host progeny if allowed to remain in the host's mouth until they are released (pers. obs.). I have also observed in the laboratory that when stripping eggs from the buccal cavity of carrying female cichlids, there are many more cuckoo catfish eggs than the number of catfish that usually emerge from the mouth when they are allowed to remain until release.

The goal of the current study was twofold. First, since larger female cichlids have been shown to have larger broods, I was interested in using the number of eggs as an indicator of reproductive potential. I wanted to compare the number of eggs between unparasitized and parasitized broods to find out if the cuckoo catfish preferred a certain size of host to parasitize, and if the number of host eggs differed between unparasitized and parasitized broods. I predicted that the number of eggs would be positively correlated with the size of the carrying cichlid, and as a result, cuckoo catfish would prefer larger cichlids to parasitize since larger eggs would provide a more nutritive meal for their fry. Also, I predicted that the act of parasitism by the cuckoo catfish would result in a decrease in the number of cichlid eggs in parasitized broods. Secondly, I used a commercially available egg tumbler as a proxy for the buccal cavity of cichlids to test differences in brood success between parasitized and unparasitized broods, much in the same way that nest success studies with birds are carried out. Since the cuckoo catfish

have been observed completely eliminating host fry, I expected that parasitism would represent a large reduction in reproductive success for parasitized broods. Also, because the number of catfish observed emerging at the end of brood care is much lower than would be expected by the number of eggs they laid, I suspected cannibalism by the cuckoo catfish.

Materials and Methods

Study Species (Fig. 3.1)

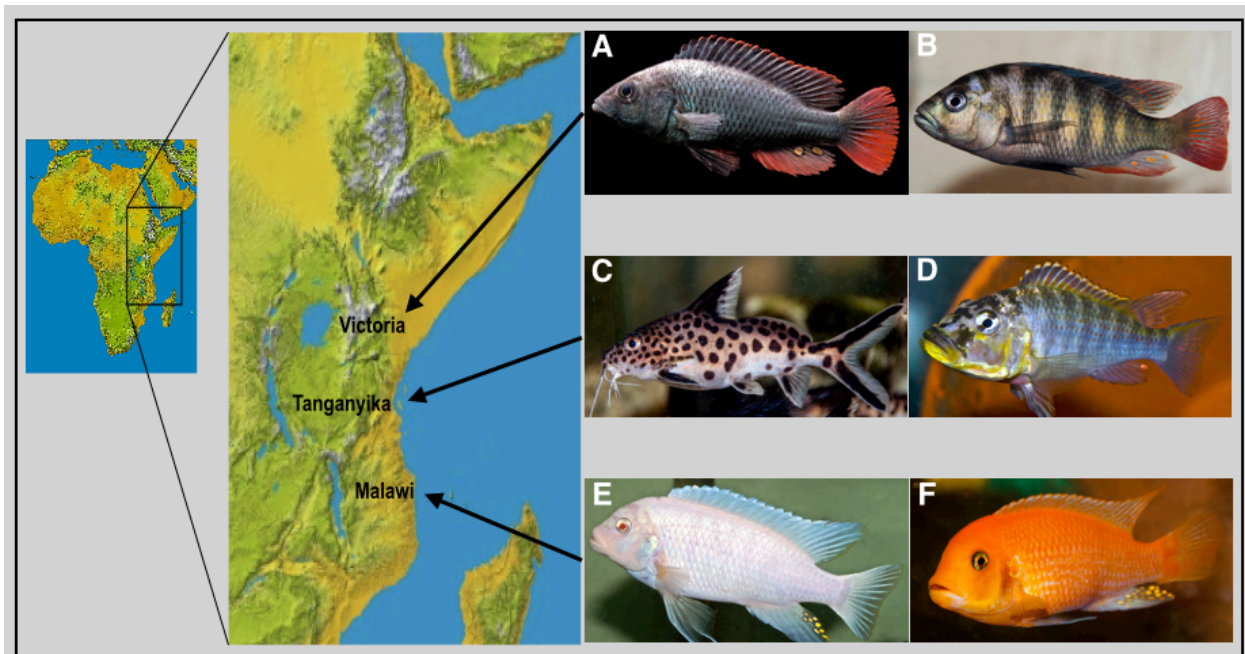


Fig. 3.1. Five African cichlid host species: (A) *H. nubilus*, (B) *H. latifasciatus*, (D) *C. horei*, (E) *M. zebra*, and (F) *M. estherae*. (C) The brood parasitic cuckoo catfish (*S. multipunctatus*). Black arrows indicate the Rift Valley lakes where the natural populations of these species are found and fish from the same area are organized by row (background map images source: NASA/JPL/NIMA).

Synodontis multipunctatus (Boulenger 1898), the cuckoo catfish, is a brood parasitic catfish in the Mochokidae family endemic to Lake Tanganyika in Africa. This catfish is a generalist brood parasite on different species of mouthbrooding cichlids, and can be distinguished from similar looking congeners by a humeral process that is narrow posteriorly (Eccles 1992). Wild-caught specimens were purchased from Old World Exotic Fish

(<http://www.oldworldexoticfish.com>), and used for experimentation along with their F1 offspring that were bred in the lab.

Ctenochromis horei (Günther 1894) is a mouthbrooding cichlid endemic to Lake Tanganyika and surrounding river tributaries, and it is a naturally occurring host of the cuckoo catfish (Sato 1986). Wild-caught specimens were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for experimentation along with their F1 and F2 offspring that I bred in the lab. Data were collected during 2006, 2007, and 2013-2015.

Metriaclima zebra (Boulenger 1899), the zebra cichlid, is a mouthbrooding cichlid endemic to Lake Malawi. The albino morphs were used in this study, as aquarists have reported using them successfully to rear cuckoo catfish. Tank-raised specimens were purchased locally from PetSmart, and used for experimentation along with their offspring that I bred in the lab. Data were collected during 2006.

Metriaclima estherae (Konings 1995), the red zebra cichlid, is a mouthbrooding cichlid endemic to Lake Malawi. Tank-raised specimens were purchased from Atlantis Tropical Fish Hatchery (<http://www.cichlids.net>), and used for experimentation along with their offspring that I bred in the lab. Data were collected from 2011 to 2013.

Haplochromis nubilus (Boulenger 1906) is a mouthbrooding cichlid endemic to not only Lake Victoria, but also surrounding smaller lakes and associated river tributaries in the greater Victoria system (Van Oijen *et al.* 1991). Tank-raised specimens were purchased locally from Golden Fish Aquarium (<http://www.goldenfishaquarium.com>), and used for experimentation along with their offspring that were bred in the lab. Data were collected from 2000 to 2002, and during 2006.

Haplochromis latifasciatus (Regan 1929) is a mouthbrooding cichlid endemic to Lake

Kyoga and Lake Nawampasa (belonging to the greater Victoria system), and little is known about the habitat and ecology of the species as it is critically endangered in the wild (Kaufman 1996). Although endangered in the wild, tank-raised specimens of this species are readily available for purchase through the aquarium trade. The tank-raised specimens for this study were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for experimentation along with their offspring that I bred in the lab. Data were collected from 2011 to 2012.

Fish Husbandry

Male and female cuckoo catfish were maintained with breeding populations of adult African cichlid species from Lake Malawi (*M. zebra* and *M. estherae*), Lake Tanganyika (*C. horei*), and the greater Lake Victoria system (*H. nubilus* and *H. latifasciatus*). These populations were housed in 110 liter (77 x 32 x 47 cm), 208 liter (122 x 32 x 53 cm), 284 liter (122 x 47 x 53 cm), and 473 liter (184 x 47 x 59 cm) aquariums. The fish were maintained at 24-26°C in a 14:10 light:dark cycle and were fed body size proportional amounts of food daily in the mid-afternoon. Catfish were fed a combination of frozen chironomid larvae and *Artemia* shrimp pellets daily, while cichlids were fed cichlid pellets or cichlid flake foods. Fifty percent water changes were conducted every other week. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was added to the tap water to remove chlorine after the tap water was added to tanks. A pH of 8.0-9.0 was maintained in the tanks by using crushed coral as the tank substrate and then buffering the pH and hardness up with a buffer mixture made from 1:1:1 sodium bicarbonate (NaHCO_3) to Epsom salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) to Instant Ocean[®] sea salt after water changes. The buffer was added in small dosages to aquariums until a dKH of 10-14 was reached, and dKH levels were monitored using a KH carbonate hardness test kit from API[®]. Terracotta flowerpots were placed in the aquariums and served as territorial

display and spawning areas.

Host Female Size and Number of Eggs and Parasitism Experimental Design

Breeding colonies of the cuckoo catfish were established with either sympatric hosts or allopatric hosts described above. The total number of fish in each tank varied based on the size of the aquarium used. Since the host species are polygynous, the cichlids were maintained at 3:1 (female to male), while the catfish were 1:1 (female to male). However the ratio of host to parasite was 2:1 (cichlids to catfish), as I found these ratios produce good breeding results.

Aquariums were monitored daily for female cichlids carrying eggs, which can be identified by their distended buccal (oral) cavity. Following identification, I carefully removed the carrying females from the tank and recorded their standard length (SL). I obtained eggs by gently holding the female's mouth open in a small volume of tank water and irrigating the oral cavity with a transfer pipette to dislodge the eggs from the mouth. The number of cichlid eggs and catfish eggs, if any, were then recorded. I obtained 100 broods from each of the five cichlid host species.

Egg Tumbler Experimental Design

I used the sympatric host, *C. horei*, from Lake Tanganyika for this experiment. After obtaining eggs from carrying female *C. horei* as described above, eggs from unparasitized and parasitized broods were placed in egg tumblers that were designed to simulate the buccal cavity of the female cichlid (Jehmco.com - item # ET1) (Fig. 3.2). The tumblers have adjustable airflow and screens to provide a gentle rolling action to simulate the normal incubation method of mouthbrooding fishes. The eggs from the brood were placed in the tumbler and monitored daily for 21 days, which is the average incubation time of cichlid fry before release for *C. horei* maintained in the lab at 24-26°C (pers. obs.). One limitation was that if some of the eggs were

initially unfertilized, or any embryos died in the tumblers before the eggs hatched (at ~5 days post fertilization), a fungus could develop and infect other eggs in the clutch. To address this issue, all tumblers were cleaned daily to prevent fungus and any unfertilized eggs or dead embryos were removed. I recorded the consumption of fry and any cannibalism by the cuckoo catfish daily for 21 days.



Fig. 3.2. Egg tumbler (Jehmco.com). Inset photo shows *C. horei* sac-fry in the tumbler.

Statistical Analyses

All statistical test were done using JMP[®] Pro 11 (64-bit). I performed a Spearman's rank correlation analysis to determine if number of eggs carried was correlated to the size of the female cichlid carrying the eggs. I used the nonparametric Spearman's rank instead of Pearson's since the two variables were not normally distributed. I used the Kolmogorov-Smirnov two-sample test to evaluate whether there was a difference in the frequency distributions of female mouthbrooder size in unparasitized versus parasitized brood. To compare the number of cichlid eggs carried between unparasitized and parasitized broods I performed a Wilcoxon two-sample test. Differences between parasitized and unparasitized broods in the tumbler experiment were also assessed using a Wilcoxon two-sample test. I used nonparametric statistics for test of parasitism differences because my data did not meet the assumptions of parametric tests.

Results

Host Female Size and Number of Eggs

I performed a Spearman's rank correlation analysis for unparasitized broods and found a positive correlation between the size of the female cichlid and the number of eggs carried in the buccal cavity ($n=355$, Spearman's $\rho = 0.431$, $p < 0.0001$) (Fig. 3.3). A Kolmogorov-Smirnov two-sample test ($n=500$, $D = 0.064$, $p > 0.05$) determined there was no difference in the frequency distributions of the sizes of female cichlids that were carrying eggs between unparasitized and parasitized broods (Fig. 3.4.).

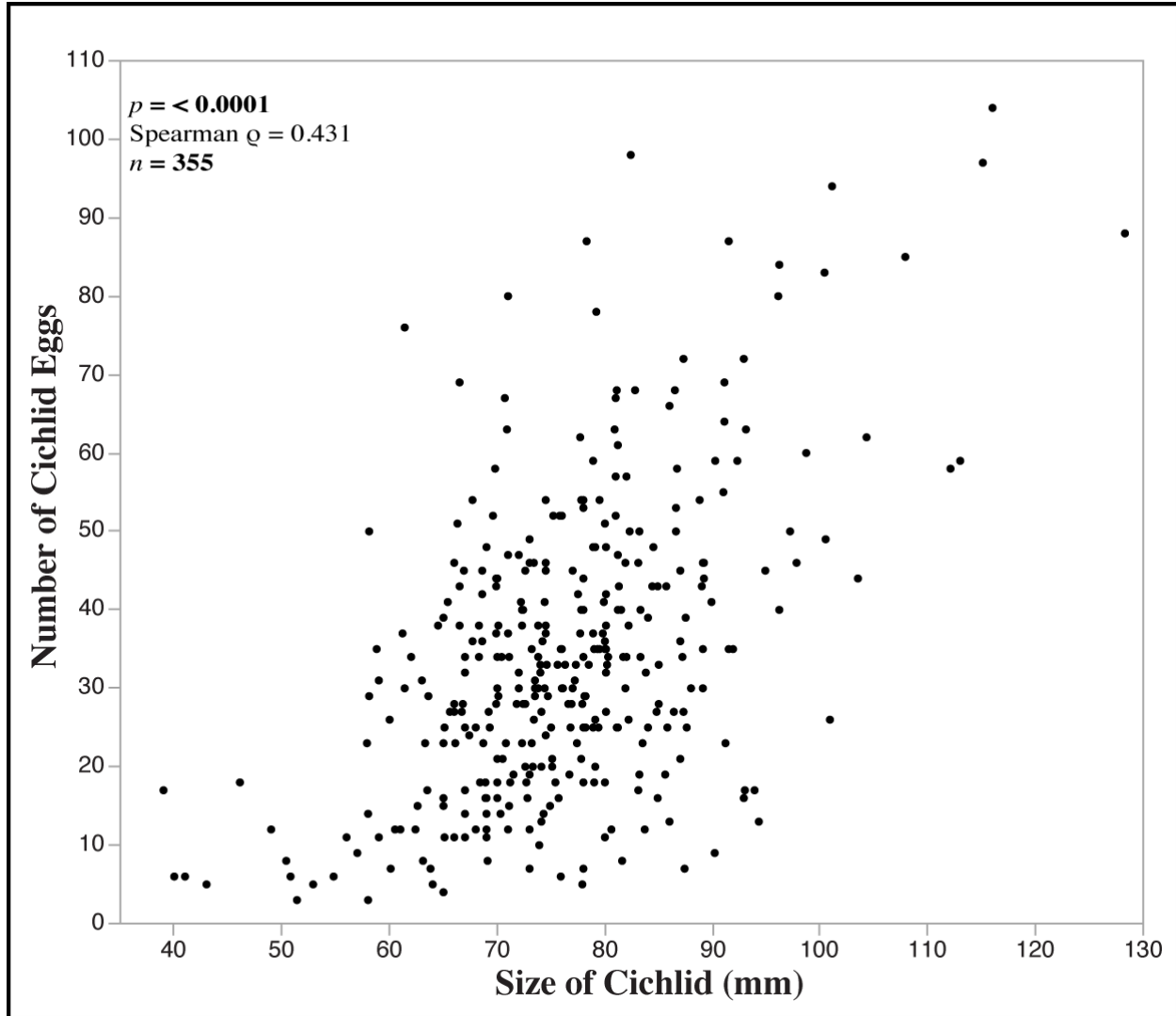


Fig. 3.3. Spearman's Rank Correlation for number of cichlid eggs carried by a female (clutch size) vs. the size of the female for all cichlid species in unparasitized broods.

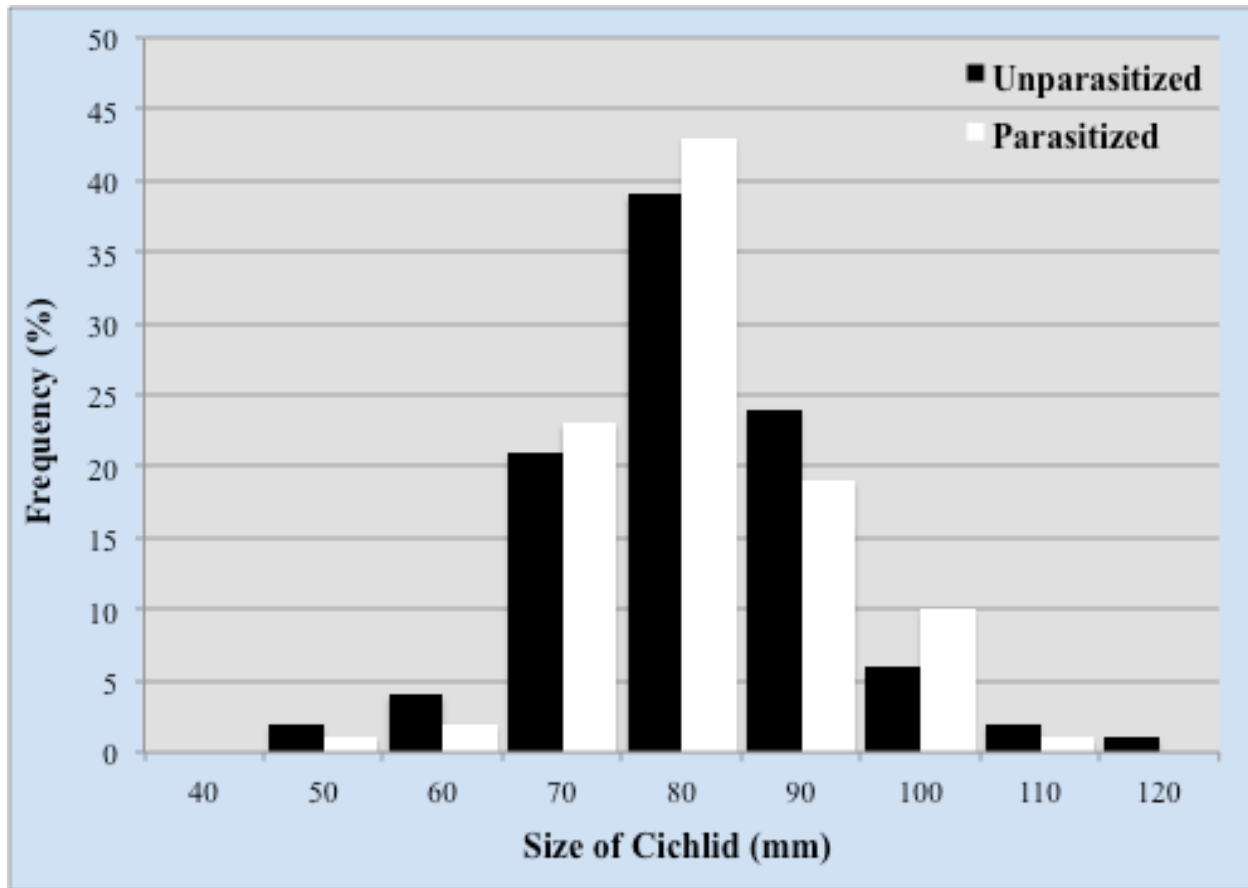


Fig. 3.4. Frequency distributions of size of female mouthbrooder carrying eggs for both unparasitized and parasitized broods.

Reduction in Number of Cichlid Eggs

In the 500 broods surveyed, I found that there was a significant reduction in the mean number of cichlid eggs carried by female cichlids in parasitized (28.5) versus unparasitized (34.1) broods by using a Wilcoxon two-sample test ($S = 32347.5$, $z = -2.71$, $p = 0.007$), with a small effect size ($r = 0.12$) (Fig. 3.5).

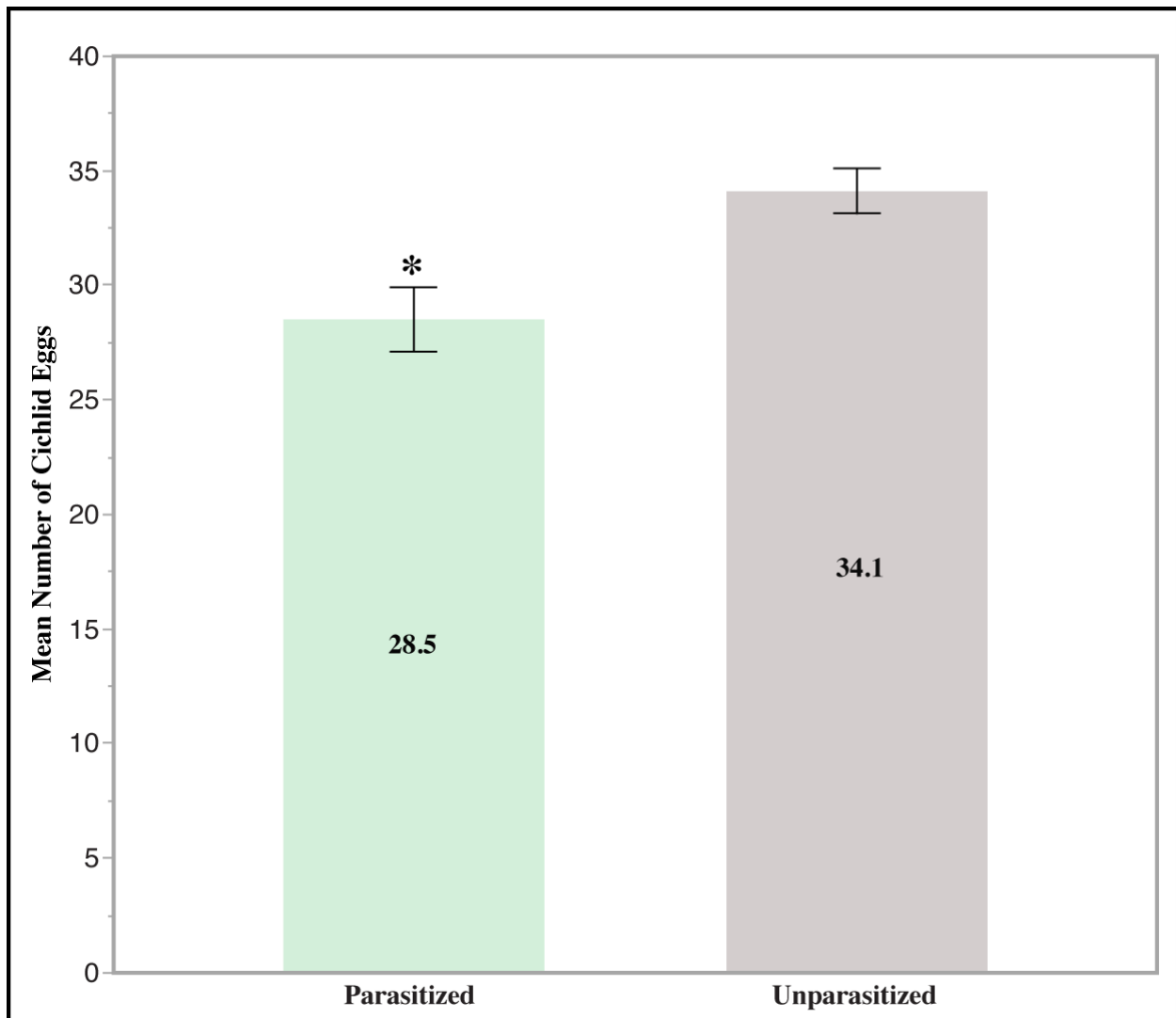


Fig. 3.5. Wilcoxon two-sample test for the mean number of cichlid eggs carried by female cichlids in parasitized vs. unparasitized broods ($n=500$). The * indicates a significant difference ($S = 32347.5$, $z = -2.71$, $p = 0.007$, $r = 0.12$). Error bars indicate SEM.

Egg Tumbler Study

Significantly less cichlids (0) survived per parasitized brood as compared to unparasitized brood (29.9) when performing a Wilcoxon two-sample test ($S = 45$, $Z = -3.96$, $p < 0.0001$), with a large effect size ($r = 0.86$) (Table 3.1). The difference in survival between unparasitized and parasitized broods represents a survival rate reduction of 69.9% following parasitism. The catfish

survival rate was 35.2%, where 2.78 catfish per brood survived the 21 days and 42 catfish were cannibalized during that time.

Table 3.1. Brood Success in Unparasitized and Parasitized *Ctenochromis horei* broods.

	Unparasitized	Parasitized (Cichlids)	Parasitized (Cuckoo Catfish)
Number of broods	12	9	-
Total eggs	513	235	71
Surviving to 21 days	359	0	25
Surviving to 21 days (%)	69.9	0	35.2
Surviving fish/brood *	29.9	0*	2.78
Total catfish cannibalized	-	-	42

*Differences between parasitized and unparasitized broods are significant for the number of cichlids surviving per brood (Wilcoxon two-sample test, $S = 45$, $Z = -3.96$, $p < 0.0001$, $r = 0.86$)

Discussion

Female Size and Number of Eggs

My results from a Spearman's rank correlation analysis found a positive correlation between the size of the female cichlid and the number of eggs carried in the buccal cavity. These results agree with other studies of cichlids that have reported a similar correlation between body size and brood size (Fryer and Iles 1972; Nelissen 1975; Kuwamura 1986; Duponchelle *et al.* 2000). These results may be related to something as simple as a larger buccal cavity provides more space for more eggs, or there may be a more complicated allocation of reproductive effort taking place. For example, research across fish taxa has shown there is often a tradeoff in clutch

size versus egg size, whereby there is a balance between producing few large or many small eggs (Duarte and Alcaraz 1989; Elgar 1990). This becomes further complicated when parental care is factored in. Because the eggs and larvae are being protected, it makes sense to provide care to fewer offspring per reproductive effort, so that they can be reared successfully to a larger independent size. African cichlids exist in an environment of high predation and competition, and therefore show a typical *K*-selected strategy of high parental investment in fewer eggs. However, there is even variability among the African cichlids, where mouthbrooders produce even fewer eggs that are larger and more yolk-filled than substrate spawning egg guards do (Fryer and Iles 1972). Therefore, egg size related to female size may be an important factor in addition to egg number, and needs to be investigated further.

Because mouthbrooders produce smaller numbers of larger eggs, and in general larger fish produce more eggs, I predicted that the cuckoo catfish would select the larger cichlids to parasitize. It seemed likely that the larger cichlids with more eggs would provide the most nutrition for the parasitic young per reproductive effort. It turns out that after performing a Kolmogorov-Smirnov two-sample test there was no difference in the frequency distributions of the sizes of female cichlids that were carrying eggs between unparasitized and parasitized broods. This result indicates that the cuckoo catfish are not discriminating between host sizes. There is likely a cost-benefit compromise between parasitizing smaller cichlids and larger ones. The smallest cichlids have a smaller buccal cavity and therefore less space for the developing catfish young, as well as fewer cichlid young for the catfish to feed on. Conversely, the larger the cichlid, the higher the risk of physical harm is to the catfish attempting to interrupt the spawning. It may actually make sense for the cuckoo catfish to prefer an intermediate size of host, whereby they can maximize buccal cavity space while minimizing risk of physical harm. My results would

likely not detect this preference, because most of the cichlids sampled fell within the intermediate size range of 70-90mm. This range is where the highest frequency of spawning events occurred regardless of whether the broods were unparasitized or parasitized. The fact that many cichlids used in this study are falling in that narrow size range may be an artifact of the experimental design. For example, *C. horei* has a maximum reported SL of 200mm in nature (Eccles 1992), and may be prevented from reaching their maximum size in an aquarium. Therefore, more work needs to be done with a larger size range of hosts to see if there is a preference for host size that I did not detect in this study.

Reduction in Number of Cichlid Eggs

For the 500 broods of cichlids examined, I found that there was a significant reduction in the mean number of cichlid eggs carried by female cichlids in parasitized (28.5) versus unparasitized (34.1) broods. This reduction in cichlid eggs is directly related to the actual act of parasitism, whereby the cuckoo catfish interrupts the cichlid's spawning and consumes some of the cichlid eggs before laying their own eggs. The removal of eggs by a brood parasite before laying their own eggs is a common practice among birds (Ortega 1998). Sealy (1992) summarized possible reasons that birds remove host eggs, including: deceiving a host that may be counting eggs, as a food source for the parasite, or removing crowding and competition from the host offspring. In the case of the cuckoo catfish it is uncertain why they are removing host eggs, and there is a delicate balance between removing some of the host eggs while leaving enough to make sure there are sufficient numbers to sustain the young catfish later on. It is unclear whether the physical act of the catfish eating cichlid eggs, or the addition of catfish eggs to the existing cichlid brood exceeded the amount of eggs the cichlids could carry, or a combination of the two caused the observed reduction in cichlid reproductive success. Assuming

that some of the catfish eggs were fertilized, I was interested in finding out what the ultimate cost to the cichlid in reproductive output was after brooding was complete.

Egg Tumbler Study

Unfortunately it is not possible to do traditional nest success experiments with mouthbrooding cichlids, where periodic searching of nests can allow one to follow a brood to fledging or failure. With mouthbrooding cichlids, once the clutch is removed from the mouth, the cichlid chooses not to pick up the eggs again or some eggs are damaged in the process of repeatedly removing them and allowing the cichlid to pick them up (pers. obs.). Therefore, an egg tumbler device was employed to simulate the buccal cavity of the mouthbrooder. It was important to use a natural host species (*C. horei*) for this experiment in order to gauge the most realistic responses to parasitism by the cuckoo catfish. Broods of *C. horei* were tracked for 21 days, which represents the average time *C. horei* brood their young before release under laboratory conditions. My results supported the prediction of total loss of host progeny followed by cannibalism by cuckoo catfish progeny once the host fry were exhausted as a food source. Other studies have shown that some avian brood parasites are brooded along with host chicks (e.g., cowbirds) and others (e.g., cuckoos, honeyguides) completely eliminate the host offspring and monopolize parental care (e.g., Davies 2000). Unlike the cuckoo catfish, siblicide has not been reported for any avian brood parasites. *Ctenochromis horei* will not feed during incubation (Kuwamura 1986; Taborsky and Foerster 2004, pers. obs.), so it is possible with different hosts that the incidence of cannibalism is not as high as what I have reported, and the catfish may have access to other sources of food than just the cichlid fry or their siblings. Kuwamura (1986) reported that roughly half of the mouthbrooding cichlids surveyed fed during incubation.

Egg Size

While working with cichlid species from the three Rift Valley lakes, I observed that the eggs of the Tanganyikan *C. horei* are noticeably larger than the eggs of the non-Tanganyikan species used (pers. obs.). Coleman (2008) collected egg data from aquarists for 70 Rift Valley cichlid species and following a method he devised found the “effective diameter” of a non-spherical egg. When Coleman’s egg data is sorted by lake, the average size of Tanganyikan eggs is 3.9 mm compared to 3.0 mm for Malawian eggs and 2.7 mm for Victorian, lending support to my observations about *C. horei*. It is unknown whether Tanganyikan species having larger eggs is an adaptation against brood parasitism or not. Larger eggs could give the young cichlids a slight advantage as the cuckoo catfish exhaust their yolk sacs and begin feeding exogenously on cichlids. I have noticed that the catfish cannot easily grasp the unhatched eggs of the cichlids, and they will wait until the cichlids hatch and then grab them by the head to consume them (pers. obs.). Thus it is crucial that the timing of yolk exhaustion by the cuckoo catfish be coordinated to when the cichlids are hatching. When the cichlids hatch they would have larger fry, and/or larger yolk sacs, thus providing bigger meals for the catfish fry. This could decrease the total number of individuals eaten by the catfish before the cichlids are free-swimming and could better escape being ingested. However, I found that the offspring resulting from these larger Tanganyikan eggs seem to be easily consumed by the cuckoo catfish in the egg tumblers. Also, the cichlid buccal cavity is much smaller than the egg tumbler, and that should make it even easier for the catfish to catch and consume cichlids. However, it is possible that the larger eggs are a step towards a parasitism counter-adaptation by Tanganyikan cichlids to thwart the cuckoo catfish. Researchers have proposed the idea that there may be an evolutionary lag in developing adaptations on the part of the host in avian systems of brood parasitism, and in a situation like a host experiencing

low rates of parasitism it could take thousands of generations for the host to evolve an adaptation against parasitism (Rothstein 1982; Brooke and Davies 1988; Davies and Brooke 1989b; Rothstein 1990). Thus, at any given time we are essentially glimpsing a snapshot of where a system is in the evolutionary arms race. In the case of Tanganyikan cichlids, we may just be detecting a step towards the development of an adaptation against parasitism. Due to the ritualistic nature of cichlid spawning and the speed at which the females pick up their eggs after oviposition, it seems unlikely that the cichlids are easily able to evolve the ability to recognize and/or reject parasitic eggs. Cichlids have been shown to be able to change the length of their care in response to the presence of a predator (Taborsky and Foerster 2004), or even change spawning to an inclined surface after being exposed to the cuckoo catfish (Wisenden 1999). Therefore, other adaptations against parasitism, like larger eggs or behavioral responses (e.g., aggression), seem plausible.

Conclusions and Future Directions

My results show that parasitism by the cuckoo catfish is extremely costly to the host. Further sampling in Lake Tanganyika is necessary to get a better idea of what the true frequency of parasitism is by the cuckoo catfish in nature. Sato (1986) performed a small survey of a handful of species, and his study represents a good starting point for finding natural parasitism frequencies. Finding the parasitism frequency for more species would allow us to better determine the effect that this brood parasite actually has on host populations. There may be so many cichlids that the effect of this parasite is negligible for any given species; however, it is also possible that these catfish represent a serious threat to the reproductive success of certain populations of cichlids. Because African cichlids can breed year round in the tropical Rift Valley lakes, occasional parasitism likely does not represent a big problem for an individual; however,

repeated loss of entire broods would be detrimental to their overall fitness. Some authors have suggested that with the spread of the brown-headed cowbird (*Molothrus ater*) across North America, naïve host species are vulnerable to parasitism in the absence of any defenses (Mayfield 1965; Rothstein 1975a; Brittingham and Temple 1983). Thus, the accidental introduction of the cuckoo catfish to new habitats could be problematic for naïve host populations. For example, the cichlids in Lake Victoria have already been victim to overharvesting and devastation from introductions of novel predators like the Nile perch (*Lates niloticus*) (Witte *et al.* 1992), so adding a brood parasite that completely eliminates host clutches could further add to the decline of these species. Obligate brood parasitism has appeared independently in several groups of birds, and given how well the cuckoo catfish has exploited mouthbrooding behavior, it is expected that brood parasitism would be more widespread in the Rift Valley lakes. However, the cuckoo catfish has certain developmental traits that allow it to be so successful. Some *Synodontis* species may not be capable of having their fry grow faster than the cichlid fry, time exogenous feeding to the hatching of the cichlids, and be large enough to consume the cichlid fry. For example, I compared juvenile cuckoo catfish to a congener (*Synodontis lucipinnis*) and found that there is a difference between the shape of their head skeletons, whereby the cuckoo catfish has a much more robust oral morphology than *S. lucipinnis* (unpub.). More research on this understudied host-parasite system is necessary to uncover the intricacies of the relationship between this unique brood parasite and their cichlid hosts.

Chapter 4

The role of visual cues in synchronizing breeding with African cichlid hosts by the brood parasitic cuckoo catfish (*Synodontis multipunctatus*).

Abstract

Here I investigate the role that vision plays in the process of breeding synchronization, and assess whether video playback is an appropriate tool to use in cuckoo catfish (*Synodontis multipunctatus*) behavioral research. The cuckoo catfish is endemic to Lake Tanganyika and parasitizes mouthbrooding cichlids. Cichlids only spend up to a few hours spawning, so it is important for the cuckoo catfish to be able to quickly recognize cichlid spawning behavior and coordinate their parasitism with cichlid oviposition. Cuckoo catfish also need to recognize mouthbrooder spawning, because cichlids that spawn on the substrate are inappropriate hosts. It is unknown how the catfish locate cichlids to parasitize. In the laboratory I gave male and female cuckoo catfish dichotomous choices using a side-association design. I tested the catfish using a live group of mouthbrooders vs. an empty tank, and video playback methods using videos of: mouthbrooder group vs. an empty tank, mouthbrooder pair spawning vs. substrate pair spawning, and mouthbrooder pair spawning vs. not spawning. Both male and female catfish spent significantly more time with a live group of mouthbrooders and video of a group of mouthbrooders, confirming that the catfish are able to use vision to locate hosts in the absence of other stimuli, while also establishing video playback as a viable behavioral research tool to use with cuckoo catfish. Female catfish spent significantly more time with video of a mouthbrooder pair spawning than a substrate spawning pair, but males did not show a preference, likely because females lead males into the cichlid spawning areas and would be responsible for recognizing mouthbrooders spawning. Neither males nor females showed a preference between a

mouthbrooder pair spawning and not spawning, possibly because the videos are qualitatively similar or are missing vital cues used by the catfish to find their hosts. Future studies are needed to find the specific visual signals the catfish are using, and the roles that other sensory modalities play need to be explored.

Introduction

The only known non-avian obligate brood parasite among vertebrates is the cuckoo catfish (*Synodontis multipunctatus* Siluriformes: Mochokidae), a species endemic to Lake Tanganyika that uses female mouthbrooding cichlids (Perciformes: Cichlidae) as hosts (Finley 1984; Sato 1986; Cruz *et al.* 2004). While the breeding behaviors of the cichlid-cuckoo catfish system have been observed by aquarists and documented by a few observational studies (Brichard 1979; Ferguson 1983; Finley 1983; Sato 1986; Wisenden 1999; Cruz *et al.* 2004), little is known about the specific interactions between host and parasite. Of particular interest is the means by which brood parasites are able to locate their hosts and successfully parasitize them.

The cuckoo catfish as a brood parasite most resembles bird species (e.g. cuckoos or cowbirds). In the case of the cuckoo catfish, they travel in large shoals around Lake Tanganyika, and when cichlid breeding is detected, a female catfish will lead one to several males into the cichlid spawning area. The catfish will then interrupt the cichlid breeding sequence and breed simultaneously with the cichlids. The female cichlid will inadvertently pick up the catfish eggs and brood them in the buccal cavity together with their own eggs. The catfish grow faster, and after they have exhausted their yolk sacs as a nutrition source, they begin to devour the host cichlid's fry while still in the host's mouth. However, unlike birds that can be parasitized for days to as long as a week (Ortega 1998), the cichlids only spawn for a couple of hours or less (Cruz *et al.* 2004). It is therefore crucial that the cuckoo catfish are capable of finding spawning

cichlids during this very short timeframe, and that they have the ability to synchronize their breeding with the cichlids. Members of the family Cichlidae are mostly either substrate spawning guarders, or mouthbrooders (Fryer and Iles 1972; Kuwamura 1986; Ochi 1993), and Lake Tanganyika is distinct from Lakes Malawi and Victoria in that there are many representative species of both breeding strategies present (Rossiter 1995). Thus, it is imperative that the catfish be able to discriminate between behaviors associated with mouthbrooder spawning and those of substrate spawning species, as the latter would not be suitable hosts since they are unable to provide proper care of the parasitic catfish young.

It is unknown what senses the cuckoo catfish are primarily using to find and exploit their hosts' spawning efforts. However, mouthbrooding cichlids have stereotypical acts associated with spawning that are conserved among species (McElroy and Kornfield 1990), thus it is likely that catfish can recognize spawning behaviors from a distance. Therefore, vision is likely important to the catfish. Furthermore, vision is thought to be a principal sense used by teleosts, suggested by the prevalence of large well developed eyes and a relatively large dorsal mid-brain region in these fishes (Schwassmann 1975). The cuckoo catfish do have large eyes measuring up to 62% of snout length (Wright and Page 2006), which may signify the importance of vision in this particular species. Experimental techniques that study the role of vision in fish can be extremely valuable, especially in a controlled laboratory setting where specific stimuli that evoke or modify behaviors can be parsed out. There are multiple approaches for investigating the role of vision in behavioral studies with fish (Rowland 1999). For example, the use of live fish as both test subject and stimulus has obvious benefits, as these scenarios most closely mimic natural conditions. However, it can be difficult to control variables associated with live fish as the stimulus, and these confounding and/or covarying factors complicate causal relationships that

might exist between the stimuli and the observed behaviors of the test subject. Thus, video playback is being increasingly used as a technique for behavioral research (Trainor and Basolo 2000), and has proven to be invaluable in assessing visual communication between and within multiple species of animals (e.g. Rosenthal *et al.* 2004; Shashar *et al.* 2005; Trainor and Basolo 2006; Van Dyk and Evans 2007; Zoratto *et al.* 2014). Specific behavioral sequences can be recorded, isolated, and then played back to experimental subjects to study their reactions to particular stimuli. Furthermore, the recent advances in the quality of video recording equipment and playback devices, as well as the advanced capabilities of modern video editing software allow for a greater sense of realism when using video sequences to replace live stimuli. However, it is important to consider that video playback technology was engineered to be viewed by human observers (Rowland 1999), so other animal subjects may perceive video in an unnatural way. Potential limitations of video stimuli in fish include color perception, flicker, depth perception, visual acuity, and interaction (D'eath 1998). In order for video to be used as a valid experimental technique, it is essential to confirm that the test subjects respond to the recordings in a comparable way to live fish stimuli.

The aim of the current study was twofold. First, I wanted to assess whether video playback could be effective as a stimulus in eliciting responses to behaviors of mouthbrooding cichlid hosts by the cuckoo catfish, as it is possible that video playback may appear differently to the cuckoo catfish as compared to live fish stimuli. Secondly, I tested the hypothesis that the breeding synchrony of the cuckoo catfish with host species is achieved at least in part via visual cues, considering the cichlid spawning sequence has repeated behaviors that are easily discernable. I used live fish as the stimulus followed by video playback stimuli to investigate whether or not cuckoo catfish males and females could discriminate between a group of

mouthbrooding cichlids vs. an empty tank, a pair of spawning mouthbrooders vs. a pair of substrate spawning cichlids, or a pair of mouthbrooders while spawning vs. not spawning. I predicted that cuckoo catfish would recognize and spend more time with groups of mouthbrooders, as well as a spawning pair of mouthbrooders for both video sets where they were used as stimuli. Together I hope to provide a foundation for using video playback methods in future studies with the cuckoo catfish, and offer a deeper understanding of the role visual cues play in the parasitism of cichlids by the cuckoo catfish.

Materials and Methods

Study Species (Fig. 4.1)



Fig. 4.1. Species used in experiments. The cuckoo catfish (*S. multipunctatus*) and mouthbrooding cichlid (*C. horei*) are endemic to Lake Tanganyika (illustrated by a black arrow). The substrate spawning convict cichlid (*A. nigrofasciata*) is native to El Salvador, Guatemala, and Honduras (illustrated as an inset map of Central America region). Background map images source: NASA/JPL/NIMA.

Synodontis multipunctatus (Boulenger 1898), the cuckoo catfish, is a brood parasitic catfish in the Mochokidae family endemic to Lake Tanganyika in Africa. This catfish is a generalist brood parasite on different species of mouthbrooding cichlids, and can be distinguished from similar looking congeners by a humeral process that is narrow posteriorly (Eccles 1992). Wild-caught specimens were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for experimentation along with their F1 offspring that were bred in the lab.

Ctenochromis horei (Günther 1894) is a mouthbrooding cichlid endemic to Lake Tanganyika and surrounding river tributaries, and it is a naturally occurring host of the cuckoo catfish (Sato 1986). Wild-caught specimens were purchased from Old World Exotic Fish (<http://www.oldworldexoticfish.com>), and used for experimentation along with their F1 and F2 offspring that I bred in the lab.

Amatitlania nigrofasciata (Günther 1867), the convict cichlid, is a substrate spawning cichlid native to El Salvador, Guatemala, and Honduras (Schmitter-Soto 2007). They inhabit the fast moving water areas of creeks, streams, and rivers, and prefer small cracks and crevices amongst the rocks (Conkel 1993). Tank-raised specimens were purchased locally from PetSmart, and used for experimentation along with their offspring that I bred in the lab

Fish Husbandry

Cuckoo catfish were maintained by themselves and with breeding populations of African cichlid species from Lake Malawi (*M. zebra* and *M. estherae*), Lake Tanganyika (*C. horei*), and the greater Lake Victoria system (*H. nubilus* and *H. latifasciatus*). These populations were housed in 110 liter (77 x 32 x 47 cm), 208 liter (122 x 32 x 53 cm), 284 liter (122 x 47 x 53 cm), and 473 liter (184 x 47 x 59 cm) aquariums. The fish were maintained at 24-26°C in a 14:10

light:dark cycle and were fed body size proportional amounts of food daily in the mid-afternoon. Catfish were fed a combination of frozen chironomid larvae and *Artemia* shrimp pellets daily, while cichlids were fed cichlid pellets or cichlid flake foods. Fifty percent water changes were conducted every other week. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was added to the tap water to remove chlorine after the tap water was added to tanks. A pH of 8.0-9.0 was maintained in the tanks by using crushed coral as the tank substrate and then buffering the pH and hardness up with a buffer mixture made from 1:1:1 sodium bicarbonate (NaHCO_3) to Epsom salt ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) to Instant Ocean[®] sea salt after water changes. The buffer was added in small dosages to aquariums until a dKH of 10-14 was reached, and dKH levels were monitored using a KH carbonate hardness test kit from API[®]. Terracotta pots were placed in the aquariums and served as territorial display and spawning areas for cichlids. Lengths of PVC pipe 5 cm. in diameter were cut to pieces 10-30 cm. long and provided for the catfish to use as hiding places.

Dichotomous Choice Trials - Live Fish.

Data were collected during 2007 and 2008. The experimental design used side-association data based on the time spent in proximity to different stimuli (Houde 1997). This method has been widely used in mate choice experiments in fishes (e.g. Bischoff *et al.* 1985; Basolo 1990; Basolo 1995; Seehausen and van Alphen 1998; Williams *et al.* 2013; Heinen-Kay *et al.* 2014). The set up consisted of a single 206 liter (122 x 32 x 53 cm) experimental tank placed in the middle, and two 110 liter (76 x 30 x 47 cm) tanks; one placed on each side (Fig. 4.2). The tanks were aligned closely so it was possible for fish to see the side tanks clearly from the center one, but did not allow for direct contact between the catfish and cichlids. This setup limited the stimulus cues to visual cues only. The 206 liter central tank contained a cinder block (15 cm^3) in the center of the tank as a place for the catfish to hide, while having an opening at

each end to allow for the catfish to see both sets of stimuli simultaneously. Terracotta pots (15 cm) were centrally located in each of the side tanks for territorial display areas and spawning by cichlids. Preference zones were marked within the 206 liter central tank on the outside of the tank 10 cm (approximately one average adult catfish body length) from each end. Opaque barriers were placed between the center tank and the side tanks to visually isolate the center tank from the stimulus tanks when trials were not being run. During trials, observers remained isolated from the testing area by using curtains and recording was done remotely using a security camera system with DVR device (Q-See, model QSNDVR4R). Experimental tanks were maintained at $24 \pm 1^\circ\text{C}$. Filtration was turned off during trials so they would not produce any sound or other unwanted stimulus. Trials were recorded between 0800 and 1700 hours, and fish were fed (as described above) following the end of testing for that day.

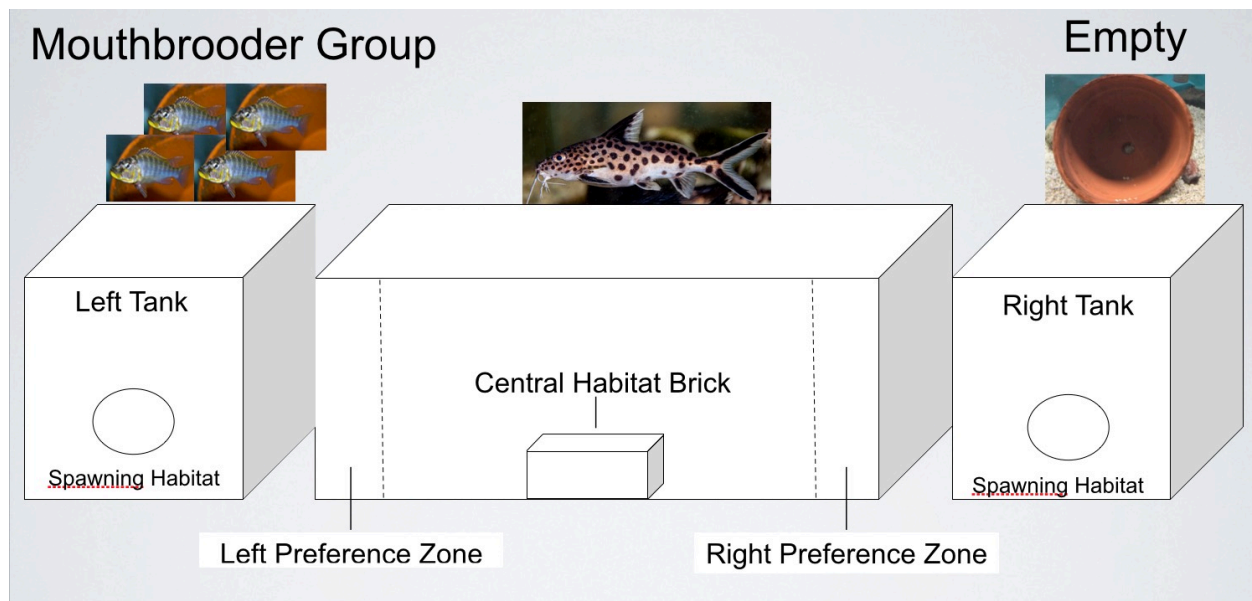


Fig. 4.2. Dichotomous choice setup for trials using live fish. Male or female cuckoo catfish (*S. multipunctatus*) were placed in the central tank (illustrated by a cuckoo catfish picture above the central tank), while a breeding group of mouthbrooding cichlids (*C. horei*) were either presented on the left or right side of the central tank, and an empty tank was presented on the opposite side (illustrated as pictures of a group of mouthbrooders above the left tank and picture of an empty tank above the right tank). The left and right tanks were then switched for a second trial.

A breeding group of adult *C. horei* (one male and three females) mouthbrooding cichlids were placed in one of the 110 liter side aquariums, and the other side aquarium was setup in an identical fashion without fish present (empty tank). The mouthbrooding group was allowed to acclimate to the aquarium and establish territories for a period of one week. These side tanks were located on stands with rolling casters, so they could be easily moved to the opposite side of the central tank between trials. A male or female catfish was selected and their standard length and sex were recorded, and then they were placed in the central tank overnight to acclimate.

A 5-minute pre-trial with the barriers in place was recorded to assess whether or not the catfish showed any preference for either side of the aquarium in the absence of a stimulus. A 15-minute trial was then recorded following removal of the barriers. Then, right tank was moved to the left side of the center tank, and the left tank to the right side of the center tank. The switching of sides was performed as a control to remove any side bias by the catfish. After the first trial, the barriers were replaced and the fish were allowed to rest for 30 minutes before running the second 15-minute trial. The number of seconds spent in each preference zone during trials were recorded, and then averaged between the two trials. A cross into a preference zone was defined as any portion of the fish passing the 10 cm line, and lasted until the last portion of the fish crossed out of the 10 cm zone. A successful trial required that the test catfish perform at least one cross into a preference zone, and any trials where the catfish did not perform were discarded. One additional trial was repeated with non-performing catfish, and if they did not perform again different catfish was tested.

Dichotomous Choice Trials - Video Playback.

Data were collected from 2008-2011. The aforementioned experimental design and methods were repeated with a single 300 liter (47 x 35 x 53 cm) experimental tank placed in the

middle containing either a female or male cuckoo catfish, but with 47 x 30.5 cm LCD flat-screen computer monitors (Dell, model 2009WT) substituted for aquariums on the sides (Fig. 4.3). The monitors were placed so that the bottom of the videos on the screen coincided with the level of the crushed coral gravel in the aquariums, and the level of water was kept equal to the level of the top part of the video on the screen. These two modifications were intended to enhance the appearance of a contiguous environment for the catfish between the end of the central tank and the monitors on the sides.

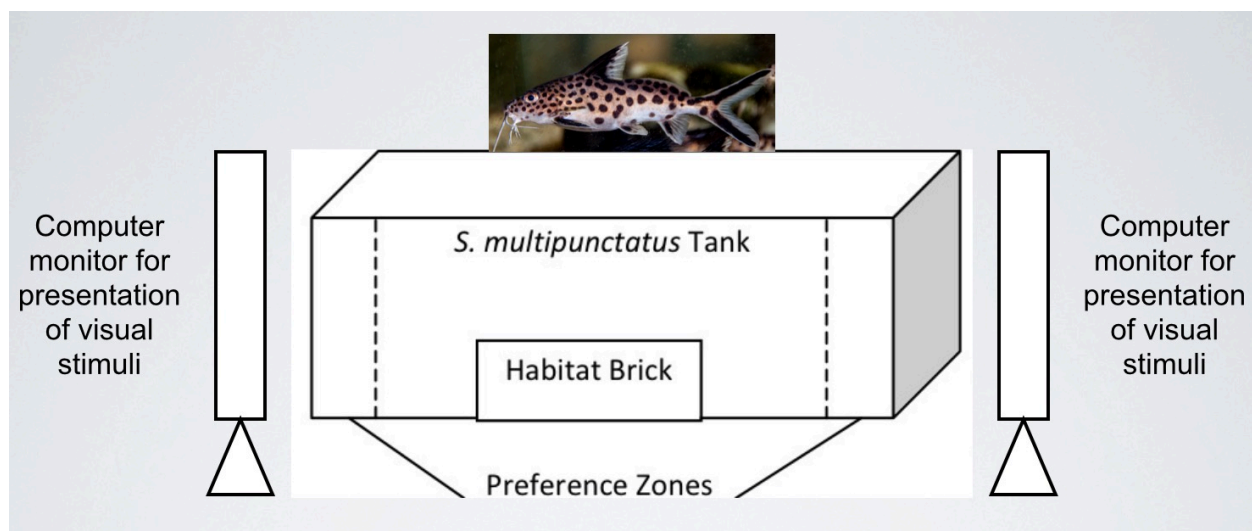


Fig. 4.3. Dichotomous choice setup for trials using video playback. Male or female cuckoo catfish (*S. multipunctatus*) were placed in the central tank (illustrated by a cuckoo catfish picture above the central tank), while different sets of video stimuli were presented on monitors on either the left or right side of the central tank. The stimuli shown on the left and right monitors were then switched for a second trial.

For recorded video sequences, Cichlids were housed in 110 liter (76 x 30 x 47 cm) tanks with a single terracotta pot in the center, and high definition video cameras (Panasonic, model HDC-HS9) were placed the same distance from each tank and set to the same zoom for all recordings, so the cichlids appeared the same size in all videos. A 20-minute sequence was recorded of a *C. horei* group consisting of one male and three females (hereafter “mouthbrooder group” video) exhibiting a variety of common behaviors (e.g. swimming, picking at the gravel,

or remaining motionless at times). This video included no spawning or aggressive behaviors. A 20-minute video was also recorded of an identical empty tank (hereafter “empty tank” video). Finally, A breeding group (3 females and 1 male for *C. horei*, and 1 male and 1 female for *A. nigrofasciata*) was placed and tanks were recorded for at least 10 hours a day until a female holding eggs in her buccal cavity for *C. horei* and eggs on the ground for *A. nigrofasciata* were found. Then the part of the recordings containing the spawning events were found and 20-minute videos of a *C. horei* pair exhibiting spawning behaviors (hereafter “mouthbrooder spawning pair” video), the spawning behaviors of a *A. nigrofasciata* pair (hereafter “substrate spawning pair” video), and a *C. horei* pair performing common non-spawning behaviors (hereafter “mouthbrooder non-spawning pair” video) were created using video editing software (Apple Inc., iMovie).

From these recorded sequences three different sets of dichotomous choice video stimuli were presented to female or male cuckoo catfish. The first set of video stimuli was mouthbrooder group vs. empty tank, the second set was mouthbrooder spawning pair vs. substrate spawning pair, and the third set was mouthbrooder spawning pair vs. mouthbrooder non-spawning pair. The trials for mouthbrooder group vs. empty tank were performed prior and separately from the trials for the other two sets of videos. The videos for mouthbrooder spawning pair vs. substrate spawning pair and mouthbrooder spawning pair vs. mouthbrooder non-spawning pair were presented in random order and in succession.

Statistical Analyses

All statistical test were done using JMP[®] Pro 11 (64-bit). I recorded the time male or female cuckoo catfish spent (in seconds) associating with a stimulus in either preference zone, and then each stimulus was presented on the opposite side for a second trial. The recorded values

were averaged between the two trials, and I compared the averages of time spent in each preference zone using Wilcoxon signed rank tests. I used nonparametric statistics because my data did not meet the assumptions of parametric tests.

Results

I found no evidence of side bias during the pre-trial recordings for any of the experiments (data not shown).

Dichotomous Choice Trials - Live Fish.

I presented 33 female and 25 male cuckoo catfish with the choice of associating with either a mouthbrooder group or an empty tank. I found that both female and male cuckoo catfish spent significantly more time with the mouthbrooder group than the empty tank (Figs. 4.4 and 4.5). Female catfish spent an average of 46.2 seconds with the mouthbrooder group vs. 20.3 seconds with the empty tank (Wilcoxon signed rank test, $S = 167$, $p = 0.002$), while the male catfish spent an average of 73.4 seconds associating with the mouthbrooder group vs. 41.1 seconds with the empty tank (Wilcoxon signed rank test, $S = 88$, $p = 0.01$).

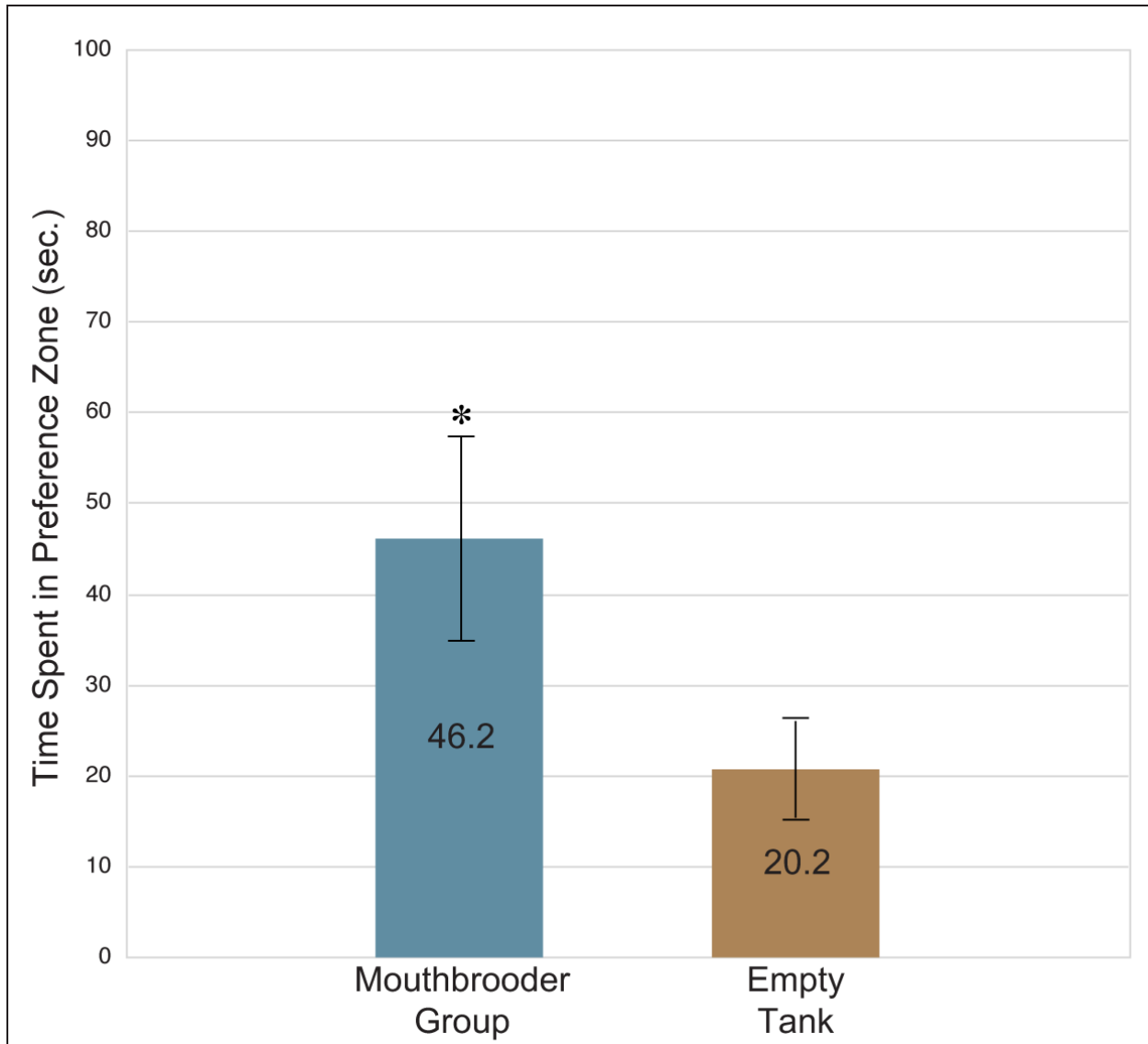


Fig. 4.4. Time spent by female cuckoo catfish (*S. multipunctatus*) with the live group of *C. horei* cichlids (mouthbrooder group) and an empty tank. The catfish spent significantly more time associating with the mouthbrooder group (indicated by *, Wilcoxon signed rank test, $S = 167$, $n = 33$, $p = 0.002$). Error bars indicate one standard error of the mean.

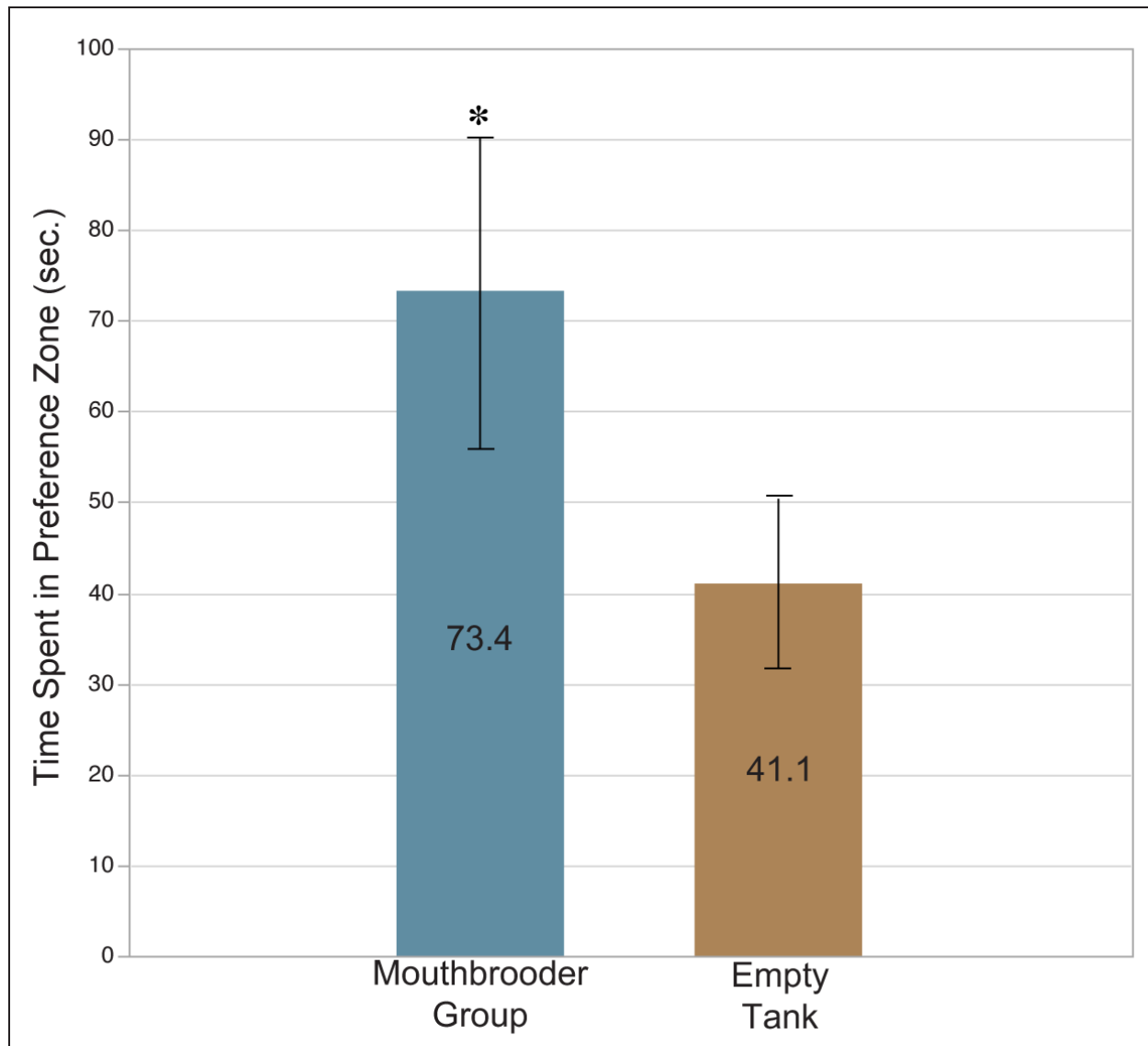


Fig. 4.5. Time spent by male cuckoo catfish (*S. multipunctatus*) with the live group of *C. horei* cichlids (mouthbrooder group) and an empty tank. The catfish spent significantly more time associating with the mouthbrooder group (indicated by *, Wilcoxon signed rank test, $S = 88$, $n = 25$, $p = 0.01$). Error bars indicate one standard error of the mean.

Dichotomous Choice Trials - Video Playback.

I gave 12 female and 12 male cuckoo catfish the choice between a video of a mouthbrooder group, and a video of an empty tank. Similar to the live trials above, both female and male cuckoo catfish spent significantly more time with video of the mouthbrooder group than they did with the video of the empty tank (Figs. 4.6 and 4.7). Female catfish spent an average of 122.6 seconds with the video of the cichlid group vs. 63.5 seconds with the video of

the empty tank (Wilcoxon signed rank test, $S = 39$, $p < 0.001$), while the male catfish spent an average of 84.5 seconds associating with the cichlid group as opposed to 38.1 seconds with the empty tank (Wilcoxon signed rank test, $S = 38$, $p = 0.001$).

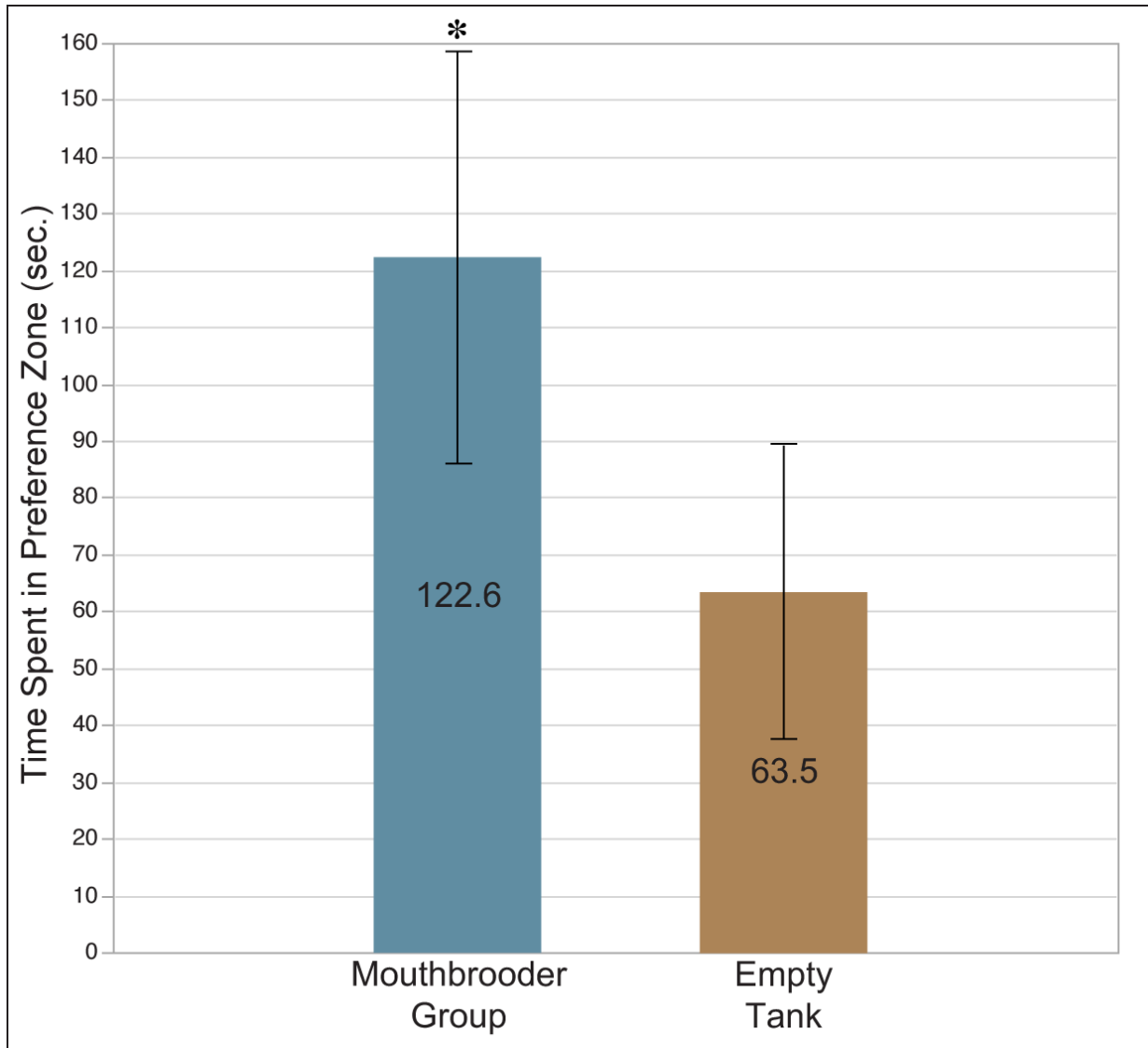


Fig. 4.6. Time spent by female cuckoo catfish (*S. multipunctatus*) with videos of a group of *C. horei* cichlids (mouthbrooder group) and an empty tank. The catfish spent significantly more time associating with the video of the mouthbrooder group (indicated by *, Wilcoxon signed rank test, $S = 39$, $n = 12$, $p < 0.001$). Error bars indicate one standard error of the mean.

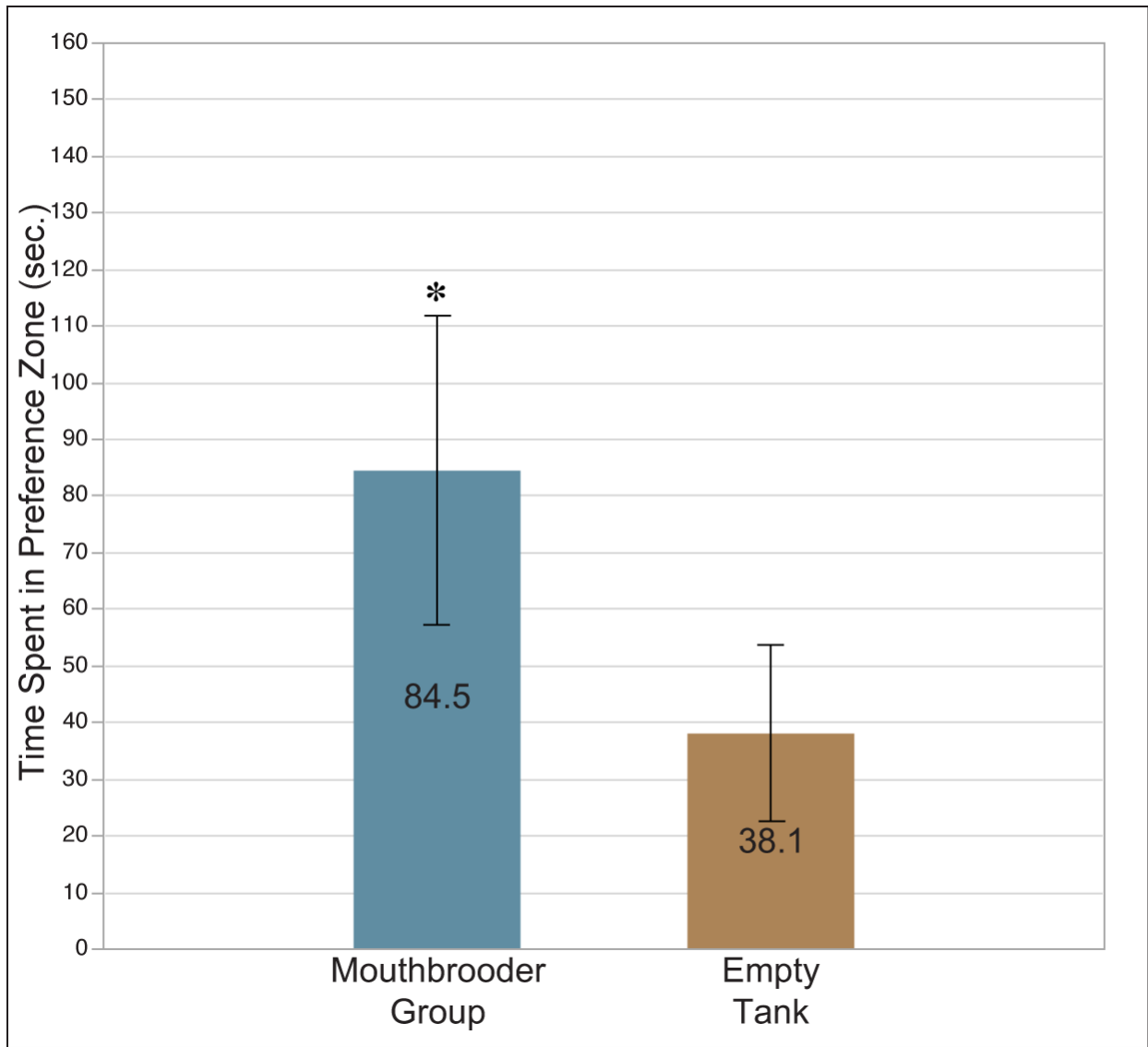


Fig. 4.7. Time spent by male cuckoo catfish (*S. multipunctatus*) with videos of a group of *C. horei* cichlids (mouthbrooder group) and an empty tank. The catfish spent significantly more time associating with the video of the mouthbrooder group (indicated by *, Wilcoxon signed rank test, $S = 38$, $n = 12$, $p = 0.001$). Error bars indicate one standard error of the mean.

32 female and 25 male cuckoo catfish were offered video of a mouthbrooder cichlid spawning pair, or the video of a substrate spawning pair of cichlids. The female cuckoo catfish spent significantly more time with the video of the mouthbrooder spawning pair than the substrate spawning pair, but the male catfish showed no significant preference for either video (Figs. 4.8 and 4.9). The female cuckoo catfish spent an average of 35.7 seconds with the video of the mouthbrooder spawning pair vs. 20.3 seconds with the video of the substrate spawning pair

(Wilcoxon signed rank test, $S = 118.5$, $p = 0.017$), while the male catfish spent an average of 32.7 seconds with the video of the mouthbrooder spawning pair compared to 26.5 seconds with the video of the substrate spawning pair (Wilcoxon signed rank test, $S = 46$, $p = 0.22$)

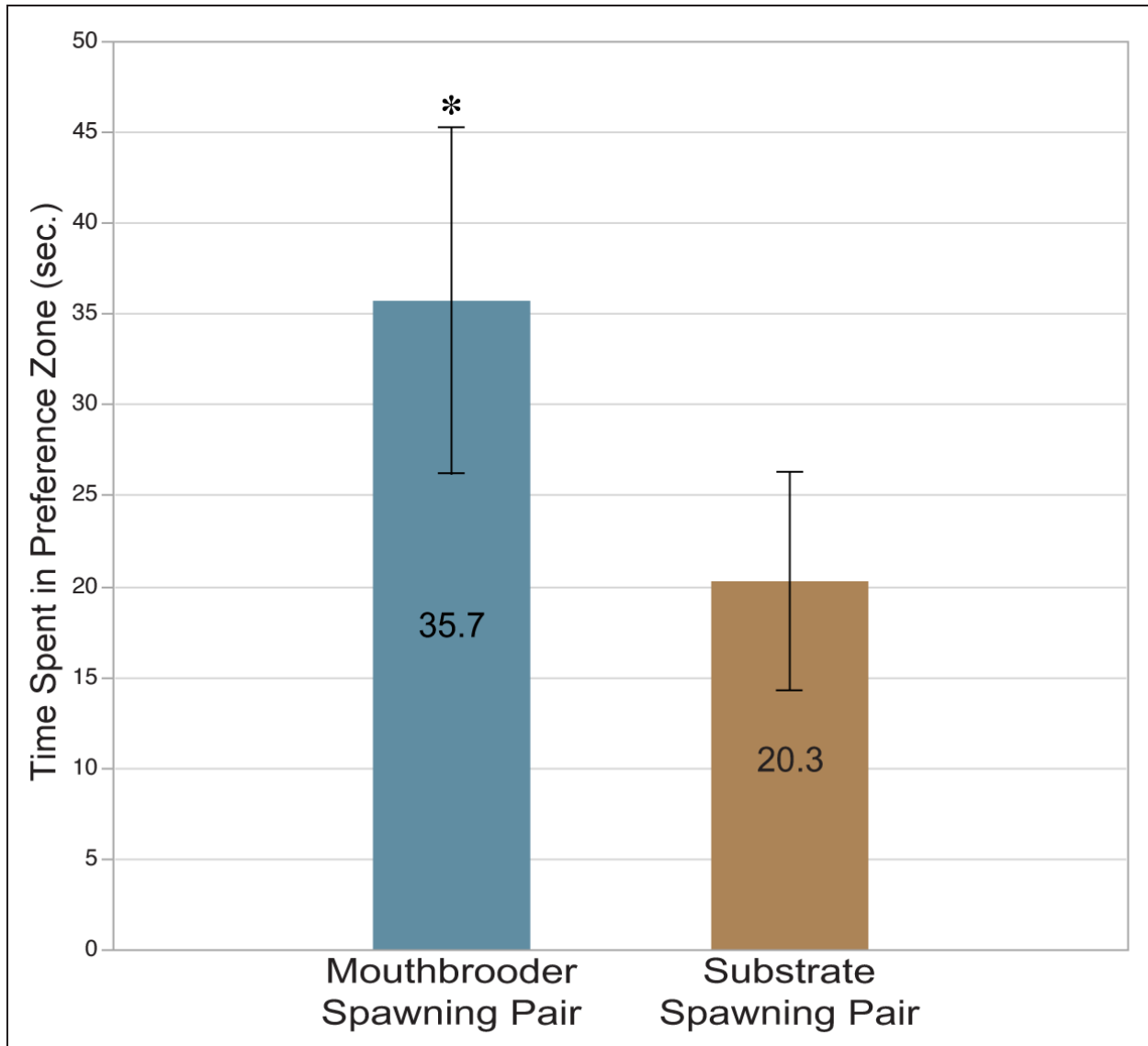


Fig. 4.8. Time spent by female cuckoo catfish (*S. multipunctatus*) with videos of a pair of spawning *C. horei* cichlids (mouthbrooder spawning pair) and a pair of spawning *A. nigrofasciata* cichlids (substrate spawning pair). The catfish spent significantly more time associating with the video of the mouthbrooder spawning pair (indicated by *, Wilcoxon signed rank test, $S = 118.5$, $n = 32$, $p = 0.017$). Error bars indicate one standard error of the mean.

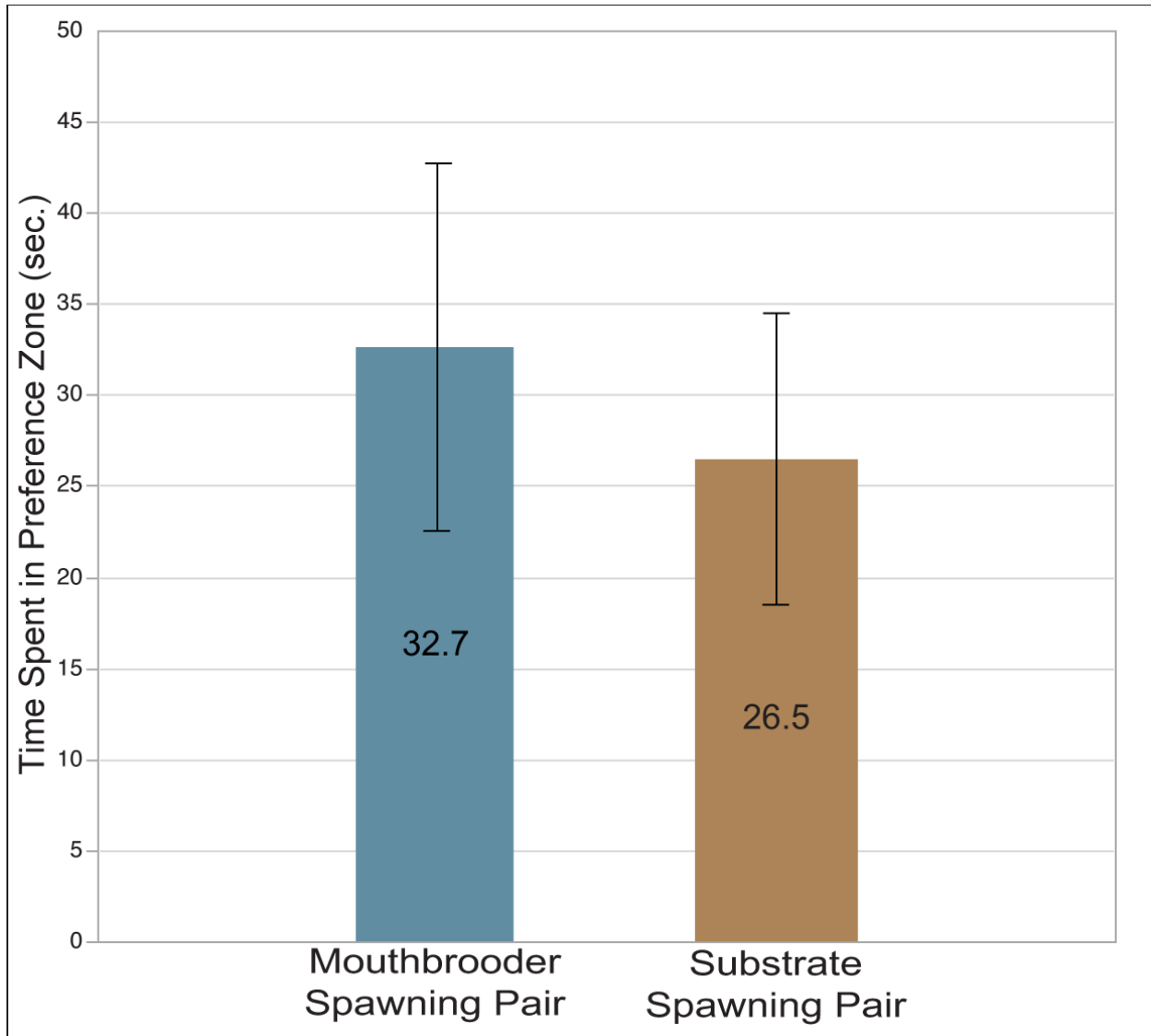


Fig. 4.9. Time spent by male cuckoo catfish (*S. multipunctatus*) with videos of a pair of spawning *C. horei* cichlids (mouthbrooder spawning pair) and a pair of spawning *A. nigrofasciata* cichlids (substrate spawning pair). The catfish showed no preference for either stimulus (Wilcoxon signed rank test, $S = 46$, $n = 25$, $p = 0.22$). Error bars indicate one standard error of the mean.

For the third and final set of video stimuli, I provided 32 female and 29 male cuckoo catfish the ability to select either a video of a mouthbrooder spawning pair of cichlids, or the video of a mouthbrooder non-spawning pair of cichlids. Both the female and male cuckoo catfish showed no significant preference towards either video (Figs. 4.10 and 4.11). The female cuckoo catfish spent an average of 19.2 seconds with the video of the mouthbrooder spawning pair vs.

20.9 seconds with the video of the mouthbrooder non-spawning pair (Wilcoxon signed rank test, $S = 30$, $p = 0.58$), and the male catfish spent an average of 27.3 seconds with the video of the mouthbrooder spawning pair compared to 29.1 seconds with the video of the mouthbrooder non-spawning pair (Wilcoxon signed rank test, $S = 25$, $p = 0.58$)

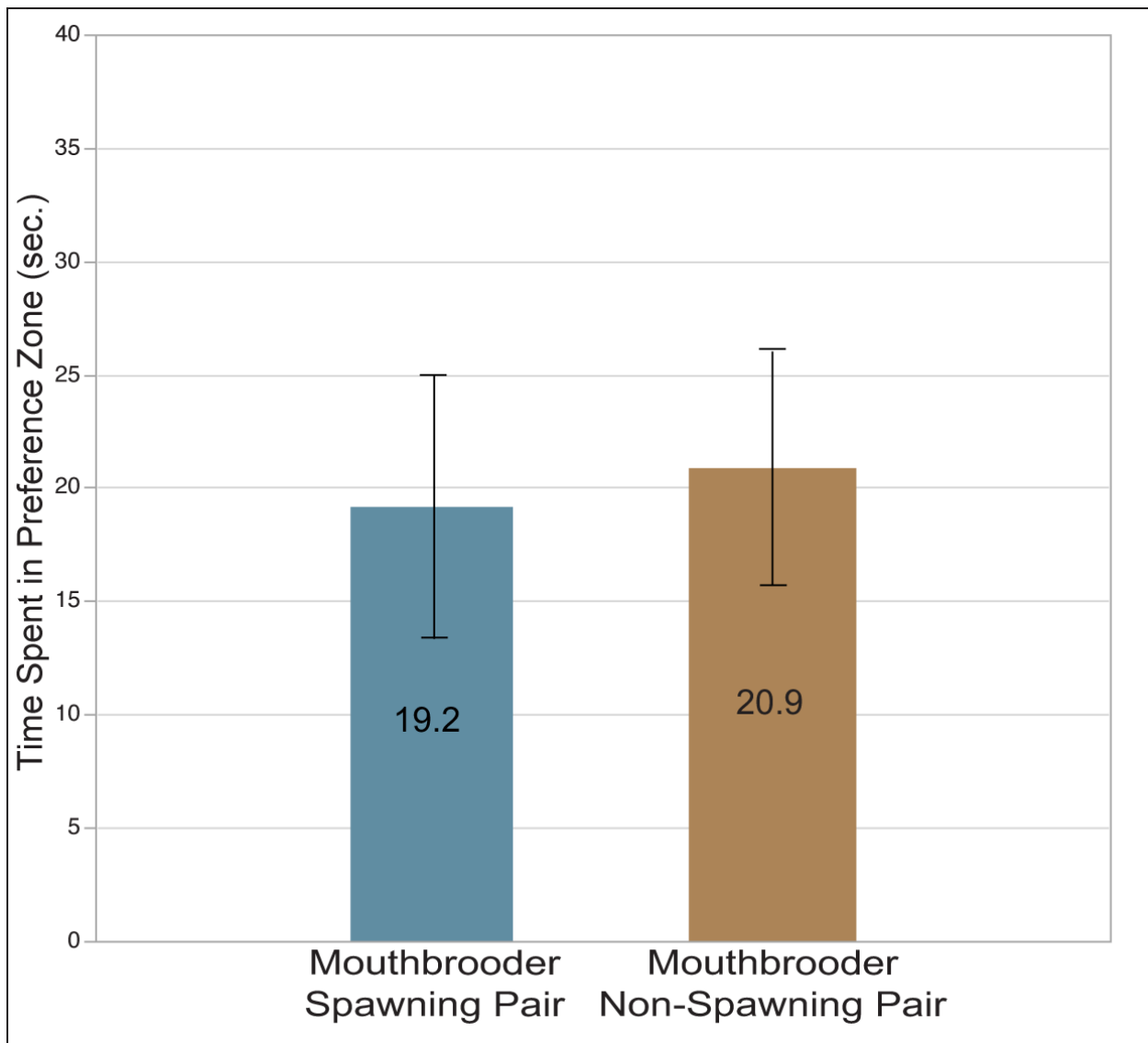


Fig. 4.10. Time spent by female cuckoo catfish (*S. multipunctatus*) with videos of a pair of spawning *C. horei* cichlids (mouthbrooder spawning pair) and a pair of *C. horei* cichlids performing non-spawning behaviors (mouthbrooder non-spawning pair). The catfish showed no preference for either stimulus (Wilcoxon signed rank test, $S = 30$, $n = 32$, $p = 0.58$). Error bars indicate one standard error of the mean.

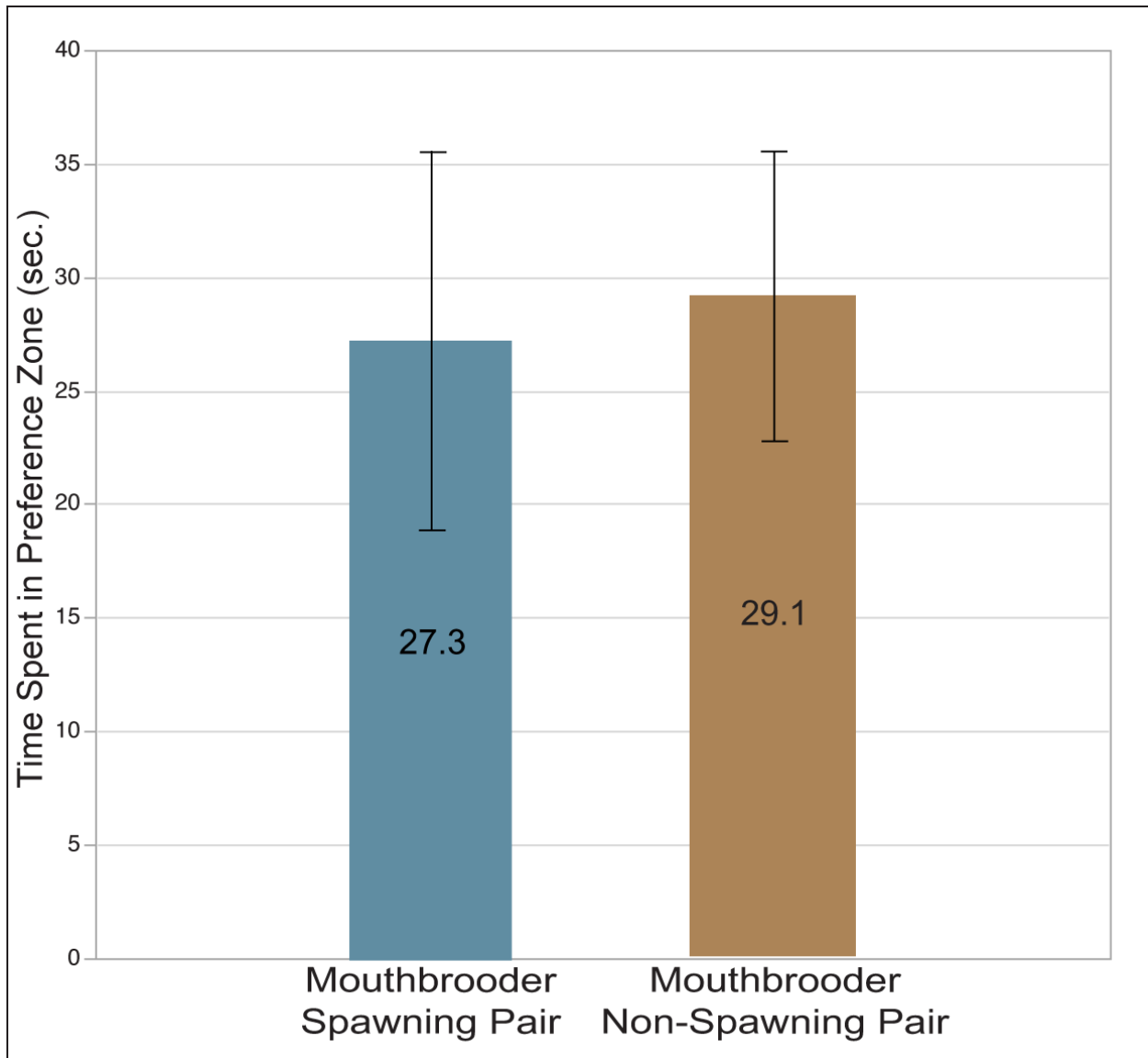


Fig. 4.11. Time spent by male cuckoo catfish (*S. multipunctatus*) with videos of a pair of spawning *C. horei* cichlids (mouthbrooder spawning pair) and a pair of *C. horei* cichlids performing non-spawning behaviors (mouthbrooder non-spawning pair). The catfish showed no preference for either stimulus (Wilcoxon signed rank test, $S = 25$, $n = 29$, $p = 0.58$). Error bars indicate one standard error of the mean.

Discussion

Dichotomous Choice Trials - Live Fish.

I found that both male and female cuckoo catfish spent significantly more time associating with a group of live mouthbrooding cichlids rather than an empty tank. This was an important finding since it established that cuckoo catfish are capable of using vision in the absence of other sensory stimuli to find potential hosts. Considering that the larger shoal of cuckoo catfish travelling around Lake Tanganyika may not be in close proximity to actively spawning cichlids, it is necessary for a pair or small group of catfish to be able to identify when to break off from the shoal and where to go to find suitable hosts to successfully parasitize. Brown-headed cowbirds also decouple breeding sites from the areas they feed in large flocks, and will often commute long distances between the two (Rothstein *et al.* 1984; Curson *et al.* 2000; Chace *et al.* 2003). Additionally, the breeding behaviors of cichlids can be quite conspicuous, and sometimes very large breeding aggregations occur that are comparable to a lek mating system in birds (Fryer and Iles 1972; McKaye 1983; Kuwamura 1986; Kotrschal and Taborsky 2010). Coupled with the fact that Lake Tanganyika is very transparent, and will only become more so as the climate changes and warming trends continue (Hecky 1991; Verburga and Hecky 2009), having high visual acuity is extremely useful for finding spawning cichlids from a distance. Although I discovered that catfish preferred a live cichlid group to an empty tank, I found that it was difficult to correlate any particular behaviors exhibited by the cichlids to any preferences shown by the catfish. Therefore, I used video playback methods in subsequent experiments to present specific behaviors as the stimulus and to parse out which behaviors elicited a response from the catfish.

Dichotomous Choice Trials - Video Playback.

Use of video playback has been successfully applied to many branches of behavioral study with fishes including: aggression, courtship, schooling behavior, and predator-prey dynamics (Clark and Stephenson 1999; Rowland 1999; Johnson and Basolo 2003). It is such a useful tool because it allows for unprecedented control over all stimuli in the experiment. However, before presenting video sequences of specific cichlid behaviors, it was important to confirm that video playback was an appropriate method to use specifically with cuckoo catfish. So, for the second experiment I presented a video of a group of cichlids and a video of an empty tank. I again found that both male and female cuckoo catfish spent significantly more time associating with a group of mouthbrooding cichlids than they did an empty tank. Thus, cuckoo catfish responded appropriately to video as the stimulus. Also, catfish responses were very similar for mouthbrooder group vs. empty tank for both live and video playback trials. The video images seemingly provided enough information to allow discrimination between the videos. Nevertheless, the results of this experiment do not reveal what specific attributes of the cichlid group the cuckoo catfish are responding to.

In the final experiment I presented two sets of video stimuli in random order and in succession to the same female and male cuckoo catfish. The first set of videos gave the catfish a choice between a video of a *C. horei* pair actively spawning (mouthbrooder spawning pair) and video of a pair of *A. nigrofasciata* spawning (substrate spawning pair), while the second set of videos allowed the catfish to select between the mouthbrooder spawning pair video and a video of a pair of *C. horei* cichlids performing non-spawning behaviors (mouthbrooder non-spawning pair). It is important to note that the sample sizes are different for cuckoo catfish males between the second and third set of videos due to the fact that, although both sets of videos were

presented in succession, the data from male or female catfish for either video set are not necessarily from the same male or female. That is, if a particular catfish would not perform in trials for either video set, those null results from that video set were not recorded and I moved on to showing the next video set to that catfish or moved on to testing another catfish until all the adult male and female catfish had been tested. It is serendipitous that the female sample sizes are identical between video sets, as there were both male and female catfish individuals that failed to perform. Since the individuals were not the same between these video sets, I did not perform any analysis to detect video order effects in the data.

For the video set of mouthbrooder spawning pair vs. substrate spawning pair I found that females spent significantly more time with the mouthbrooder pair than the substrate pair, but males showed no significant preference. This is not surprising since the female cuckoo catfish are the ones leading males from the larger shoal into the cichlid spawning areas (pers. obs.), and the females need to be able to recognize the correct spawning behaviors by the cichlids, while males can simply pay attention to wherever the female goes. Females of many species of brood parasitic birds are also choosing which host nests to parasitize, but often do so well in advance of laying (Davies 2000). In contrast, female cuckoo catfish must make a decision about when to parasitize a host quickly as cichlid spawning bouts occur over hours, not days as in birds (Ortega 1998).

I predicted that when presented with the choice of videos between a spawning mouthbrooder pair vs. a non-spawning mouthbrooder pair that they would be able to distinguish between the two and prefer the video with spawning activities. However, I found that neither males nor females showed a preference for either video sequence. These results may indicate that the video sequences I chose in the final video set were similar enough that they did not elicit a

differential response from the cuckoo catfish. For example, I noticed that the mouthbrooder pair was much more active in both videos (i.e. whether they were spawning or just swimming around) than the substrate spawning pair's overall activity level was during their video (pers. obs.). I chose *A. nigrofasciata*, a Central American species, as the substrate spawner in this study since they are easy to breed, of comparable size, and have a relatively similar pattern (e.g. multiple dark vertical bars on a lighter background) to *C. horei*. Tanganyikan substrate spawning species that met the same criteria were difficult to find, nevertheless, the spawning sequence of *A. nigrofasciata* may not be representative of Lake Tanganyika substrate spawners. If, however, the courtship activities of substrate spawners in Lake Tanganyika are also qualitatively different enough from mouthbrooder displays, then it is likely sufficient for the cuckoo catfish to simply recognize large aggregations of spawning mouthbrooding cichlids and investigate further until they find an appropriate spawning pair to parasitize. Also, it is well established that the cuckoo catfish is a host generalist, and although endemic to Lake Tanganyika it has been able to parasitize cichlids from other Rift Valley lakes as well as South American mouthbrooding cichlids in laboratory settings (Loiselle 1998; Wisenden 1999). Therefore, the cues that attract the catfish to breeding mouthbrooders are apparently highly conserved within the family Cichlidae, and so it seems likely that *S. multipunctatus* is able to differentiate between potential host and non-host cichlid species based on behavioral cues alone. Another factor to consider is that these videos were all zoomed in on the small spawning area of just a pair or handful of cichlids, and it is possible that these videos are missing some important signals used by the catfish. In an environment as vast as Lake Tanganyika the cuckoo catfish would have many other contextual cues at their disposal to find and parasitize hosts. Vision may not be the primary sense used once the catfish are in close proximity to actively spawning cichlids.

In addition to vision, cuckoo catfish may be using audition, chemosensory reception (gustation/olfaction), electroreception, or a combination of these. Since cuckoo catfish are otophysan fishes, their swim bladder is attached to their inner ear via the Weberian Apparatus, and as such they have improved hearing sensitivity (Ladich 1999; Lechner and Ladich 2008). Lechner and Ladich (2008) found a species of *Synodontis* catfish had very good hearing in the range of frequencies between 50-300 Hz. Many species of cichlids have been shown to produce sounds during courtship and mating that fall well within the aforementioned 50-300 Hz range (Nelissen 1978; Amorim *et al.* 2003; Simões *et al.* 2008). Nelissen (1978) found that Tanganyikan cichlids in the genus *Tropheus* seem to be more adapted for acoustic communication than visual communication, possibly due to their peak activity levels occurring at night. Therefore, in certain circumstances the catfish may be orienting to sounds breeding cichlids produce entirely in the absence of any available visual cues. Catfish are also highly adapted to using chemosensory cues, as their barbels have highly concentrated numbers of taste buds on them, and they also have taste buds over the entire body surface (Atema 1971; Peters *et al.* 1974; Davenport and Caprio 1982). I have collected preliminary data that shows cuckoo catfish orient towards the smell of cichlid water (data not shown). Furthermore, a Japanese marine catfish has recently been shown to use chemoreception in a remarkable way, whereby these catfish have the ability to detect minute changes in pH from prey respiration (Caprio *et al.* 2014). Another study found that several *Synodontis* are weakly electric and may be using electrical signals for communication (Hagedorn *et al.* 1990). Other studies have suggested the importance of multimodal signaling and cross-modal influences of sensory stimuli in fish behavioral responses (Lugli 1997; Smith and van Staaden 2009; Verzijden *et al.* 2010; Sturmbauer *et al.* 2011; Estramil *et al.* 2014; Maruska and Sisneros 2015). Furthermore, it is

likely that fish use different ways of communicating depending on the situation or distance of the communication (Sturmbauer *et al.* 2011). Therefore, applying a multisensory approach testing not only vision, but also audition, chemoreception, and/or electroreception should be considered.

Conclusions and Future Directions

My results show that video playback is an effective technique for studying visual preferences by the cuckoo catfish, and that these catfish are able to use vision in the absence of any other sensory modality to orient towards potential hosts. Video playback could be used in future experiments to further refine the aspects of the spawning repertoire employed by mouthbrooding cichlids to discover which behaviors the catfish are specifically recognizing. For example, the ritualistic aspects of mouthbrooder spawning behaviors (e.g. males quivering, male and female circling, and spawning positioning) vary little between species (McElroy and Kornfield 1990). Isolating specific behaviors may provide clues as to the activities or behaviors that are eliciting a visual response. Use of a technique like animation could be a powerful way to present particular behaviors. Animation is especially effective because the same individual can be used, with only one feature modified. One possibility would be to create identical videos of computer models of a natural host species (e.g. *C. horei*), and then animate them to present very precise behaviors as stimuli to cuckoo catfish. Little is known about the complicated social behaviors of the cuckoo catfish. Unfortunately, the complex nature of their movements in pairs and groups did not lend itself well to the dichotomous choice design used in the present study, however, additional research into the behavior of the catfish is needed to further address their social dynamics. The results of the present study contribute to our growing knowledge of host-parasite interactions, and lay the groundwork for future experiments aimed at teasing apart the specific stimuli that cuckoo catfish are using to tightly synchronize their spawning with hosts.

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