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Evolutionary Trickery: Brood Parasitism in *Synodontis multipunctatus*

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Evolutionary Trickery: Brood Parasitism in *Synodontis*
multipunctatus

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Undergraduate Honors Thesis

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Abstract

Obligate brood parasitism, the act of reproducing by exploiting the parental investment of a host species to raise young, is fairly well known among bird species (Cruz et al. 2004 *Encycl. Anim. Behav.* ed. M. Bekoff. Greenwood Press, Westport, Conn). There is, however, only one known fish species that is an obligate brood parasite: *Synodontis multipunctatus* from Lake Tanganyika in Africa (Cruz et al. 2004 *Encycl. Anim. Behav.* ed. M. Bekoff. Greenwood Press, Westport, Conn). *S. multipunctatus* was identified as an obligate brood parasite by Sato in 1986 (A brood parasitic catfish of mouthbrooding cichlids in Lake Tanganyika. *Nature*. 323, 58-59), yet the ecology of the system is still understudied. A review of the literature indicates that knowledge of the precise cues that allow *S. multipunctatus* to spawn at the correct time could contribute to a deeper understanding of both the ecology and the evolution of the system. Yet, the cues are currently not fully understood. In a series of three experiments, where the amount of time that *S. multipunctatus* spends in preference zones in a bilateral visual preference setup is recorded to demonstrate visual preference, this paper aims to examine the nature of these visual cues.

An experiment first evaluates the use of video playback as a valid research technique with *S. multipunctatus*. Results indicate that *S. multipunctatus* can see and respond to video as a stimulus in a way that is statistically comparable to live fish as a stimulus. Video playback is then used to compare *S. multipunctatus* preference for the visual spawning sequence of a host (*Ctenochromis horei*) versus a non-host (*Amatitlania nigrofasciata*) species, with no significant differences found. Finally, live fish are used as stimuli to compare the preference between two endemic host species, *Ctenochromis horei* and *Simochromis diagramma*, that are naturally parasitized to differing degrees, with no significant differences. There are many potential explanations for the lack of significant results, including limitations in the experimental design; further experimentation is therefore necessary to reach definitive conclusions. Results did indicate that *S. multipunctatus* physically moves significantly more often when in a pair than when alone, indicating that the social structure of *S. multipunctatus* plays an important role in the parasitic system.

Introduction

Brood parasitism, the act of exploiting the parental investment of a host species to raise young, is the ultimate form of evolutionary trickery. Generally, providing parental care is a biologically costly endeavor for parents (Clutton-Brock 1991). Brood parasitism exploits the investment of non-parent individuals to reduce these costs (Clutton-Brock 1991). While this behavior is fairly well known within bird species, there is only one known fish species able to reproduce exclusively through brood parasitism (Cruz et al. 2004). This obligate brood parasite, *Synodontis multipunctatus*, is commonly known as the cuckoo catfish (Sato 1986). *S. multipunctatus* is found exclusively in Lake Tanganyika in Africa where the endemic species is



Figure 1: A juvenile *Synodontis multipunctatus* fry consuming a host cichlid egg. (M. Hawkins 2008)

able to precisely synchronize its egg-laying and fertilization with that of mouthbrooding cichlid hosts (Sato 1986). As the host pair spawns, a *S. multipunctatus* pair swims into the breeding area and begins to eat the cichlid eggs, while simultaneously releasing their own sperm and eggs.

The cichlid female host then scoops up fertilized catfish eggs of the parasite along with her own eggs into a buccal cavity within her mouth. She then incubates and protects both species' eggs for approximately three weeks, the standard incubation period for the cichlid host. The catfish eggs, however, hatch first and the juvenile catfish fry consume the cichlid

host's own young for their initial source of nourishment as can be seen in Figure 1. Thus, the catfish completely exploit the costly parental investment of their hosts (Cruz et al 2004).

This fascinating and unique breeding mechanism is currently understudied. For both theoretical and practical reasons, the *S. multipunctatus* system is highly biologically interesting. The sequence of behaviors and interactions between species represents an invaluable opportunity to understand more about the fields of ecology and animal behavior. Evolutionarily, the system can add academic understanding to the specifics of how closely associated species have evolved. A working understanding of this system is also critical in the development of any successful conservation plan in the African Rift Valley Lakes.

Nonetheless, the specific cues that allow *S. multipunctatus* to spawn are currently not well understood and the biological implications of this system have yet to be fully investigated. Thus, an experiment to explore the specific visual cues involved in *S. multipunctatus* breeding behavior was designed to increase understanding of this significant system. In order to understand the value of such a study, however, a review of the available information about the ecology, evolution, and conservation implications of brood parasitism is required. Thus, a review of the literature is included here to synthesize current understanding about brood parasitism in order to elucidate subtleties that may have been overlooked in the primary studies.

I. Background to Brood Parasitism

Before one can fully examine the impacts of the *S. multipunctatus* breeding system, it is necessary to understand the general effects of brood parasitism on reproduction. From a biological standpoint, life is all about successful reproduction. Reproduction, however, can itself be detrimental at times. It imposes a cost on an individual when a current reproductive attempt reduces the potential reproductive output of that individual in the future (Smith and Wootton

1995). There are many costs associated with reproducing. The production of eggs and sperm is energetically costly, immune function can be reduced due to hormones that regulate reproduction, and finding and procuring a mate can be a challenging task (Smith and Wootton 1995). For many species however, parental care is by far the most costly aspect of reproduction (Smith and Wootton 1995). Providing parental care can reduce the survival of the parent. Exposure to predators or limits on the parents' ability to procure food, for example, often directly reduces parental survival. The breeding frequency of the parent can be reduced if reproduction cannot continue while parental care is provided and the ability to acquire a mate can be reduced if mate choice is based on physical condition that is negatively impacted by parental care. Nonetheless, about 20% of bony fishes in the teleost group engage in some form of parental care, presumably because the benefits of parental care outweigh the costs (Blumer 1982). In a study examining the effects of hypoxia on the parental behaviors of the species *Pseudocrenilabrus multicolor* native to swampy areas near the Rift Valley Lakes in Africa, for example, it was found that females with young have limited access to oxygen as available oxygen must be portioned between the mother and the young (Reardon and Chapman 2010). Thus, females incubate their young for an optimal time to maximize the benefits of parental care while reducing costs.

Within the family Cichlidae, nearly all species provide an advanced form of parental care to their offspring, although methods for care and variations in which sex is the primary caregiver vary (Gonzalez-Voyer *et al.* 2008). Because cichlids have two sets of jaws, true jaws at the front of their mouths and pharyngeal jaws in their throats, they are extremely dexterous with their mouths. Thus, many cichlid species are able to use their mouths to dig pits or build nests in the substrate and guard their eggs until the young have hatched. This form of parental care is termed

substrate spawning (Barlow 2000). When total numbers of differences in DNA sequences were used to model the evolutionary history of all cichlids, substrate spawning was found to be the ancestral form of parental care (Goodwin *et al.* 1998). A more extreme form of brood care that evolved more recently, termed mouthbrooding, occurs when a parent takes eggs into the mouth and protects the eggs and hatched young within what is termed a buccal cavity. It is estimated that mouthbrooding has evolved independently 10-14 times within the phylogenetic history of cichlids (Goodwin *et al.* 1998).

This latter form of parental care is especially costly for the brooding parent. In an experiment with the maternal mouthbrooding cichlid *Cyphotilapia frontosa*, for example, gut fullness was measured as an index of feeding (Yanagisawa and Ochi 1991). Mouthbrooding females were found to have a gut fullness that was only 19-28% that of non-brooding females, indicating that the females hardly ate while incubating young (Yanagisawa and Ochi 1991). When *Pseudocrenilabrus multicolor victoriae* females were exposed to environments with lowered oxygen levels, the brooding females spent additional time at the surface of the water and, therefore increased their risk of predation compared to their non-brooding counterparts (Correi *et al.* 2008). In *Pseudocrenilabrus multicolor*, brooding females also expended 15.7% more energy than females without their brood as measured by oxygen consumption (Mrowka and Schierwater 1988). In fact, it has been found that female mouthbrooding individuals eat less, grow less, and have to wait a greater amount of time before breeding again due to the intense parental care they provide (Smith and Wootton 1995).

Cost-benefit analysis can be applied to parental care as it can generally be assumed that cumulatively detrimental behaviors would likely be purged from a population by natural selection over large stretches of evolutionary time. When variations in modes of parental care

exist, only those modes that increase the relative success of an individual will remain in the population. Therefore, in a successful species, the benefits of parental care must outweigh the costs (Clutton-Brock 1991). Because there are so many costs associated with advanced parental care in the family Cichlidae, it can therefore be assumed that their parental care is also highly successful at increasing the survival of young. There are several successful methods of parental care that use different strategies to maximize the benefits depending on the specific ecology of the species. In a 1985 study, Carlisle found that the level of parental care in a cichlid species was varied to minimize the costs and maximize the benefits of parental investment in every situation. Within a substrate-spawning species, *Aequidens coeruleopunctatus*, the female stayed by her nest for an increased amount of time and returned to her nest more quickly after her brood was threatened when the size of the brood was experimentally increased. In their 1988 paper, Mrowka and Schierwater used energy consumption rates in *Pseudocrenilabrus multicolor* to conclude that mouthbrooding is a low-cost strategy in this species as females profit from investments made in the period before incubation of the young. These results indicate that parental behavior is carefully balanced to provide the most reproductive success.

It would, however, be especially advantageous to gain successful parental care for offspring while simultaneously vastly reducing parental reproductive costs. Brood parasitism accomplishes this feat. By definition, brood parasitism occurs whenever a non-parent contributes parental care to young. Within fish, brood parasitism most commonly occurs as an optional, facultative act within a single species (DeWoody and Avise 2001). A conspecific adult of the same species lays eggs in a nest where it is not providing parental care. In the paper, “Genetic Perspectives on the Natural History of Fish Mating Systems”, the authors used molecular analysis to determine the maternity and paternity of offspring in nests of several

species of fish that engage in parental care (DeWoody and Avise 2001). The authors found that approximately 33 percent of all nests included young that were not sired by the caregivers and that, of the parasitized nests, nest-guarding adults parented only 70 to 95 percent of the individuals receiving their care. Within the one mouthbrooding cichlid species included, *Pseudotropheus zebra*, the amount of intraspecific parasitism, where host and parasite are of the same species, was slightly lower than the average for the study, but still existed. Thus, potential hosts of *S. multipunctatus* have been found to engage in brood parasitism within their own species.

In facultative brood parasitism, the adults who parasitize others maintain their own nests. Thus, intraspecific brood parasitism can be considered a form of elective “bet hedging”. Defined as dispersing investments to increase the chances of success in at least one investment, bet-hedging theory addresses how individuals should increase their fitness in varying and unpredictable environments (Kozlowski and Stearns 1989). Bet hedging has been identified as a successful method for increasing reproductive success in variable environments for several bird species (Kozlowski and Stearns 1989). It is beneficial to the parents to produce the largest number of fertilized eggs they can successfully care for during a single breeding attempt. This optimal brood size may change unpredictably based on shifts in environmental conditions. Thus, both birds and fish may produce more zygotes than they can care for to insure that the number of offspring receiving parental care can be flexibly adjusted downward to the optimum number for that breeding attempt (Kozlowski and Stearns, 1989). The extra zygotes they produce may then be deposited in non-parental nests. If something should happen to the primary parental nest, the extra zygotes serve as insurance that some parental genes will survive in the next generation. Even within intraspecific brood parasitism, there is a detrimental effect on the success of the

surrogate parents. Adults that raise unrelated individuals incur the same costs of parental care but gain no genetic benefits from the unrelated young who survive.

A more extreme form of brood parasitism occurs between individuals of different species. Such interspecific brood parasitism can be facultative, where the parasitic species maintains its own nest but lays some eggs in other nests, similar to the form of parasitism observed within one species (Robert and Sorci 2001). Interspecific brood parasitism can also be obligatory, however. This occurs when parasitic species are only able to lay eggs in the nests of other species and are no longer able to provide parental care to their own young. Obligate brood parasitism is fairly well known among birds, occurring in approximately 1% of all bird species (Robert and Sorci 2001). For example, the brown-headed cowbird (*Molothrus ater*) is an obligate brood parasite of many species of songbirds in North America (Lichtenstein and Sealy



Figure 2: *Molothrus ater*, Brown-headed cowbird juvenile being fed by a host species, the Reed Warbler (*Acrocephalus*) Photo: H. Olsen

1998). The cowbird greatly reduces the success of its host species. The parasitic young are much larger than the host young and they beg much more intensely (Olsen 2000). Because begging requires energy output from the young, it is normally used as an honest signal of juvenile need within one species. Parents can accurately assess the condition of their young and feed them accordingly. However, parasitic cowbird young beg a great deal more than the honest host young (Olsen 2000). The cowbirds are therefore fed more even though their actual need

may not be as great. Thus, the cowbird young consume a large proportion of the feeding investment of the parents. This directly reduces the survival of the host young (Lichtenstein and Sealy 1998). Because of the differences in the signaling of the young, obligate brood parasites can use a disproportionately large amount of the parental input. Thus, this latter form of brood parasitism is typically more costly to the hosts than any interspecific form (Lichtenstein and Sealy 1998).

Due to the costs the host species incur, many adaptations must have evolved to reduce brood parasitism. However, no studies have yet been completed that examine adaptive behaviors of the cichlid hosts in the *S. multipunctatus* system. Therefore, analogous bird systems currently provide the only information about adaptive defenses against brood parasitism. These adaptations include nest guarding, increased aggression towards parasitic species, egg rejection behaviors, and nest abandonment behaviors (Robertson and Norman 1976). In a 1976 paper, Robertson and Norman identified increased aggression as the preferred mechanism to avoid parasitism, as parents who used this mechanism were able to successfully fledge the highest number of offspring. In their study, host species of the brown-headed cowbird were markedly more aggressive towards intruders near their nests. The host species were also able to distinguish between the parasitic species and other birds near their nests and attacks were especially violent towards brown-headed cowbird females. However, if the cowbird approached the nest with a bowed head, a behavior that induced preening and reduced aggression, the host species were significantly less aggressive. Therefore, Robertson and Norman (1976) found that the aggressive line of defense often failed.

When a nest is parasitized, the host eggs may be ejected from the nest (Clark and Robertson 1981). Counting and identification of one's own eggs, with both learned and innate

components, is necessary to successfully eject host eggs. Species with eggs that are markedly different from parasitic eggs (i.e. the American Robin, *Turdus migratorius*) are often able to eject the parasitic eggs (Clark and Robertson 1981). Other species may notice their nest has been parasitized but may be unable to distinguish or eject only the parasitic eggs. Many such species have evolved nest abandonment strategies where parents leave their nests to begin anew. One species, the yellow warbler (*Dendroica petechia*), engages in nest burial behavior, where parents build another layer over a parasitized brood (Clark and Robertson 1981). In a 1981 study, 41% of yellow warbler broods were parasitized. The success of a parasitized nest that was buried was significantly greater than nests in which parasitic young hatched (Clark and Robertson 1981). Selection favors hosts that best avoid parasitism and, paradoxically, also favors parasites that can best exploit their hosts. The brood parasite-host interrelationship is, therefore, a dynamic system that can shed important light into behavioral ecology and co-evolutionary patterns.

II. Brood Parasitism in the *Synodontis multipunctatus* system

In 1986, Sato discovered that *Synodontis multipunctatus* was an obligate brood parasite, making it the only known obligate brood parasite that is a fish. Since the discovery of the reproductive scheme of this fish, a surprisingly sparse body of research has developed to describe the unique and fascinating breeding biology. Filling this void is crucially important to expand the biological understanding of ecology, evolution, and conservation related to the breeding system. Similar to obligate brood parasitism in birds, *S. multipunctatus* adults are only able to reproduce by laying their eggs in the nests of their hosts (Sato 1986). The ‘nests’ of the hosts are within buccal cavities inside of the cichlid female’s mouth, however. Therefore, *S. multipunctatus* adults must spawn in carefully timed synchrony with their hosts so that their eggs

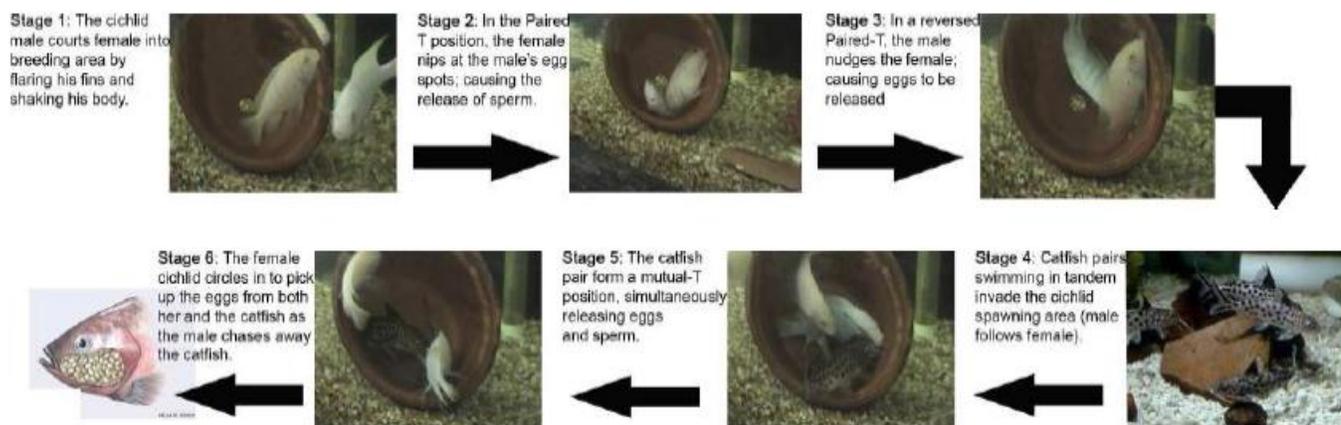


Figure 3: A diagram detailing the stages involved in the parasitism of a cichlid host by *S. multipunctatus* (M. Hawkins 2008)

are incorporated into the mouth of the host. Sato (1986) found that the developmental stages of the *S. multipunctatus* young closely matched that of the cichlid young, indicating that both species of eggs were deposited at only one time. This same behavior has been observed in laboratory settings. Observation of developmental sequences revealed that the *S. multipunctatus* young hatch in advance of the cichlid young so that they are at a developmental stage that allows them to ingest the cichlid young (Hawkins 2008). This is further evidence for the importance of precisely timed *S. multipunctatus* spawning. However, there is currently little known about the specific cues that allow *S. multipunctatus* to spawn at the correct time.

Exploratory research by Wisenden (1999) indicates that the catfish may be cuing into visual signals used in the spawning sequence of the cichlid hosts to time their own reproduction. In a laboratory setting, Wisenden (1999) found successful parasitism by the cuckoo catfish in not only sympatric species naturally occurring in the same location in Lake Tanganyika, but also in naturally geographically separated, or allopatric, species from other Rift Valley Lakes. The catfish were even able to parasitize mouthbrooding cichlid species naturally found in South America. Thus, it can be concluded that the proximate cues used by *S. multipunctatus* to identify potential hosts during spawning are highly conserved and widespread, even though they remain

unidentified to this day. In 2004, the sequence of spawning was further described by Cruz *et al.* The authors found that the adult *S. multipunctatus* pair uses a combination of visual and chemical, pheromonal cues to identify spawning hosts at the correct time. A catfish pair then enters the spawning area, swimming in tandem with the female leading the male. The catfish begin to eat the cichlid eggs and release their own eggs and sperm concurrently. The female cichlid then takes both species of egg into her mouth to be incubated. Figure 3 summarizes these steps.

S. multipunctatus females are known to lay eggs in small numbers of about 30 at a time over a period of several days (Cruz *et al.* 2004). This adaptation has been termed fractional spawning. Unlike bird brood parasites that have up to a week to lay their eggs in a host nest, *S. multipunctatus* adults have a window of less than an hour in which to lay eggs while the host is spawning. Thus, this fractional spawning adaptation presumably increases the probability that some of the catfish eggs will be successfully incorporated into a host's brood (Cruz *et al.* 2004). Even with the discovery of this adaptation, the timing of the spawning must be so precisely coordinated that additional research into the specific cues that allow synchronous spawning to occur remains a biologically pertinent goal.

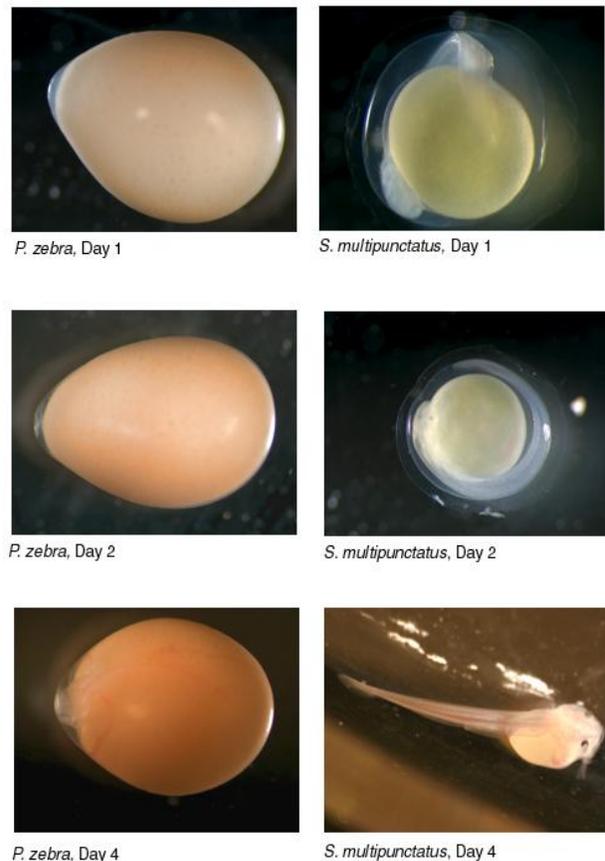


Figure 4: Comparative photographs of the developmental sequence of *S. multipunctatus* (on the right) and a host species, *Pseudotropheus zebra* (on the left) (Hawkins 2008)

S. multipunctatus eggs hatch first within the buccal cavity and the juvenile catfish feed on the cichlid young for their initial source of nourishment (Sato 1986). Thus, the catfish fully exploit the parental investment of their hosts at a level that is even greater than is known in analogous bird systems; the juveniles directly predate on the host young instead of lowering host success through competition. For this reason, adaptations to avoid parasitism could be very important in long-term success of the cichlid host species. Sato's initial paper indicated differing rates of parasitism among different host species (1986). For example, 15% of *Ctenochromis horei* broods were parasitized and 7.7% of *Simochromis diagramma* broods were parasitized (Sato 1986). The reason for these differences in parasitism rates is unknown as nothing is currently known about any reproductive adaptations of the hosts to avoid parasitism. Larval size and skeletal development between a sympatric and an allopatric species parasitized in laboratory settings were compared in a study by Rosario, Hawkins and Cruz (2008). There were no significant differences found between the different cichlid species examined, and thus no evidence for developmental adaptations to avoid parasitism. The catfish eggs are known to be significantly smaller and differently colored in comparison to the eggs of their hosts (2.78 mm compared to that of the host eggs at 4-6 mm) (Sato 1986). There are, however, no known cases of egg rejection behavior within the cichlids. Mouthbrooding females are apparently in such a rush to scoop up their own eggs during spawning to protect them that they occasionally even pick up rocks and bits of coral. Thus, it is unlikely that females distinguish at all between the species of egg that they take into their buccal cavities.

One species of cichlid, *Simochromis diagramma*, displays interruptions during its spawning sequence that could potentially offer some defense against brood parasitism (Martin 2009). Preliminary results indicate that *S. diagramma* females release eggs one at a time and

males completely clear the spawning area of debris before spawning occurs. These actions may make *S. diagramma* especially efficient at scooping eggs into the buccal cavity and may, therefore, reduce parasitism rates (Martin 2009). However, additional research is necessary on the topic of host adaptations to brood parasitism. Cichlid adaptations to brood parasitism have yet to be fully investigated or explained. Even if no significant host adaptations were to be discovered, additional research is still necessary to understand the evolutionary explanations for a lack of anti-parasitic adaptations.

III. Evolution of Brood Parasitism

S. multipunctatus is endemic to Lake Tanganyika, a Rift Valley Lake in Africa. As a group, the Rift Valley Lakes (Malawi, Tanganyika, and Victoria) are the paramount model system for the study of evolution (Brichard 1989). There is a tremendous amount of biodiversity in each lake. In fact, it is thought that there may be more species of fish in the rift lakes than in the entire Atlantic Ocean (A. Cruz, personal communication). According to Conservation International, 69.1% of the freshwater fishes are endemic to the region. Cichlid fishes form the majority of fish species in the Rift Valley Lakes, with an estimated 1,800 species in Lake Tanganyika alone (Kolbmuller *et al.* 2006). The geology of the lakes is such that the timing of historic events can be modeled rather accurately (Kornfield and Smith 2000). Therefore, it is known that the lakes underwent periods of explosive speciation and adaptive radiation in geologically recent times. The rapidity of the radiation of the cichlid fishes has been associated with habitat complexity, lake level fluctuation, reproductive characteristics, trophic polymorphism, and sexual selection within the cichlids (Kornfield and Smith 2000). All cichlids maintain high levels of parental care. Within Lakes Victoria and Malawi, 100% of the cichlid species are mouthbrooders. Within Lake Tanganyika, 33% of the cichlid species are mouthbrooders (Brichard 1989).

Synodontis multipunctatus belongs to the greater genus of squeaker catfishes (*Pisces*, *Mochokidae*, *Synodontis*). These fish are widely distributed throughout Africa and inhabit a biogeographic range similar to that of the cichlids (Brichard 1989). In 2006, Kolbmüller *et al.* used mitochondrial DNA to reconstruct the phylogeny of *Synodontis* species. The authors identified six distinct lineages within the phylogenetic clade of related East African catfishes. Most interestingly, the authors found that *S. multipunctatus* diverged about 2 million years ago, approximately the same time that the mouthbrooding haplochromines, the hosts of *S. multipunctatus*, diverged. Additionally, an accelerated rate of molecular evolution was identified for *S. multipunctatus* in comparison to the other major *Synodontis* lineages. In a similar study in birds, it was found that a faster rate of DNA sequence evolution exists for brood parasites in African finches compared to non-parasitic lineages (Sorenson and Payne 2001). This accelerated rate of evolution may be a result of co-evolutionary processes between the parasite and the host species. The precise adaptations that would drive this expedited “evolutionary arms race” are unknown in the *S. multipunctatus* system. Because the adaptations that allow the catfish to parasitize its host have not been described, the underlying evolutionary processes cannot yet be fully investigated.

In 2001, Robert and Sorci described the evolution of obligate interspecific brood parasitism in birds. The authors used a clutch optimization model; only birds within a single species that lay more eggs than they can provide parental care for will parasitize other nests. The authors then postulated that species that experienced pressures to lay more eggs and to provide less parental care would eventually begin to engage in obligate interspecific brood parasitism. However, this explanation for the evolution of brood parasitism cannot explain brood parasitism within the catfish as no *Synodontis* species provides parental care to its own offspring. All

Synodontis species except for *S. multipunctatus* engage in 'broadcast spawning' where large numbers of eggs are laid, fertilized, and then abandoned (Robert and Sorci 2001). The young receive no parental care. Thus, the evolution of the breeding system of *S. multipunctatus* must have arisen in a very different way in comparison to the analogous bird system. The evolutionary history of obligate brood parasitism in fish can only be described with additional understanding of the specific mechanisms that allow parasitism to occur. Thus, to understand how such a unique breeding system could have evolved, additional research into the breeding system itself is required.

IV. Implications of Brood Parasitism and the Importance of Effective Conservation Strategies

Brood parasitism has implications on the dynamics of an entire community. In healthy ecosystems with native brood parasite populations, hosts and parasites exist in a fragile state of constant evolutionary competition. An evolutionary arms race drives coevolution, where adaptations in one species evolve in response to adaptations in the other (Kus 2002). Over evolutionary time, these adaptations prevent both groups from being completely decimated. However, this fragile balance is easily upset. The influences of humans often contribute to ecological interruptions that threaten the continued existence of species (Knight and Turner 1998). Because of the large costs of parasitism on host species, the presence of an unchecked brood parasite can grossly reduce host population success (Knight and Turner 1998).

Within bird species, there are numerous examples of the detrimental effects of unimpeded brood parasitism on host populations. Because of anthropogenic influences on habitats, brood parasite populations can grow far beyond their natural levels. For example, the

brown-headed cowbird prefers open areas on the edges of forests (Chace *et al.* 2003). Habitat fragmentation due to human development increases the amount of this kind of open habitat. In Boulder, Colorado for example, it was found that the presence of the brown-headed cowbird increased significantly as the distance from the urban/wildland interface decreased (Chace *et al.* 2003). Thus, human activities can increase the success of the brood parasite and cause the population size to boom. When this occurs, even native host populations can be negatively impacted by brood parasitism (Kus 2002). In another example, the endangered least bell's vireo (*Vireo bellii pusillus*) has significantly lower levels of successful reproduction when parasitized by the brown-headed cowbird (Kus 2002). The least bell's vireo has a nest abandonment adaptation that, in natural conditions, increases the breeding success of the parents (Kus 2002). However, the population of brown-headed cowbirds in the least bell's vireo's habitat has reached such a high level that 43% of all nests are parasitized (Kus 2002). Broods are often parasitized in several subsequent breeding attempts and nest success quickly drops to zero. Since 1980, when the species was added to the endangered species list, its numbers have dropped to less than 300 individuals primarily due to the effects of brood parasitism (Kus 2002).

Additionally, brood parasites have been introduced or have expanded into areas where they were previously unknown. This can have a devastating effect on native, naïve populations with no experience with brood parasites. For example, in the West Indies, the shiny cowbird (*Molothrus bonariensis*) only became widespread within the last century (Cruz *et al.* 1985). Because native avian species did not coevolve in the presence of a brood parasite, the shiny cowbird is extremely successful at exploiting nests in the region (Cruz *et al.* 1985). In mangrove forests in Puerto Rico, it was found that 61% of native passerine bird species experienced brood parasitism and that the parasitism depressed nest success below that of non-parasitized nests

(Cruz *et al.* 1985). The shiny cowbird has been tied to the decline of several endemic house wrens on Grenada, the yellow warbler (*Dendroica petechia*) on Barbados, the Martinique oriole (*Icterus bonana*), and the yellow-shouldered blackbird (*Agelaius xanthomus*) on Puerto Rico (where 94.2% of nests assayed were parasitized), among others (Weaver 1985). Conservation efforts for many species of birds, therefore, depend on understanding the role of brood parasitism. This same concept can easily be extended to fish brood parasitism in the African Rift Valley Lakes.

Although there are currently no known devastating effects of *S. multipunctatus* on African cichlid populations, the cichlid populations in the African Rift Valley Lakes are definitely in danger (Seehausen *et al.* 1997). Unless conservation strategies are implemented, this intricate host-parasite behavior may be destroyed before it can even begin to be understood. Anthropogenic activities are seriously damaging lake populations (Seehausen *et al.* 1997). For example, pollution and increased turbidity and sedimentation in Lake Victoria have been linked to the disappearance of many Haplochromine cichlids (Seehausen *et al.* 1997). Many cichlid species in the Rift Valley Lakes have ecologically similar habitats. Often, sexual selection based on male coloration defines species and acts as a reproductive barrier to inbreeding. Sexual selection based on coloration and visual display is, in fact, one defining factor that helps to explain the extremely high species number in the lakes (Turner 1999). In areas that have become turbid as a result of anthropogenic actions, dull fish coloration, few color versions, and low species diversity are all found. By constraining the color vision of the fish, both turbidity and algae growth due to eutrophication interfere with mate choice, relax sexual selection, and block the reproductive isolation that differentiates species (Knight and Turner 1998). Therefore, loss

of visibility due to anthropogenic events is apparently responsible for a reduction in diversity that has resulted in an overall decline in Haplichromine numbers (Knight and Turner 1998).

Invasive species have also been recognized in greatly reducing cichlid numbers in the Rift Valley Lakes. For example, the Nile perch (*Lates niloticus*, a voracious predator that can reach 2 meters in length) was introduced into Lake Victoria in the 1950s (Ogutu-Ohwayo 1990). Since its introduction, the perch has contributed to a massive decline in populations of Haplichromine cichlids and a complete restructuring of the food web in the lake (Ogutu-Ohwayo 1990). The Nile perch has not only had a devastating effect on the ecology and species composition of Lake Victoria, however, but the introduced species is also partially responsible for famine, fishery-related political upheaval, and war in the region. Because of their large size and quality meat, the Nile perch and other introduced species form the basis of the current fishing industry in Uganda, Kenya, and Tanzania (Kitchell *et al.* 1997). However, a large amount of the fish that is caught is exported to European nations for profit; many locals are consequently unable to afford fish and, thus, a valuable source of protein is unavailable (Kitchell *et al.* 1997). Ironically, the success of the fish industry can be tied to famine in the region. In the documentary, *Darwin's Nightmare*, Hubert Sauper (2004) found that the same cargo planes that transport Nile perch often carry Russian ammunitions to the volatile African nations surrounding Lake Victoria. Sauper stated, "In the Eastern Congo alone, the casualties of war on each single day equal the number of deaths of September 11 in New York." Although it may initially seem like an unlikely consequence, the introduced species of fish in Lake Victoria is having a direct impact on human wars in Eastern Africa. Thus, there are human reasons beyond just preservation of the environment to develop effective conservation strategies.

It is believed that the ecosystem of Lake Victoria cannot be restored to its stable, ancestral condition (Balirwa *et al.* 2003). Many of its endemic species have already gone extinct, habitats are being irreversibly altered through anthropogenic events, and the fishing industry continues to decimate remaining species of large cichlids for food (Balirwa *et al.* 2003). However, the fishing industry based on Nile perch could potentially have some hidden benefits for species of smaller prey cichlids. In a 2003 study, Balirwa *et al.* found that overfishing of the Nile perch for export is allowing a small comeback of native fish. However, Lake Victoria can still be considered one of the most extreme cases of mass extinction documented in the 21st century (Balirwa *et al.* 2003). In order to halt the decimation of Lake Victoria and to conserve the endemic species that remain, a radical conservation plan is needed.

Although Lake Tanganyika does not currently exhibit the same level of ecological devastation as is seen in Lake Victoria, similar pressures plague the future of this important biological site (Balirwa *et al.* 2003). In designing any high-quality conservation plan to preserve Lake Tanganyika, it seems that the role of *S. multipunctatus* must be fully understood. Because of the grave impact that analogous avian brood parasitic systems have had on host populations, understanding the specific ecology of *S. multipunctatus* as a brood parasite is critical to the continued success of all native mouthbrooding hosts. Although the catfish is a native of Lake Tanganyika, human-caused changes in the lake environment could potentially cause an increase in population numbers which could, in turn, be devastating to host populations of cichlids. In addition, brood parasitism within a fish species is so rare that efforts must be made to maintain *S. multipunctatus* populations for ecological research.

Successful conservation plans cannot exist in a vacuum; all aspects of the natural biology of a system must be considered in order to reduce the effects of humans and conserve the

ecosystem for the future. There is currently an artificial division between animal behavior research and conservation biology. William Sutherland argues that behavioral studies are crucial both for generating support for conservation and for creating successful conservation plans (1998). Therefore, attaining a deeper understanding of *S. multipunctatus* reproductive strategies has lasting implications in understanding novel biological phenomena and also in practical, conservation plan development.

Experimental Section

I. Introduction

It is clear that there is a need to engage in research to begin to more fully understand the implications of brood parasitism in the *Synodontis multipunctatus* system on ecology, evolution, and conservation. Although many questions remain unanswered about the system, perhaps the most glaring is how *S. multipunctatus* is able to spawn in such close synchrony with its cichlid hosts. The answer to this question should clarify the close behavioral association between the brood parasite and its hosts, a relationship that is especially intriguing in that it elucidates the breeding ecology of a number of biologically important species. Understanding the specifics of how *S. multipunctatus* is able to parasitize cichlid hosts will also potentially expose evolutionary co-adaptations and direct further research into the evolutionary history of this unique breeding system. Finally, any effective conservation plan must consider the visual behaviors involved in this brood parasitic system to predict and plan how best to preserve the environment.

The present investigation, therefore, aimed to explore the role of visual cues in catfish breeding biology in a series of three related experiments that answered the questions 1) Can video playback be used as a valid research technique for *Synodontis multipunctatus*? 2) Can *Synodontis multipunctatus* visually distinguish between the spawning sequence of a host species and a closely related, non-host species? and 3) Why are different species of mouth brooding cichlid hosts differentially parasitized by *Synodontis multipunctatus*?

First, the use of recorded video as a visual stimulus was established as a valid experimental technique in the *S. multipunctatus* system. Vision is the dominant sense in many fish species (Rowland 1999). The relatively large size of the eyes in comparison to body size in many

species, including *S. multipunctatus*, can be used as an indicator of the importance of vision. For this reason, experimental techniques to study the role of vision in fish can be extremely valuable. Ideally, studying visual behavior in a controlled setting can allow the experimenter to tease apart the specific stimuli that evoke or modify behaviors. There are currently many different approaches used to investigate the behavioral role of vision in fish.

Using live fish as stimuli has many ethological benefits. Interactions between live fish are clearly the most natural. In a 1948 study, Tinbergen used observations of live fish to conclude that a series of visual cues between male and female three spine sticklebacks creates a chain of behaviors that eventually results in spawning. Observation of unaltered, live fish is one of the least intrusive methods to study the behavior of fish. However, it can be difficult, if not impossible, to control variables to allow valid conclusions about the causation of behaviors. For this reason, the use of video playback has recently increased in popularity (Trainor and Basolo 2000).

Particular behavioral sequences can be recorded and played back to experimental subjects to study behavioral reactions to isolated events. Video playback offers many opportunities for manipulating visual stimuli; it provides for control of physical characteristics such as body shape and body size as well as allowing for isolation and manipulation of specific behaviors (Trainor and Basolo 2000). Using recorded video as a stimulus has been established as a valid research technique to study fish behavior in several species. For example, in 2000 Trainor and Basolo compared the response of female green swordtail, *Xiphophorus helleri*, when presented with live and video playback stimuli. They found that the females responded equally to both methods of stimuli presentation. Video-editing programs and animations can allow for even greater experimenter control. In a recent study, computer animation was paired with video playback

with the cichlid *Pelvicachromis taeniatus* (Baldauf *et al.* 2009). The experimenters investigated elements of the complex nuptial coloration within the species and concluded that computer animation can be a flexible and useful investigational tool with cichlids.

There are, however, potential downfalls of using video playback experimentally. Video playback technology was engineered specifically for viewing by human observers (Rowland 1999). For this reason, 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 important to confirm that the subject responds to the image in a comparable way to the live fish stimulus. Because there is a possibility that the video playback may appear differently to *S. multipunctatus* in comparison with a live stimulus, the present experiment aimed to investigate video playback as a research technique for *S. multipunctatus* specifically.

Synodontis multipunctatus was presented with a bilateral visual preference setup with video playback stimuli of *Ctenochromis horei* on one side and video playback stimuli of an empty tank on the other side. It was predicted that the catfish would spend a significantly greater amount of time in the preference zone near the video of the fish versus in the preference zone near video of an empty tank as these results had been previously established using live fish as stimuli in research completed by Marcus Cohen. If similar results were obtained using video playback, it could therefore be assumed that the catfish responds to video stimuli in a similar way to live stimuli.

Second, preference of the parasite for the visual spawning sequence of an endemic natural host species versus a related, non-host species was assayed. *Synodontis multipunctatus* is a host

generalist known to parasitize mouthbrooding cichlids from around the world (Brichard 1989). Although the catfish is endemic to Lake Tanganyika in Africa, it has been able to parasitize South American mouthbrooding cichlids in laboratory settings (Brichard 1989). Therefore, the cues that attract the catfish to cichlid spawning are apparently highly conserved within the cichlid family. However, cichlid species that protect their fry within a buccal cavity (mouthbrooders) and provide their own eggs as food for the catfish fry are the only ones that can serve as effective surrogate parents for the catfish fry. The species that do not protect their young in this way make nests (substrate spawners) and cannot be parasitized by the catfish (Ochi 1993). In Lake Tanganyika, both mouthbrooding cichlids and substrate spawning cichlids are present. In a preliminary study, Kuwamura (1986) identified 17 substrate-brooding species and 31 mouthbrooding species along the East-middle coast of the lake. In fact, the lake is known for harboring the one of the most ecologically, morphologically, and behaviorally diverse assemblages of cichlid fishes (Salzburger *et al.* 2002). One explanation for this impressive example of adaptive radiation may be rooted in behavioral diversification (Salzburger *et al.* 2002). Therefore, it seems likely that *S. multipunctatus* is able to differentiate between potential host and non-host cichlids based on behavioral cues.

To test the validity of this assumption, the visual preference of the catfish when presented with the potential host spawning sequence of *Ctenochromis horei* versus a non-host spawning-sequence of the convict cichlid, *Amatitlania nigrofasciata* was tested. The convict cichlid is a substrate spawning species that is visually similar to natural host species but is naturally found in Central America. If a visual preference is shown for the potential host species, the spawning sequences can be analyzed for differences and the specific visual behaviors that attract the catfish. The spawning sequence of a host species *Ctenochromis horei* and a non-host species

Amatitlania nigrofasciata was first captured on video. *S. multipunctatus* preference between the spawning sequences was determined. After determining the preference, the spawning sequences were comparatively analyzed for differences and more information about the visual behaviors that attract the catfish.

Lastly, *S. multipunctatus* visual preference for two mouthbrooding endemic host species that are naturally parasitized in differing ratios was assayed. It was assessed whether this discrepancy could possibly indicate a difference in the visual aspect of the spawning sequence that attracts the catfish unequally and may indicate different visual host adaptations to avoid brood parasitism. In nature, two host species of mouthbrooding cichlid are parasitized in differing ratios. Sato (1986) found that *Simochromis diagramma* broods were parasitized 7.7% of the time while *Ctenochromis horei* broods were parasitized 15.0% of the time. There are many potential confounding variables that may have affected these results. For example, 284 broods were examined for *S. diagramma* and only 20 for *C. horei*. These two cichlid species, furthermore, have slightly different microhabitats with *C. horei* preferring a vegetated zone surrounded by sand, pebbles, and rocks (Ochi 1993) and *S. diagramma* preferring cliffs and sandy soils (Nelissen 1974). These differences in preferred habitat could make one species more vulnerable to parasitism than the other.

It is possible, however, that the above-mentioned difference in parasitism rates indicates a difference in the visual aspect of the spawning sequence that attracts the catfish unequally. Perhaps there is an intrinsic factor of the two cichlid species such as developmental sequencing, host egg size, or number of eggs that a female can carry in her buccal cavity that makes one species a more desirable host for the catfish. Thus, an experiment was designed to investigate

the possibility of differential parasitism rates that might provide clues as to catfish host preference by comparing visual preference between two host cichlid species, *Ctenochromis horei* and *Simochromis diagramma*.

Brood parasitism in *Synodontis multipunctatus* represents a unique and fascinating biological system. Because so little is known about the system and its broader biological implications, this example of obligate brood parasitism in a fish species is a remarkable opportunity to elucidate theoretical and practical issues in biology. These experiments about the visual cues used in synchronizing breeding between species are necessary and pertinent to begin to fill the substantial knowledge gap. Understanding in this arena should lead to a deeper understanding of several significant issues in the field of biology.

II. Methods

A bilateral setup using video playback was first used to compare *S. multipunctatus* visual preference of video recording as a stimulus. On one side, the catfish were presented with a 15-minute video of a breeding group (3 females and 1 male) of a natural host species, *Ctenochromis horei*. In the recording, no spawning behavior was present and no aggressive behavior was observed. Instead, the fish exhibited a variety of common behaviors including swimming, picking at the gravel, and remaining motionless for a time. On the other side, the catfish were presented with video of an empty tank. This set-up mimicked a previous study completed by Marcus Cohen that used live *Ctenochromis horei* and an empty tank as stimuli.

A five minute pretrial was completed with no stimuli to insure that the catfish did not have an inherent preference for one side of their tank over the other. Next, the catfish were

presented with the video stimuli and the 15-minute trial was filmed. The amount of time the catfish spent in 10-cm preference zones on each side of their tank was recorded. Then, the sides of the stimuli were switched to minimize any effects of side bias, the catfish were given a 30-minute “cool-down” period, and the process was repeated. The data were then analyzed to determine if the catfish showed preference for the video of the *C. horei* over the video of the empty tank.

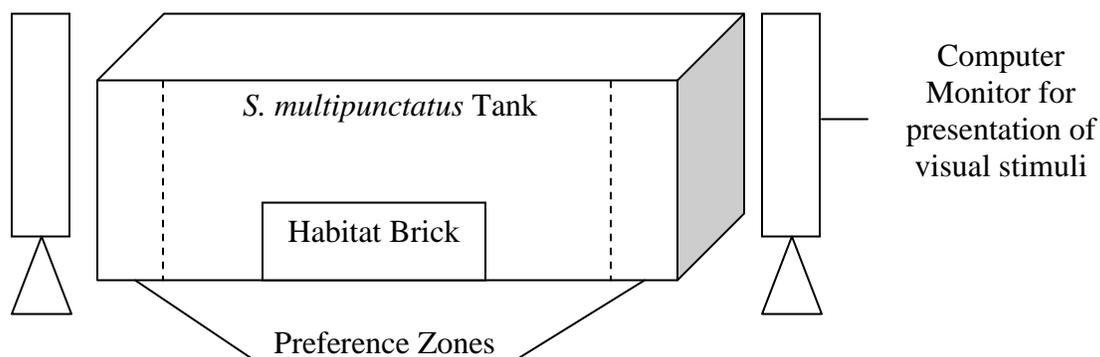


Figure 5: Experimental setup showing central habitat tank and computer monitors for presentation of visual stimuli. The time spent in 10cm preference zones was used to indicate *S. multipunctatus* visual preference.

Next, the spawning sequence of *C. horei* and *A. nigrofasciata* cichlid species was captured on video. This was accomplished by placing a breeding group (3 females and 1 male for *C. horei* and 1 male and 1 female for *A. nigrofasciata*) in a tank with a flower pot as a central habitat and spawning area. A high definition video camera was then used to record the behavior of the fish at least 10 hours a day. This was continued for about 3 weeks until a female was found holding eggs in her buccal cavity for *C. horei* and eggs were observed on the ground for *A. nigrofasciata*. Then, the video was edited using iMovie (Apple, Inc.) to focus on spawning behavior. For both species of cichlid, the resulting video sequence was approximately 25

minutes long. The following ethogram details how the time was distributed in both video sequences.

Ethogram 1: Video Spawning Sequences of a host species, *C. horei* and non-host species, *A. nigrofasciata*

Behavior	Approximate Percent of Video	
	<i>C. horei</i> (Total time: 24:56)	<i>A. nigrofasciata</i> (Total Time = 27:10)
No fish visible in frame	1%	2.5%
One or more fish in frame, no visual spawning activity	1%	5.5%
Male displays alone (vigorously shakes caudal fin and/or flares gill slits)	0%	9%
Male picks up gravel or makes motion to pick up gravel without actually moving anything	0%	36%
Female in flower pot while male guards (swims in front and above the pot)	26%	54%
Mutual T-position (male or female vibrates on side while genitalia orally stimulated)	62%	0%
Eggs released	5%	1%
Eggs retrieved by female	5%	0%

A bilateral visual preference setup was used to determine catfish preference for visual spawning behavior. After a 5-minute pretrial, *Synodontis multipunctatus* was presented with repeated

video of the breeding sequence of *C. horei* on one side and repeated video of the breeding sequence of *A. nigrofasciata* on the other side for 15 minutes. The amount of time the catfish spent in the preference zones on each side of the tank was recorded and then the sides of the stimuli were switched to minimize side bias. A minimum of a 30-minute “cool-down” period was implemented between trials. The data were then analyzed to determine if the catfish showed preference for one visual sequence over the other.

Finally, a bilateral visual preference setup was used to determine the catfish preference for host species *Ctenochromis horei* versus host species *Simochromis diagramma* using groups of live fish as stimuli. After a 5-minute pretrial, *Synodontis multipunctatus* was presented with a breeding group (1 male and 3 females) of *Ctenochromis horei* in a tank placed to one side and a breeding group of *Simochromis diagramma* in a tank placed on the other side for 15 minutes. The amount of time the catfish spent in 10-cm preference zones on each side of the tank was recorded and then the sides of the stimuli were switched to minimize side bias. A minimum of a 30-minute “cool-down” period was implemented between trials. The data were then analyzed to determine if the catfish demonstrated preference for one species of fish over the other. The diagram below summarizes the experimental set-up.

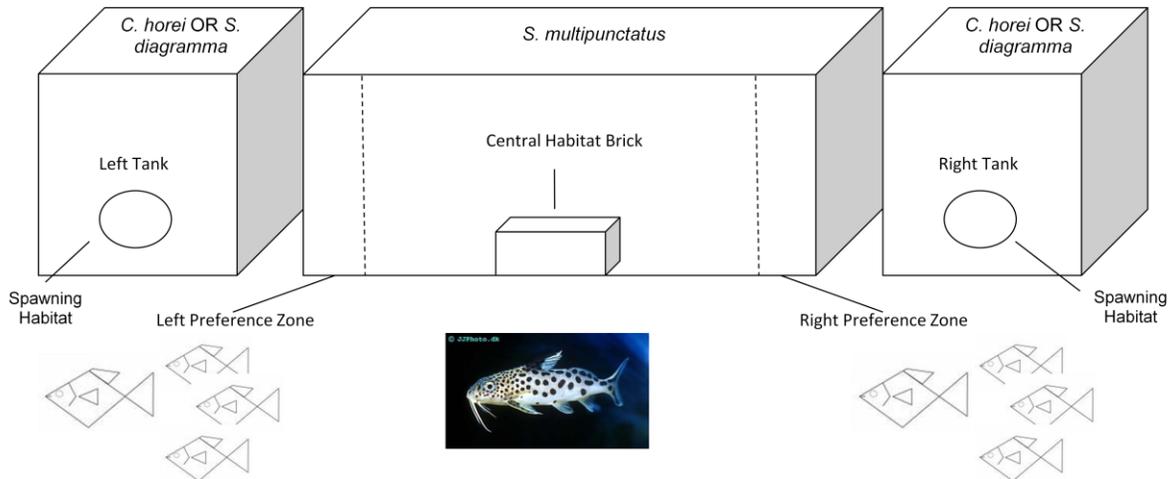


Figure 5: Experimental setup showing central habitat tank and tanks for presentation of visual stimuli. The time spent in 10cm preference zones was used to indicate *S. multipunctatus* visual preference for behaviors of *S. diagramma* and *C. horei*.

III. Results

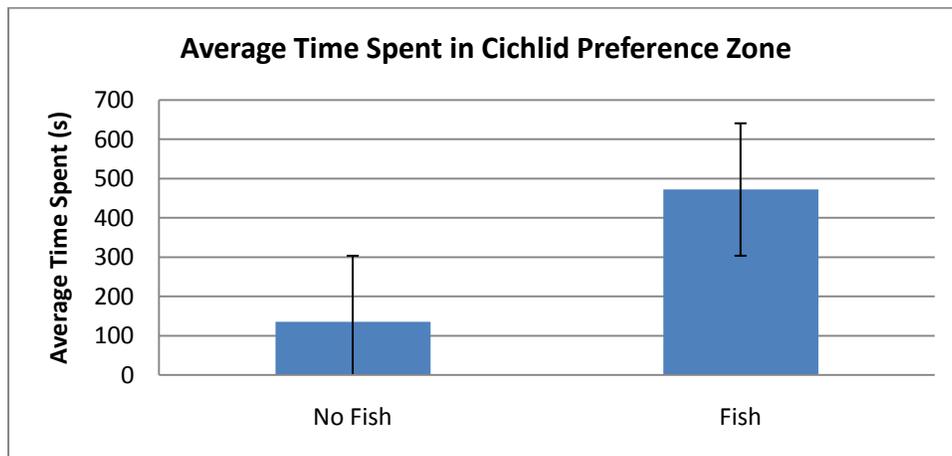


Figure 6: Mean Time Spent in Fish Zone = 135s, Mean Time Spent in No-Fish Zone = 472s, N=12, V=77, P<0.001)

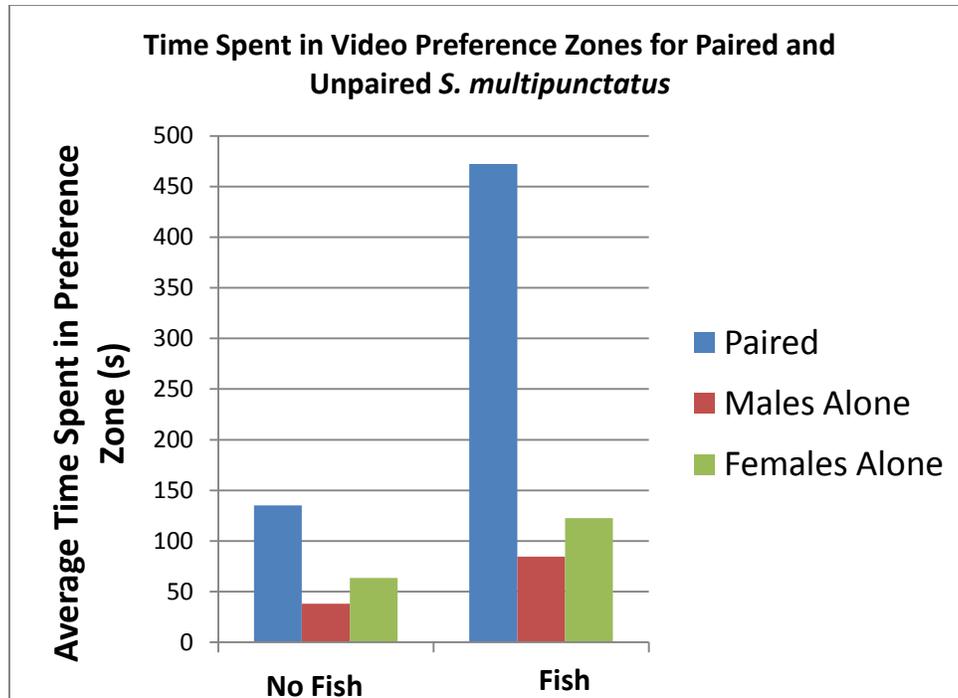


Figure 7: Time Spent in Video Preference Zones for paired and unpaired *S. multipunctatus*. Notice that paired catfish spent more time in preference zones than females or males alone (ANOVA, $F= 8.57$, $p<0.001$)

A test for normalcy found that the data were non-parametric. Thus, a Wilcoxon signed-rank test was used to compare the amount of time spent in each preference zone. With a sample size $N=12$, it was found that *Synodontis multipunctatus* significantly visually preferred video of fish over video of no-fish ($p<0.001$). These results use video recordings to statistically emulate those obtained by Marcus Cohen when live fish were used as stimuli.

Unfortunately, there were differences in the video quality of the *C. horei* video versus the *A. nigrofasciata* video. The *A. nigrofasciata* video had a blue tint and the frame was slightly different, causing the fish to appear larger.



Figure 8: A screenshot of the spawning video of *C. horei* (left) and *A. nigrofasciata* (right)

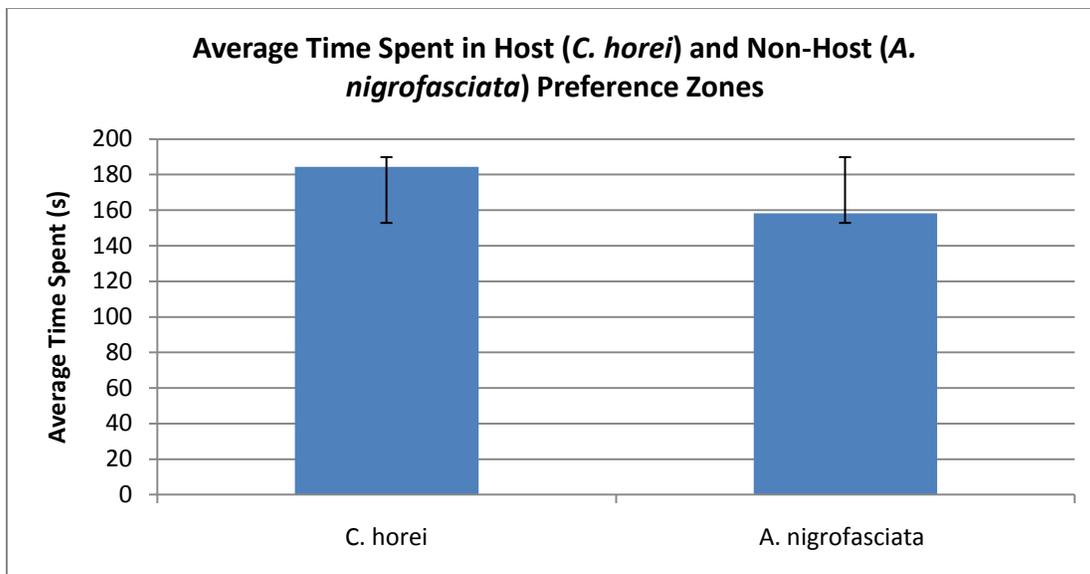


Figure 9: Mean Time Spent in *C. horei* Zone = 184s, Mean Time Spent in *A. nigrofasciata* = 158s, N=33, V=216.5, P=0.257)

A test for normalcy found that the data were non-parametric. Thus, a Wilcoxon signed-rank test was used to compare the amount of time spent in each preference zone. With a sample size N=33, it was found that *Synodontis multipunctatus* showed no difference between the time spent in the preference zone for a potential host and a non-potential host ($p=0.257$). Because there were no differences in *S. multipunctatus* preference for *C. horei* versus *A. nigrofasciata*, no statistical analyses were completed on the behavioral ethograms.

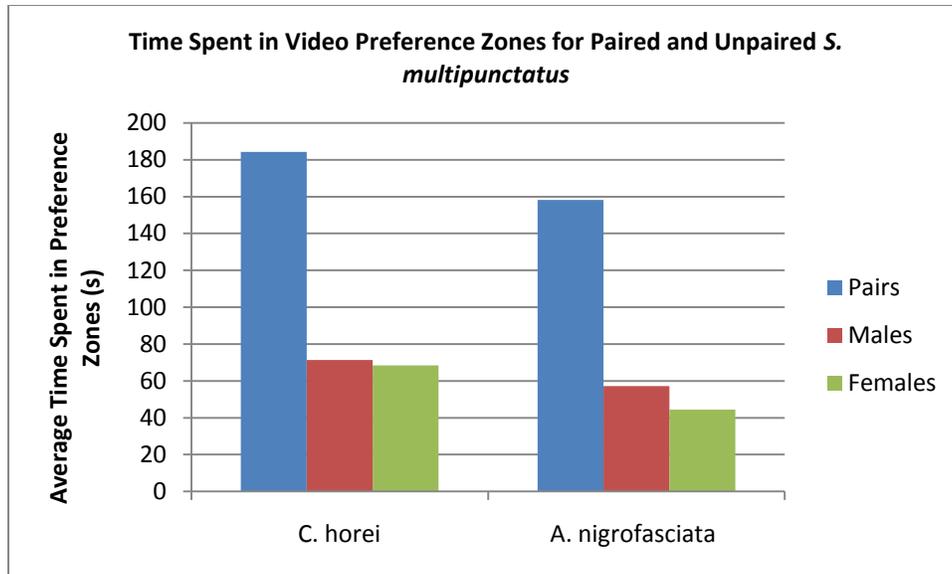


Figure 10: Time Spent in Video Preference Zones for Paired and Unpaired *S. multipunctatus*. Notice that paired catfish spent more time in preference zones than females or males alone (ANOVA, $F= 8.835$, $p<0.001$)

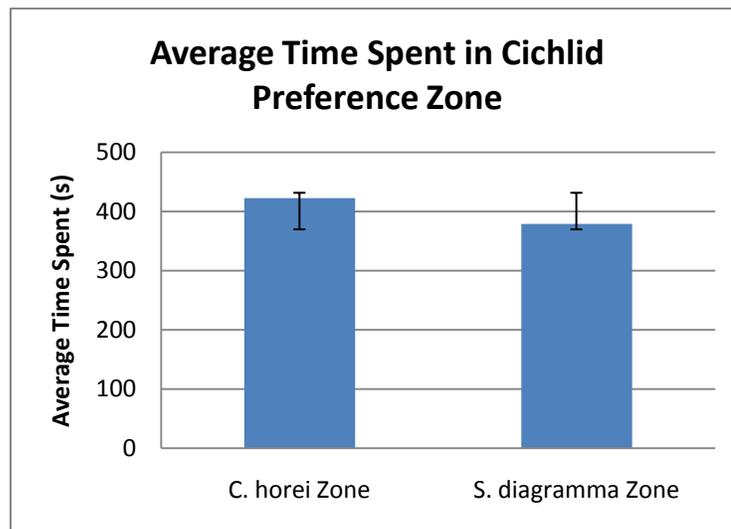


Figure 11: Mean Time Spent in *C. horei* Zone = 423s, Mean Time Spent in *S. diagramma* Zone = 379s, $N=15$, $Z=-.220$, $P=0.826$

A test for normalcy found that the data were non-parametric. Thus, a Wilcoxon signed-rank test was used to compare the amount of time spent in each preference zone. For a sample size $N=15$, there was no statistical difference between the amount of time *S. multipunctatus* spent

in the preference zone for *C. horei* in comparison to the time spent in the preference zone *S. diagramma* ($p=0.83$).

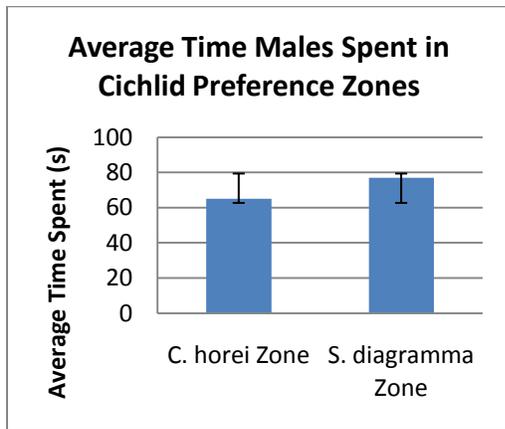


Figure 12: Mean Time Spent in *C. horei* Zone=140s, Mean Time Spent in *S. diagramma* Zone=120s, N=15, Z=-.785, P=0.433

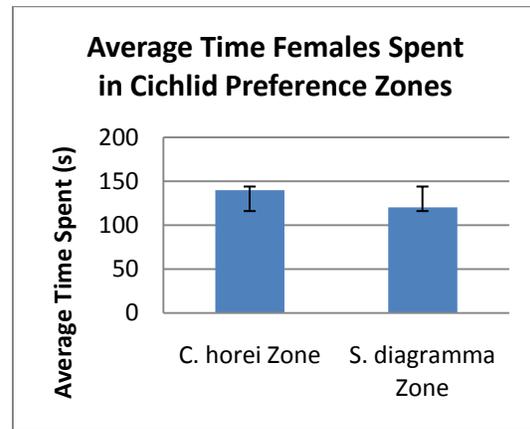


Figure 13: Mean Time Spent in *C. horei* Zone=65s, Mean Time Spent in *S. diagramma* Zone=77s, N=15, Z=-1.293, P=0.196

Neither paired fish, nor females alone ($p=0.433$), nor males alone ($p=0.196$) significantly preferred one species of cichlid over the other.

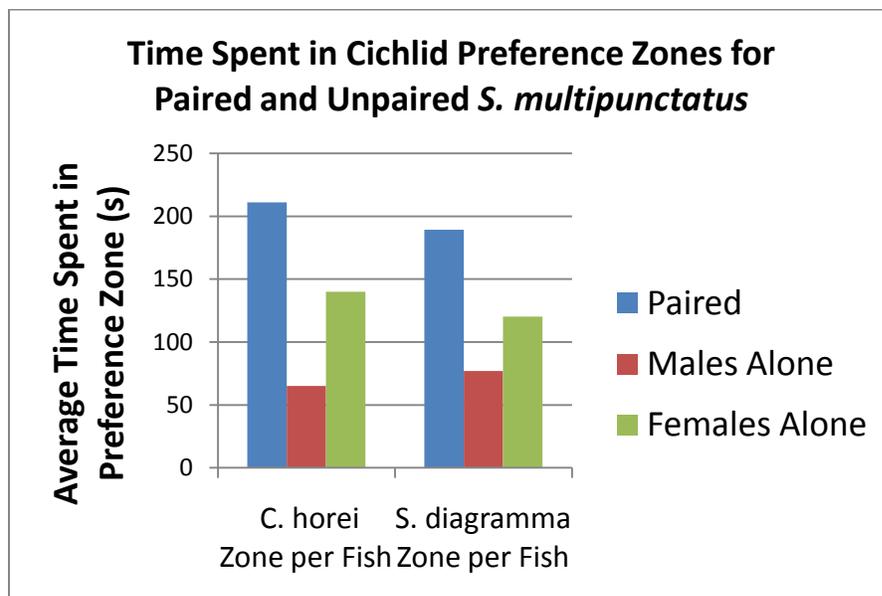


Figure 14: Movement of Paired and Unpaired Fish (ANOVA, F= 9.12, $p<0.001$)

Synodontis multipunctatus was significantly more active when paired with a fish of the same species and the opposite gender than when alone. Seventy-six percent of the movement that occurred during paired trials occurred with the two fish swimming in tandem as a pair. Females were significantly more active alone compared to males and were generally in front of the male during paired movement.

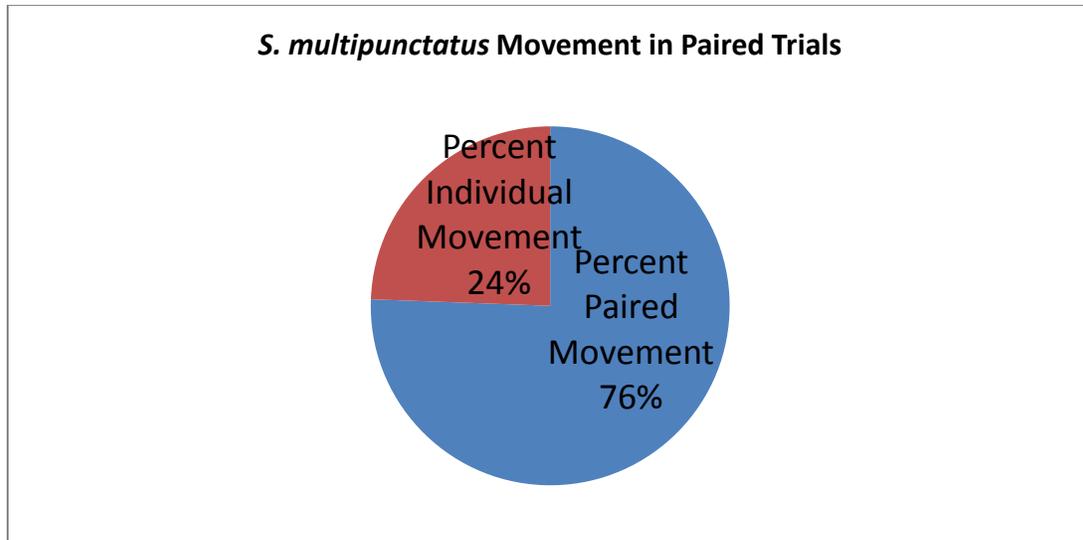


Figure 15: Movement in paired trials

IV. Discussion

Synodontis multipunctatus visually preferred video of fish over video of an empty tank. Some element of the visual behavior of the cichlids must therefore attract the attention of the catfish. These results support the hypothesis that vision plays an important role in the synchronization of *S. multipunctatus* spawning behaviors with cichlid hosts. Additionally, these results are statistically equal to those obtained by Marcus Cohen who completed a similar experiment using live fish instead of recorded video as a stimulus. It can therefore be assumed that *S. multipunctatus* can see and respond to video playback in a manner that approximates the way the catfish respond to live stimuli. Video playback is a valid research technique for *S.*

multipunctatus. The isolation of specific cichlid behaviors is possible on video and additional experimenter control is gained. These conclusions validate recording and playing back the spawning sequences of a host species and a non-host species in order to achieve a more nuanced understanding of brood parasitism in this catfish species.

Synodontis multipunctatus showed no visual preference for the spawning sequence of a potential host species, *Ctenochromis horei*, compared to a non-potential host species, *Amatitlania nigrofasciata* in the present study. There are several potential explanations for this observation. First of all, the differences in quality of the video may very well have skewed the results. As the videos were tinted a different color and the size of the fish appeared in a different way in the two clips, any actual preference for one spawning sequence over the other may have been lost in the confounding variables. New footage is needed to exclude the possibility that the results are compromised. Additionally, *A. nigrofasciata* could have been a poor choice for an experimental subject. Because the latter fish is native to Central America, the spawning sequence may not be representative of sympatric substrate spawners. A species native to Lake Tanganyika would therefore make a superior subject. Further experimentation is necessary to rule out visual preference between the spawning sequences of the two cichlid species.

Synodontis multipunctatus did not visually prefer *S. diagramma* or *C. horei*. There are several potential explanations for these results. First of all, it is possible that the breeding groups of cichlids did not exhibit any spawning or other visually attractive behavior during the trial period. The fact that live fish were used in this investigation instead of video made it impossible to control the exact behaviors of the cichlids. Without further experimentation, it is not possible to rule out visual preference between the spawning sequences of the two cichlid species.

It is also possible that similar results would be observed even if the presence of spawning behavior were more controlled. It is well established that *S. multipunctatus* is a host generalist (Brichard 1989). The inequalities observed in the original study by Sato (1986) may have simply been a consequence of the limited sample size as the inequalities were not a main focus of his paper. Differences in the locations of the spawning acts between the two species of cichlid could make one species more prone to parasitism and could explain Sato's results. Finally, Sato's results may be caused by differences in aggressive or evasive displays of the cichlids instead of due to *S. multipunctatus* preference. Perhaps, one of the species of cichlids has a behavioral adaptation to reduce parasitism. Martin (2009) determined that *S. diagramma* exhibits interruptions in its spawning sequence that may provide defense against brood parasitism. In comparable bird species prone to brood parasitism, behavioral adaptations to prevent parasitism are common. For example, willow flycatchers (*Empidonax traillii*) actively chase off brown-headed cowbirds that could parasitize their nests and, therefore, have a lower level of parasitism in comparison to other sympatric species that do not display nest-guarding behaviors (Uyehara and Narins 1995). In short, the limitations of the present study make it impossible to draw any firm conclusions. Additional research into the specifics of the breeding ecology of the cichlid hosts is clearly necessary to explain the differences in parasitism rates.

Although it was not the focus of this investigation, the data obtained here reveal new elements of *Synodontis multipunctatus* social behavior. The fish left their central habitat brick significantly more often as a pair than alone in all three experiments. These observations are consistent with what little is known about the social dynamics of *S. multipunctatus*. In the wild, the catfish generally exist in large schools of up to 200 individuals as can be seen in the National Geographic Documentary *Jewel of the Rift* (1997). Because the fish live in large groups

naturally, it is likely that they would be more active in a group setting. The data obtained here also tentatively indicate that female catfish are the more dominant sex, as the females generally lead the paired movements. The female alone was also more active than the lone male, in the experiment with live fish as stimuli reinforcing this conclusion. Additional research into the behavior of the catfish is needed to further address these social dynamics.

In conclusion, video playback is an effective research technique for *Synodontis multipunctatus*. Additional experimentation into *S. multipunctatus* visual preference between species of cichlids can therefore be completed in the future using video playback as the research technique. This further experimentation may inform understanding of the visual aspects of the unique and fascinating biological system. Results obtained here also indicate that investigation into the social system of *S. multipunctatus* is warranted to more fully comprehend this system of brood parasitism in a fish species.

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