EXOTIC ARMORED CATFISHES IN TEXAS: REPRODUCTIVE BIOLOGY, AND EFFECTS OF FORAGING ON EGG SURVIVAL OF NATIVE FISHES (*ETHEOSTOMA FONTICOLA*, ENDANGERED AND *DIONDA DIABOLI*, THREATENED)

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Sandra L. Cook-Hildreth, B.S.

San Marcos, Texas
December 2008

EXOTIC ARMORED CATFISHES IN TEXAS: REPRODUCTIVE BIOLOGY, AND EFFECTS OF FORAGING ON EGG SURVIVAL OF NATIVE FISHES (*ETHEOSTOMA FONTICOLA*, ENDANGERED AND *DIONDA DIABOLI*, THREATENED)

Committee Members Approved:

_____
David G. Huffman, Chair

_____
Timothy H. Bonner

_____
Alan Groeger

_____
Joe N. Fries

Approved:

_____
J. Michael Willoughby
Dean of the Graduate College

ACKNOWLEDGEMENTS

I am very grateful to Dr. Huffman, my advising professor, and the other members of my committee for all of their invaluable input, support and encouragements. Thanks to Randy Gibson and Joe Fries, as well as the rest of the staff at the NFHTC for all their help and positive input. Thanks to Chad Thomas for all of his editorial comments and moral support! Thank you to the many kind volunteers that donated time and energy to this project to help with specimen collection. And thanks to my loving, understanding, and supportive family for all their patience and all the sacrifices they made to help me accomplish my dreams!

This manuscript was submitted on October 22nd, 2008.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

EXOTIC ARMORED CATFISHES IN TEXAS: REPRODUCTIVE BIOLOGY, AND EFFECTS OF FORAGING ON EGG SURVIVAL OF NATIVE FISHES (*ETHEOSTOMA FONTICOLA*, ENDANGERED AND *DIONDA DIABOLI*, THREATENED)

by

Sandra L. Cook-Hildreth, B.S.

Texas State University-San Marcos

December 2008

SUPERVISING PROFESSOR: DAVID G. HUFFMAN

Armored catfishes (Loricariidae), native to Central and South America, were introduced into North American waters through the aquarium trade, and became established in Texas waters in 1964. There has been concern that breeding populations of these exotics could affect native species through dietary overlap, egg predation, and other factors. This study focused on two related topics: 1. the reproductive biology of

Hypostomus in the San Marcos River, Texas; and 2. the potential effect of the foraging activities of the exotic on the egg survival of two native central Texas fishes (the endangered fountain darter, *Etheostoma fonticola*, and the threatened Devils River minnow, *Dionda diaboli*). Based on the oocyte diameters and estimated mean fecundity (2,109) of the exotics, the *Hypostomus* population in the San Marcos River is reproducing as well in their new habitats as could be expected, and is not yet suffering from the effects of crowding. Photoperiod was the only environmental influence that could be associated with a peak in spawning activity, which occurred during the months of March through September. Size-frequency distribution plots of oocyte diameters from Ripe ovaries revealed two distinct modal diameters, suggesting a modified group-synchronous mode of ovarian development. Potentially deleterious effects of *Hypostomus* foraging on Devils River minnow and fountain darter eggs were assessed experimentally to evaluate the threat to these native species. The survival rate for fountain darter eggs was substantially lower when exposed to foraging *Hypostomus*, and three whole eggs were found in the digestive tract of the two experimental *Hypostomus*. These findings suggest that the exotic is positively trophotaxic toward fountain darter eggs, and may substantially reduce the breeding success of the darter in shared habitats. In contrast, egg survival rates for the Devils River minnow were only slightly lowered by foraging *Hypostomus*, and no eggs were recovered from the digestive tracts of the experimental *Hypostomus*.

INTRODUCTION

Concerns Regarding the Potential Effects of Exotic Taxa

There are many reports of the damage and long-term negative effects caused by the introduction of exotic species, and almost every ecosystem in the world has been affected (Westbrooks 1953; Burdick 2005; Leland 2005; Terrill 2007). A study done by Pimentel et al. (2004) indicated that there have been approximately 50,000 alien-invasive species of plants, vertebrates, and invertebrates introduced into the USA.

The methods by which exotic species are introduced to new environments can vary substantially. Sometimes exotics are introduced intentionally, such as that of pigs to the islands of Hawaii, introduced as a food source by Europeans and Polynesians during the 1800s (Diong et al. 1982). Sometimes the introductions are unintentional, such as zebra mussels into the Great Lakes Basin, most likely carried as larva in ballast water in a cargo ship traveling from the Black Sea (McMahon 1996).

The introductions of exotic species into these new habitats often have a broad range of effects on the native species they encounter. For example, the brown tree snake (*Boiga irregularis*), introduced into the island of Guam in the 1960's, is thought to be responsible for the disappearance of wild populations of many native forest birds and lizards (Campbell 2004). Another noteworthy example is the introduction of fire ants (*Solenopsis* spp.) into North America, which have been cited as responsible for the decline of several native ant species, including the harvester ant (Hook and Porter 1990).

Introduction of Armored Catfishes and Related Effects

Armored catfishes (Loricariidae) are native to Central and South America (Burgess 1989; Robins et al. 1991) and are included in the list of exotic species in USA waters are (Hoover et al. 2004). They were brought to the USA by the aquarium trade and were apparently released by amateur and professional aquarists.

Populations of armored catfishes have now been reported in Nevada, Hawaii, Texas, Arizona, Colorado, Connecticut, Louisiana, Florida, and Pennsylvania (Courtenay and Deacon 1982; Hoover et al. 2004), and were reported in the San Antonio River (Texas) as early as 1964 (Barron 1964). The documented presence of juvenile armored catfish in some central Texas streams (Barron 1964; Hubbs et al. 1978) suggests that reproducing populations have been established in central Texas. Armored catfishes may have been established in the San Marcos River in the early 1990's (T.H. Bonner, personal communication 2005), and there is now a thriving population of an unidentified *Hypostomus* species in the upper spring run of the San Marcos River.

Armored catfishes are known to influence their environment by causing biological and physical alterations. The physiology of armored catfishes can alter nutrient cycles due to their high dietary requirement for phosphorus (Hood et al. 2005) which they need to maintain the armored plates covering their body (Vanni et al. 2002). Most of this dietary phosphorus is sequestered, while much of the dietary nitrogen is released back into the environment as soluble compounds, thereby altering the local ratio of available phosphorus to nitrogen. Although I have seen no reports of armored catfishes specifically affecting the environment due to phosphorus sequestering, there are reports of zooplankton altering the species composition of local algal and phytoplankton

communities by sequestering phosphorus (Elser et al. 1988). Thus, algal and phytoplankton communities sharing nutrient cycles with a colony of armored catfishes could be likewise affected by the phosphorus metabolism of the armored catfishes.

In addition to these potential effects to the local algal and phytoplankton communities, native fishes could be affected by the foraging activities of armored catfishes in several ways. The egg survival of native fishes that lay eggs on surfaces foraged by armored catfishes could be directly threatened by the foraging behavior of armored catfishes. Additionally, native algivorous fishes could be indirectly affected through competition with the armored catfishes for food, potentially resulting in competitive exclusion of the native algivores (Cohen et al. 2008). Surfaces frequently foraged by armored catfishes show low sediment accumulation which can in turn alter the structure of the invertebrate communities dependent on this accumulation and have secondary effects on native insectivorous fishes (Powers 1990; Flecker 1992).

In addition to these biological effects, armored catfishes have also been known to have physical effects on the environment. They dig nesting burrows as long as 1.5 m into stream banks (Burgess 1989), and can form nesting colonies with dozens of such burrows close together (Nikolsky 1963; Burgess 1989). These burrowing activities not only compromise bank stability, but also re-suspend otherwise stable sediments, thus increasing local turbidity (Nico 2000) and downstream siltation rates.

Despite the fact that armored catfishes are known to influence their environment in many ways, I have found very little information regarding how the foraging activities or the reproductive behavior of armored catfishes might actually be affecting the native fishes of Texas.

Armored Catfishes in Texas, a Cause for Concern

There are several native fishes listed as "species of concern" by state and federal agencies. Two examples of native fishes in Texas that are listed as species of concern are the fountain darter (*Etheostoma fonticola*), an endangered species endemic to the San Marcos River in Hays County and the Comal River in Comal County (U.S. Fish and Wildlife Service 1974; Schenck and Whiteside 1976) and the Devils River minnow (*Dionda diaboli*), a threatened species found in San Felipe Creek in Val Verde County, the Devils River, Sycamore Creek, and Pinto Creek (U.S. Fish and Wildlife Service 1999; Lopez-Fernandez and Winemiller 2005).

Reproducing populations of *Hypostomus* (a genus of armored catfish) have been reported to occur in the entire range of the fountain darter (Hubbs et al. 1978; Whiteside and Berkhouse 1992), and in the San Felipe Creek portion of the range of the Devils River minnow (Lopez-Fernandez and Winemiller 2005). Thus, there is growing concern that the expanding populations of *Hypostomus* in these habitats might further endanger or threaten these native fishes.

One potentially direct effect of *Hypostomus* could come from *Hypostomus* foraging activities interfering with the reproduction of these two species of concern. The fountain darter is a phytolithophilic spawner, and the Devils River minnow is a lithophilic spawner. Fountain darters have been observed depositing adhesive eggs on filamentous algae in aquarium settings (Strawn 1955), and they seem to prefer natural habitats with filamentous algae (Schenck and Whiteside 1977). The Devils River minnow, in captivity, spawns over gravel with the eggs sinking down to just below the surface (Gibson et al. 2003). Thus, eggs of either of these species could be damaged by foraging

Hypostomus as they scrape algae and periphyton from surfaces such as rock, gravel, and macrophytes.

In addition to the threat of diminished reproductive potential that foraging *Hypostomus* could pose to the Devils River minnow, there is also some concern over dietary overlap and competition for food. The Devils River minnow, an herbivore (Hulbert et al. 2007), might be threatened with competitive exclusion by the introduction of *Hypostomus*, also an herbivore (Pouilly et al. 2006). Indeed, Lopez-Fernandez and Winemiller (2005) reported seeing very few Devils River minnows in areas of San Felipe Creek where *Hypostomus* populations were dense; possibly indicating that competitive exclusion is already taking place in that ecosystem.

The Management of Armored Catfishes in the San Marcos River

Controlling the populations of exotic *Hypostomus*

Ideally, the exotic *Hypostomus* populations could be eliminated without harming other organisms. However, given that this is probably an unrealistic goal, remaining options are constraining growth and range expansion of the existing populations. Among the many factors that could be exploited in attempts to control the growth and expansion of *Hypostomus* populations, knowledge concerning the reproductive biology of the species would be of paramount importance. Fecundity, spawning cycles, and reproductive behavior of *Hypostomus* and other armored catfishes have been studied in their native habitat of South America (Mazzoni and Caramaschi 1997a,b; Duarte and Araújo 2002). However, it is uncertain how much of this information is transferable to exotic populations living in the northern hemisphere where the seasons are inverted and many of the natural biological controls that have co-evolved with armored catfishes in

their native habitats may not be operating to constrain the exotic populations. Consequently, any attempts to control populations of *Hypostomus* in the northern hemisphere should involve studies on reproductive behavior of the species in this region.

Protection of the native species

Management programs designed to protect the endangered fishes in central Texas were established long before the introduction of *Hypostomus*. Now that *Hypostomus* populations have been established in central Texas, knowledge of their effects on native fishes and the survival of native fish eggs are critical to the success of management programs designed to protect the native fishes.

It is not known how *Hypostomus* responds to native fish eggs encountered while foraging. Two trophotaxic responses are possible: (1) positively trophotaxic – *Hypostomus* seek eggs of the native species, in which case the reproductive success of the native species is likely to be severely affected by foraging *Hypostomus*; (2) neutrally trophotaxic – *Hypostomus* is not influenced by eggs of the native species, in which case the eggs may be in danger of incidental ingestion during *Hypostomus* foraging activities.

Goals and Objectives

I have two goals for this project. My first goal derives from the need for information that may be useful to control *Hypostomus* populations, and will involve an assessment of the reproductive biology of *Hypostomus* in the San Marcos River. Objectives for this goal include; (1) estimating fecundity, (2) developing a subjective visual-based macro-structural scale of ovarian maturity, and (3) detecting evidence of seasonality in the spawning behavior of the San Marcos River *Hypostomus* population.

My second goal derives from the need to know how *Hypostomus* foraging activities affect the survival of native fish eggs, and this goal specifically targets egg survival of the endangered fountain darter and the threatened Devils River minnow. Objectives for this goal are to; (1) determine if *Hypostomus* is positively trophotaxic in the presence of the eggs of the two targeted native fishes, and (2) determine if there is any evidence that *Hypostomus* will actually ingest eggs of the targeted native species when the eggs are present during foraging.

MATERIALS AND METHODS

Study Site

The headwaters of the San Marcos River, a tributary of the Guadalupe River, arise from a cluster of flowing springs in San Marcos, Texas (Hays County). The San Marcos springs have been impounded since the construction of Spring Lake Dam in 1849. The spring run of the river flows for about 7 km before it is joined by the intermittent waters of the Blanco River. This study will be restricted to the 300 meter reach of the San Marcos River downstream from Spring Lake Dam, which includes the recreational area of Sewell Park.

Reproductive Biology of Hypostomus

The questions regarding the reproductive biology of *Hypostomus* in the San Marcos River were addressed by estimating fecundity, by developing a subjective visual-based macro-structural scale for ovarian maturity, and by searching for any evidence of seasonality in spawning behavior.

Fifty-one female *Hypostomus* were collected for the reproductive study (gender was determined after dissection). The fish were collected by divers at a rate of 6 to 10 fish per month from January to December of 2005. Fish were weighed (grams), measured for total length (mm), and euthanized by severing the spinal cord. After euthanization, *Hypostomus* were dissected by making an incision from the pharynx to the

vent. Ovaries were extracted and weighed (g), and preserved in a solution of 10% buffered formalin for later study.

Hypostomus ovaries were examined and assigned to ovarian maturity stages by two methods. One method used a visual-based macro-structural scale and the other method used a micro-structural scale based on the size/frequency distribution of oocyte diameters.

For the visual-based macro-structural scale, ovaries were classified into one of five maturity stages (Mature 1, Mature 2, Ripe, Recovering, and Resting) based on subjective criteria. The subjective criteria employed in my macro-structural scale were adapted from Mazzoni and Caramaschi (1997a,b) and included (1) color (opalescent to opaque yellow), (2) gross surface texture (grainy to smooth), and (3) an attempt to estimate (by eye) the percentage of the abdominal cavity occupied by the ovary (Table 1). Any ovary that did not fall into one of these four maturity stages, and was obtained from a fish that was at least 180 mm in total length, was considered to be in a Resting state wherein there is no obvious sign of oocyte development.

Table 1. Macroscopic characteristics used to visually assess the maturity stage of *Hypostomus* ovaries.[†]

Stages	External appearance
Mature I	Ovaries occupy less than 15% of body cavity, pale cream color with subtle granulation. Oocytes barely visible to the naked eye.
Mature II	Ovaries occupy up to 50% of body cavity, yellow color with light vascularization. Large light yellow oocytes visible to the naked eye.
Ripe	Ovaries occupy up to 85% of body cavity, orange in color with thin ovarian walls and strong vascularization. Large yellow oocytes present.
Recovering	Ovaries occupy less than 20 to 40% of body cavity, translucent, flaccid, and slightly vascularized.
Resting	Ovaries occupy less than 10% of the body cavity, white transparent and smooth. Oocytes not visible to the naked eye.

[†] Adapted from Mazzoni and Caramaschi (1997a,b).

According to Naumov (1956), macro-structural scales of gonad maturity are subjective by nature, and the criteria used for such scales to assign gonads into maturity stages should be validated by a more objective micro-structural scale. In this study, the size/frequency distribution of oocyte diameters (a subset of Naumov's criteria) was used as the objective reference to validate the accuracy of the macro-structural scale. If the macro-based visual scale actually reflects a progression in ovarian maturity, then a typical oocyte from Ripe ovaries would be expected to be larger than a typical oocyte from Mature 2 ovaries. This expected progression would be corroborated if the most frequently occurring oocyte diameter (modal diameter) from Ripe ovaries were larger than the most frequently occurring oocyte diameter from Mature 2 ovaries. To test this expectation, I plotted oocyte diameters from Mature 2 ovaries in a size-frequency graph and then compare the modal diameter of that graph to the modal diameter of a similar

graph using oocytes from Ripe ovaries. Ovaries in the other two maturity stages were not used in the validation procedure because the oocytes from these ovaries were too small to extract reliably with available equipment.

Four Mature 2 ovaries and 17 Ripe ovaries were used as sources for the validation oocytes. Approximately 100 (45-106) oocytes that were visible under a dissecting microscope were excised from each of the 21 ovaries. The diameters of these oocytes were then measured to the nearest 100 micrometers using a dissecting microscope equipped with an ocular micrometer. These diameters were used to develop two size/frequency graphs: one representing Mature 2 ovaries, and the other representing Ripe ovaries.

In addition to using the collected oocyte diameter data to validate the macro based maturity scale, modes of ovarian development and number of spawns per individual fish during a breeding season were also investigated by creating oocyte size frequency distribution graphs for the four fish with Mature 2 ovaries and seventeen fish with Ripe ovaries.

After ovaries were assigned to maturity stages, the fecundity (reproductive potential) of *Hypostomus* from the San Marcos River was estimated by examining 17 ovaries that had been assigned to Ripe. The fecundity estimates followed the McGregor (1922) sub-sampling-by-weight technique recommended in the International Biological Programme Handbook No. 3 (Ricker 1968).

Seasonality in the reproductive behavior of *Hypostomus* was studied by examining seasonal variation in the gonadosomatic index (GSI). The GSI was determined by expressing the gonad weight as a percentage of body weight. Several

environmental factors (monthly rainfall totals, photoperiod, and discharge rates of the San Marcos River) were investigated for potential influences on seasonal variation in monthly GSI.

Foraging Experiments

The question regarding how *Hypostomus* foraging activities affect the survival of native fish eggs, and specifically targets egg survival of the endangered fountain darter and the threatened Devils River minnow, was addressed with trophotaxy experiments.

There are two possible trophotaxic scenarios that can be investigated experimentally: positive trophotaxy and neutral trophotaxy. If *Hypostomus* is positively trophotaxic towards native fish eggs, then egg survival will be considerably lower in treatments exposed to foraging *Hypostomus* than in non-foraged controls, and eggs may be recoverable from the gut of experimental fish. On the other hand, if *Hypostomus* is neutrally trophotaxic towards native fish eggs, then the egg survival rate for treatments exposed to foraging *Hypostomus* could be comparable to the egg survival rates in the non-foraged controls and eggs may, or may not be likely to be found in the gut of experimental fish.

Effects of *Hypostomus* foraging on survival of fountain darter eggs

In order to determine if *Hypostomus* is positively or negatively trophotaxic towards fountain darter eggs while foraging, I simultaneously exposed three treatment groups to foraging *Hypostomus*. The treatments were prepared on half-round segments of 3″ PVC pipe approximately 8 - 12 cm long and all “half-rounds” used in this study were prepared at the NFHTC. The three treatments, which were exposed to foraging *Hypostomus*, are as follows:

1. two half-rounds colonized with algae prior to egg deposition;
2. two half-rounds NOT colonized with algae prior to egg deposition; and
3. two half-rounds colonized with algae, but without darter eggs.

Fountain darter eggs were obtained from the San Marcos National Fish Hatchery and Technology Center (NFHTC) which maintains a captive stock of breeding fountain darters for research and genetic preservation purposes. The NFHTC maintains the darters in shallow troughs. Each trough normally contains 2 or more half-rounds. The darters deposit their eggs onto the algae-free surface of the PVC half-rounds provided.

The two half-rounds used in the eggs-plus-algae (Treatment 1) were colonized with algae prior to egg deposition. This was accomplished by placing the half-rounds in shallow, highly eutrophic outdoor ponds at the NFHTC for approximately 5 weeks. This resulted in filamentous algae covering approximately 60 to 70% of the surface of the half-rounds prior to transferring them to a breeding trough. Two half-rounds with no algae used in the eggs-only treatment (Treatment 2) were added to a separate breeding trough at the same time.

After the half-rounds had been incubated in the breeding troughs for 3 days, the "pre-experiment" egg count for each half-round was determined. The half-rounds were transferred to small aquaria filled with clean well water (two half-rounds per aquarium), and all surfaces of each half-round were inspected for eggs under the illumination of a 100-watt lantern. The eggs are quite refractive and were easily detected in the bright light.

The experiment was executed in a Living Stream® unit at the NFHTC. The Living Stream® unit received well water ranging in temperature from 18 - 21 °Celsius.

The bare channel of the Living Stream® unit was partitioned into equal upstream and downstream sections by a plastic divider screen (Figure 1).

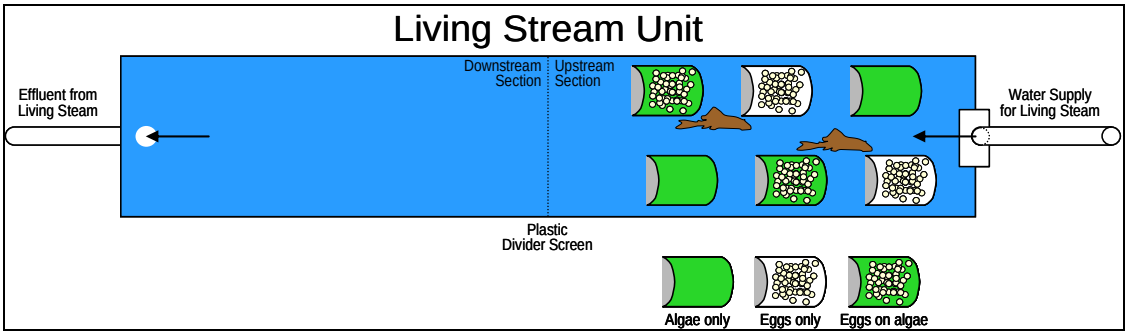

Figure 1. Feeding preferenda of *Hypostomus* provided with fountain darters eggs and algae in three treatment combinations in the Living Stream® unit.

Hypostomus for the experiment were collected from the study area by divers using hand capture and small nets. The fish ranged in length from 250 - 350 mm. Immediately following capture, the fish were transferred to 5-gallon buckets containing fresh river water, and taken to the NFHTC where two of these fish were added to the upstream section of the Living Stream® unit. For approximately 30 days, the fish were maintained in the experimental section of the Living Stream® unit and fed four Hikari® algae wafers every other day. Accumulated sediment and waste were removed twice weekly.

In order to improve the likelihood that the fish would be willing to forage soon after the introduction of the six half-rounds, algae wafers were withheld from the fish for two feeding periods (4 days). At the time the second feeding would have occurred, all six half-rounds representing the three treatments were placed simultaneously into the upstream section of the Living Stream® unit with the two fasted *Hypostomus* (Figure 1).

After the half-rounds had been exposed to the *Hypostomus* for 48 hours, the half-rounds were removed and inspected (as above) for eggs. The number of eggs that remained attached on a half-round was recorded as the “post-experiment” count for that

half-round. The ratio between the "post-experiment" and "pre-experiment" egg counts represented the egg survival rate during the 48-hour incubation period.

Although foraging *Hypostomus* could potentially have caused all observed egg losses during the incubation period, one cannot assume that other factors were not also contributing to egg losses independently of the experimental *Hypostomus*. In order to estimate the proportion of the total experimental egg losses that might have been caused by other factors, a separate egg-loss control procedure was executed after the experiment.

The setup for the egg-loss control procedure consisted of four 2-gallon aquaria supplied with water tapped from the pipe supplying the Living Stream® unit (Figure 2). Standpipes were used to maintain the aquaria at 75% full. The standpipes were fitted with fine-mesh nylon to catch any eggs that might detach and become suspended. Two aquaria were established as experimental aquaria (with fish), and the other two were established as control aquaria (without fish). Positions of experimental and control aquaria were alternated in sequence in an attempt to randomize position effects (Figure 2).

The *Hypostomus* used in the egg-loss control procedure were the same two *Hypostomus* used in the earlier experiment. For several days while this procedure was being set up, the fish remained in the Living Stream® unit and were maintained on the wafer diet as above. The eggs used in the procedure were supplied on four half-rounds obtained from the fountain darter breeding colony at the NFHTC. Eggs on the half-rounds were counted as above to establish "pre-control" egg counts for each aquarium.

Once the four aquaria had been set up and stabilized, the two *Hypostomus* were each transferred from the Living Stream® unit to one of the experimental aquaria and

allowed to acclimate for 24-hours. After the acclimation period, one of the above half-rounds was added to each of the four aquaria (Figure 2) and incubated for 48 hours.

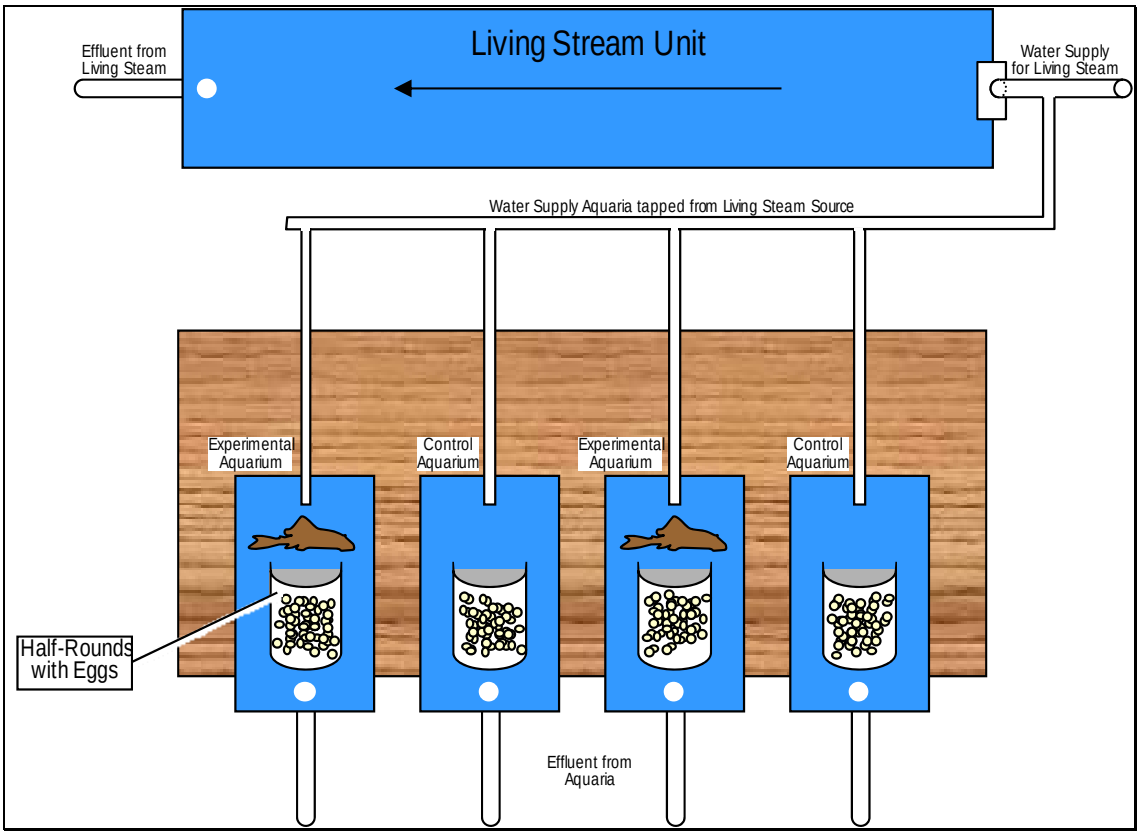

Figure 2. Set up for the egg-loss control procedure.

Immediately following the incubation period, the two *Hypostomus* were removed, euthanized, and preserved in 10% buffered formalin for later dissection, and the four half-rounds were transferred from the four aquaria to smaller containers for counting as above. After the water had settled in the aquaria, each aquarium was examined for detached eggs using the lantern procedure described above.

The "post-control" egg count for each aquarium was then established by adding the number of eggs found detached in that aquarium to the count of eggs remaining on the half-round from that aquarium.

Effects of *Hypostomus* foraging on Devils River minnow eggs

To determine if *Hypostomus* is positively or negatively trophotaxic toward Devils River minnow eggs while foraging, I simultaneously exposed three experimental trays of gravel to foraging *Hypostomus*, with each tray representing one of three treatment levels. One control tray of gravel was maintained outside the range of the foraging *Hypostomus*. The four trays represented the following conditions:

Treatment 1 - A mix of gravel with algae and gravel with eggs;

Treatment 2 - A mix of clean gravel (no algae) and gravel with eggs;

Treatment 3 - Gravel with algae, but no gravel with eggs; and

Control - Same mix of gravel as Treatment 2, but not exposed to foraging *Hypostomus*.

The trays measured approximately 10 cm by 10 cm by 1.5 cm deep. The gravel used in the experiment came from the same source of gravel that the NFHTC had used successfully in the Devils River minnow captive breeding program. This gravel was graded as medium to coarse, with a particle diameter ranging from approximately 1-2 cm.

Algae-colonized gravel for the experiment was prepared in advance by adding one layer of clean gravel to each of two clean trays, and then placing both trays into a shallow, highly eutrophic outdoor pond at the NFHTC for approximately 5 weeks. This resulted in growth of filamentous algae on the gravel in both trays sufficient to cover approximately 60 to 70% of the exposed particle surfaces.

Eggs for the experiment were obtained from breeding colonies of Devils River minnows at the NFHTC. These colonies are maintained in large flow-through stream-simulating units containing many macrophytes. Two to three breeding trays of clean

gravel were placed in four units containing breeding colonies of Devils River minnows in an attempt to obtain eggs. Breeding trays were checked every 2 to 3 days for egg deposition. However, unlike the fountain darter, which spawns readily at the hatchery during all seasons, eggs of the Devils River minnow were not continuously available. Attempts to induce the minnows to breed by manipulating conditions were much less predictable, and persuading the fish to deposit eggs in the breeding trays required several weeks of trial and error.

When eggs were found in a breeding tray, that breeding tray was carefully removed and placed into a large pan of water. All pieces of gravel from that breeding tray were individually removed with forceps and inspected for eggs using a large overhead lamp. Gravel pieces with eggs attached were then carefully transferred to a holding container for subsequent redistribution. Gravel pieces without eggs attached were set aside to be used later as clean gravel. After all gravel had been removed from the breeding tray, eggs that had settled to the bottom of the breeding tray were also counted.

The four trays used in the experiment were then prepared as follows:

Treatment-1 Tray - One of the two trays of gravel on which algae had been cultivated was established as the Treatment-1 tray. Some of the algae-colonized gravel particles were removed and replaced by gravel particles with eggs attached. These particles represented approximately 33% of the particles that had been removed earlier from the breeding tray. After all gravel particles had been placed, additional eggs were individually pipetted in between the gravel particles in the Treatment-1 tray. These

pipetted eggs amounted to approximately 25% of the eggs that had remained in the bottom of the breeding tray.

Treatment-2 Tray - The original breeding tray, in which Devils River minnow eggs had been deposited, but from which all gravel had been removed, was established as the Treatment-2 tray. This tray was empty except for the eggs which had settled to the bottom of the breeding tray. Approximately 50% of these eggs were removed for use in other trays, and the remaining 50% of these eggs were left undisturbed in the bottom of the Treatment-2 tray. The tray was then refilled with gravel particles to one layer deep from two separate sources. One source supplied gravel particles with eggs attached. These particles amounted to approximately 33% of the gravel particles that had been removed earlier from the breeding tray and stored in the holding container. The other source supplied clean gravel particles from those which had been set aside earlier. These particles were added in sufficient quantity to complete a single layer of gravel in the Treatment-2 tray.

Treatment-3 Tray – The second of the two trays of gravel on which algae had been cultivated was established as the Treatment-3 tray. All of the original algae-colonized gravel particles were left in the tray. No eggs were added to the Treatment-3 tray.

Control Tray – A clean tray with no gravel was established as the Control Tray. The tray was filled to one layer deep with gravel particles from two separate sources. One source supplied gravel particles with eggs attached. These gravel particles amounted to approximately 33% of the gravel particles that had been removed earlier from the breeding tray and stored in the holding container. The other source supplied clean gravel

particles in sufficient quantity to complete a single layer of gravel in the Control tray. After all gravel particles had been placed, additional eggs were individually pipetted in between the gravel particles in the Control tray. These pipetted eggs amounted to approximately 25% of the eggs that had remained in the bottom of the breeding tray.

After the four trays had been prepared, the total number of eggs attached to the gravel pieces in a tray was added to the total number of eggs in the bottom of that tray. This sum for each tray was established as the "pre-experiment" egg count for that tray.

The two *Hypostomus* in this experiment had been maintained in the downstream section of the Living Stream® unit for several weeks, but were moved to the upstream section several days prior to this experiment. They were deprived of food for 2 days prior to the introduction of the treatments.

The three treatment trays were placed into the upstream section of the Living Stream® unit, while the control tray was simultaneously placed into the downstream section of the Living Stream® unit devoid of fish. The two sections of the Living Stream® unit were partitioned by a screen (Figure 3). The screen was sufficiently strong to prevent the fish from moving from the experimental section into the control section, and the mesh of the screen was sufficiently fine to prevent contamination of either section with eggs dislodged from the other section. The trays were incubated for a period of 48 hours.

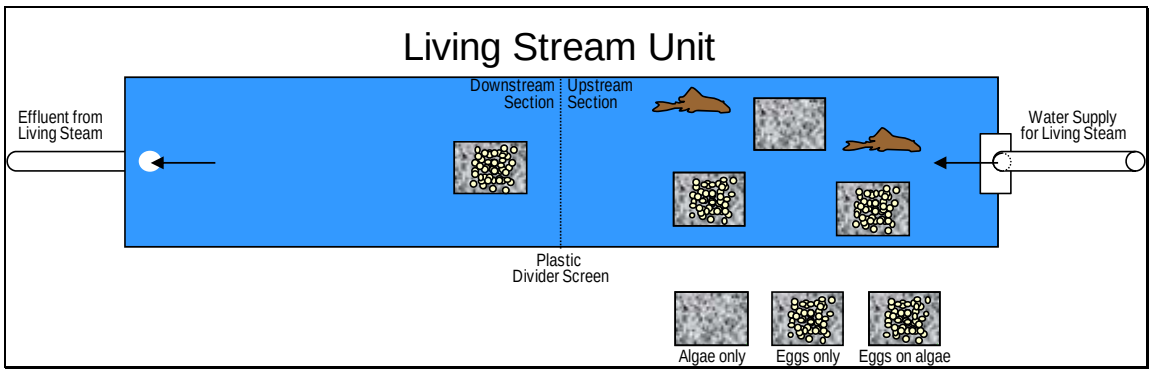

Figure 3. Feeding preference test of *Hypostomus* provided with Devils River minnow eggs and algae in three treatment combinations in a Living Stream® unit.

Following the 48-hour incubation period, all trays were removed and set aside temporarily, and the two *Hypostomus* were removed, euthanized, and preserved in 10% buffered formalin for later dissection.

The trays were then examined for surviving eggs. Surviving eggs were counted as above to establish the "post-experiment" count for each tray. The "post-experiment" counts were subtracted from the "pre-experiment" counts to determine the number of eggs lost from each tray.

RESULTS

Reproductive Biology

Visual-based ovarian classification

Eighty-seven *Hypostomus* were collected for the reproductive study from January to December of 2005. The minimum number of fish collected per month was six, and the maximum 10, except for February, when no fish were collected. Fifty-one of the 87 fish were females, but only 44 of these 51 females had ovaries that I could confidently assign to one of the five stages of ovarian maturity (as defined in Table 1). The frequencies with which these 44 females were assigned to the five maturity stages are reported in Table 2.

Seven of the 51 females had ovaries, which I could not confidently assign to one of the four stages of ovarian maturity. One of these remaining females was only 137 mm in total length, which is 43 mm shorter than the shortest of the 44 females with recognizably mature ovaries, and was therefore considered to be immature. However, the remaining six females were still considered to be sexually mature because the shortest of them (221 mm TL) was over 40 mm longer than the shortest of the other 44 females with recognizably mature ovaries (180 mm TL).

Validation for visual-based scale

In order to validate the macro based scale the micro based scale was created by examining a subset of approximately 100 oocytes from each of the four Mature 2 and 17 Ripe ovaries (2,058 oocytes; actual range 45-106 per ovary). The estimated mean

diameter of vitellogenic oocytes for the four Mature 2 ovaries ranged from 0.98 mm to 2.31 mm, while the corresponding range for the 17 Ripe ovaries was 2.09 mm to 3.74 mm.

Table 2. Frequency of occurrence of the five ovarian maturity stages among 50 females, as assigned by the visual-based scale.

Visual-based Assignment Criteria		
Appearance	% Volume of Gut Cavity	Frequency of Maturity Stages
yellow/pink, opaque/grainy	<10 to 20	Mature 1 (*n*=16)
yellow/white, transparent/smooth	21 to 30	Mature 2 (*n*=5)
yellow/pink/orange, transparent/smooth	31 to 75+	Ripe (*n*=16)
light yellow/white, transparent/grainy	<10 to 20	Recovery (*n*=7)
white, transparent/smooth	<10	Resting (*n*=6)

The diameters of the 341 oocytes from the four Mature 2 ovaries were pooled separately from the 1,717 oocyte diameters from the 17 Ripe ovaries, and these two sets of oocyte diameters were then cast into separate size/frequency-distribution graphs (Figure 4). The range of oocyte diameters from these two developmental stages overlapped considerably (0.68-3.91 mm for Mature 2 oocytes and 1.3-4.5 mm for Ripe oocytes), however, there appeared to be one distinct modal diameter for oocytes in Mature 2 ovaries and two modal diameters for oocytes in Ripe ovaries. The two peaks in oocytes diameter for Ripe ovaries were concentrated around 2 mm and 3.3 mm. The

largest modal diameter of oocytes from the Ripe ovaries was substantially larger that the modal diameter of oocytes from Mature 2 ovaries (2.1 mm).

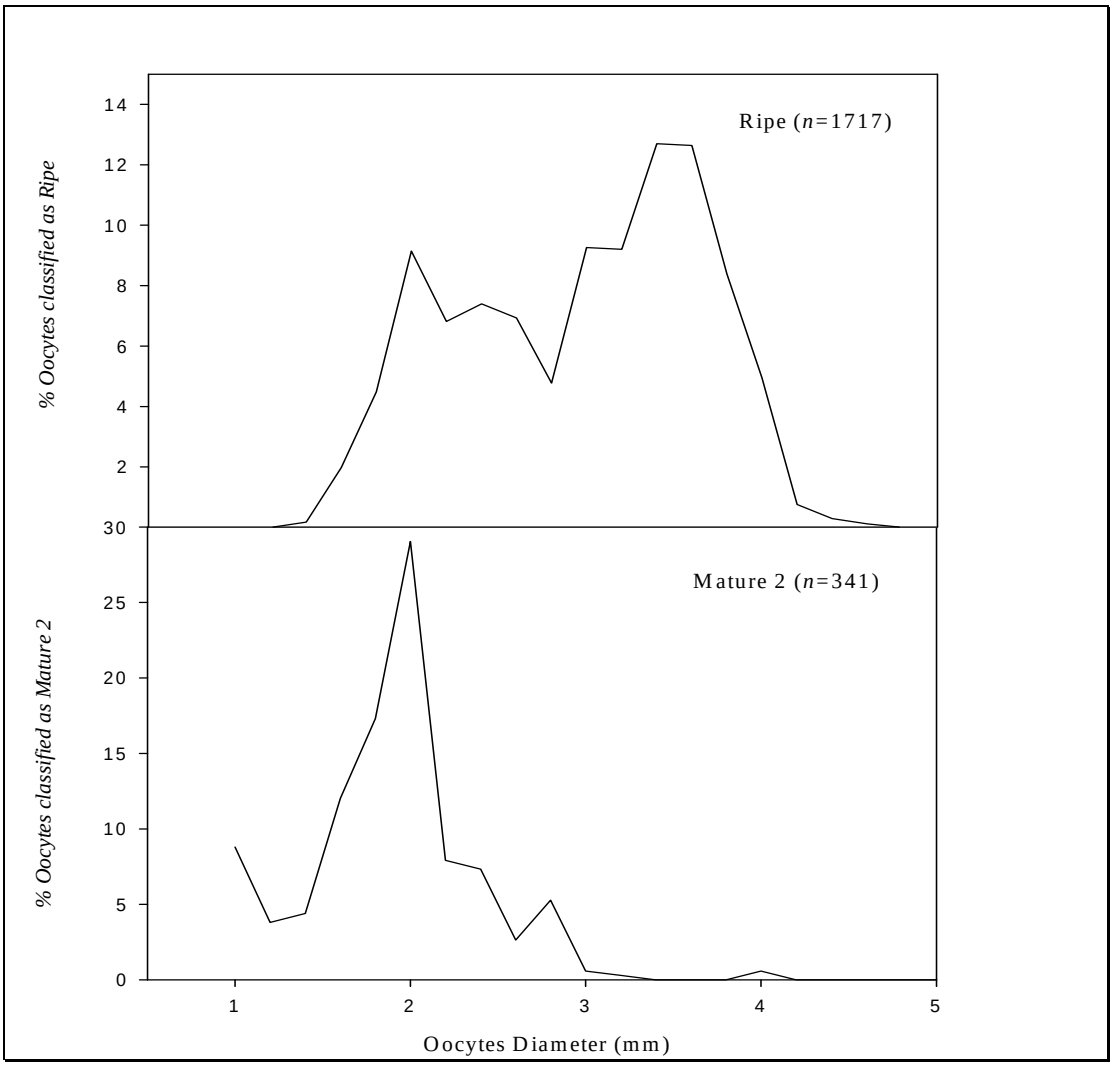

Figure 4. Size-frequency distribution of oocytes taken from *Hypostomus* ovaries classified into the Mature 2 (bottom) and Ripe (top) stages.

Seasonal variation in *Hypostomus* spawning activity

The mean monthly GSI values for female *Hypostomus* having ovaries classified as Mature 1, Mature 2, Ripe, or Recovery are reported in Figure 5. In general, GSI values were higher in the months of March to September than in other months.

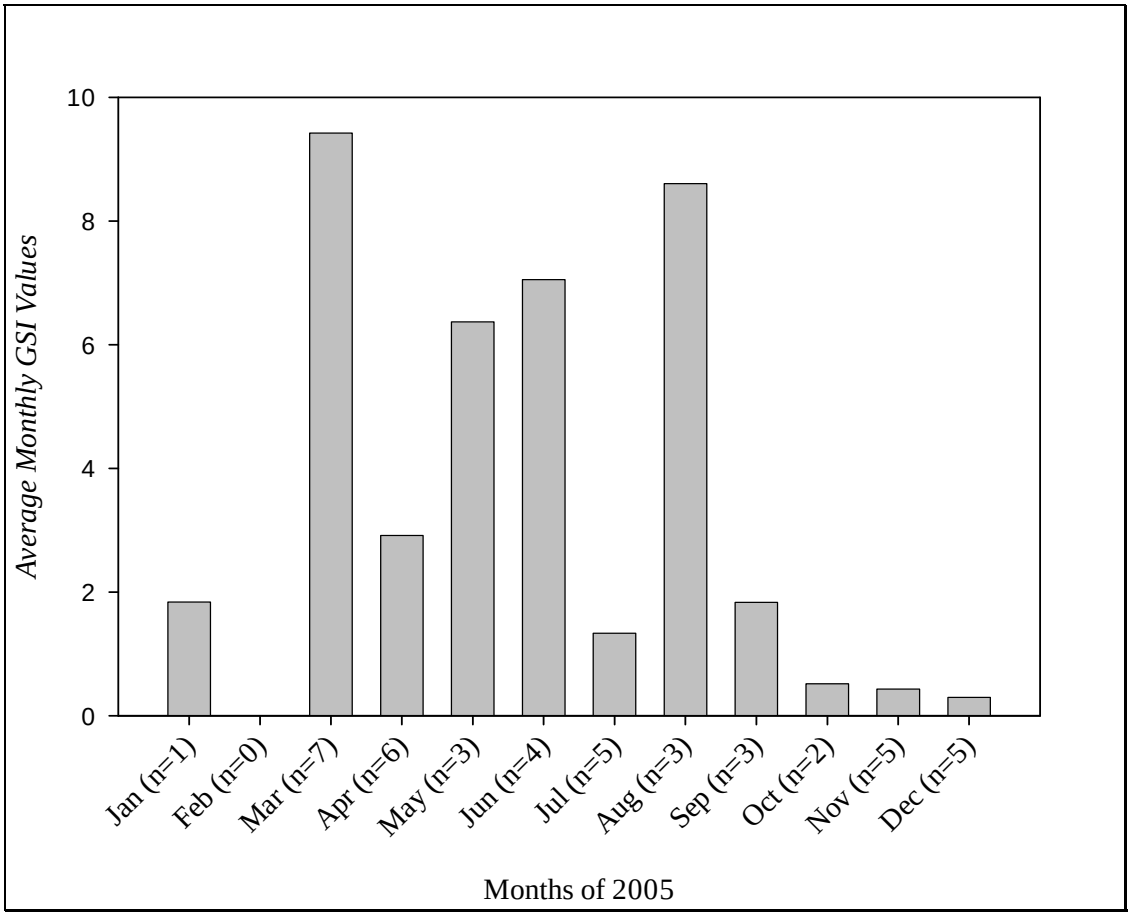

Figure 5. Monthly variation in the mean GSI for all female *Hypostomus* with ovaries classified as Mature 1, Mature 2, Ripe and Recovery (*n*=44).

The monthly variation in the results of the visual-based classification scale is reported in Figure 6. Because of very small representation of the Mature 2 stage in several months, I decided to pool the data for the Mature 1 and Mature 2 stages each month. Note that Ripe ovaries only occurred from March through September.

Seasonal and environmental factors affecting *Hypostomus* spawning activity

Monthly GSI and percent frequencies of maturity values did not appear to vary with rainfall totals, river discharge rates, or air temperature (Figure 7). Water from the San Marcos Springs fluctuates very little in temperature throughout the year and therefore was not considered to be a factor that would influence changes in *Hypostomus*

reproductive behavior. The larger GSI values, which occurred in the spring and summer months, occurred in the same seasons that longer photo periods occur. Although there are some variations in the pattern of monthly photo periods when compared to the monthly GSI values, photo period was the only environmental factor investigated in this study that increased and decreased in the same seasons that GSI values increased and decreased.

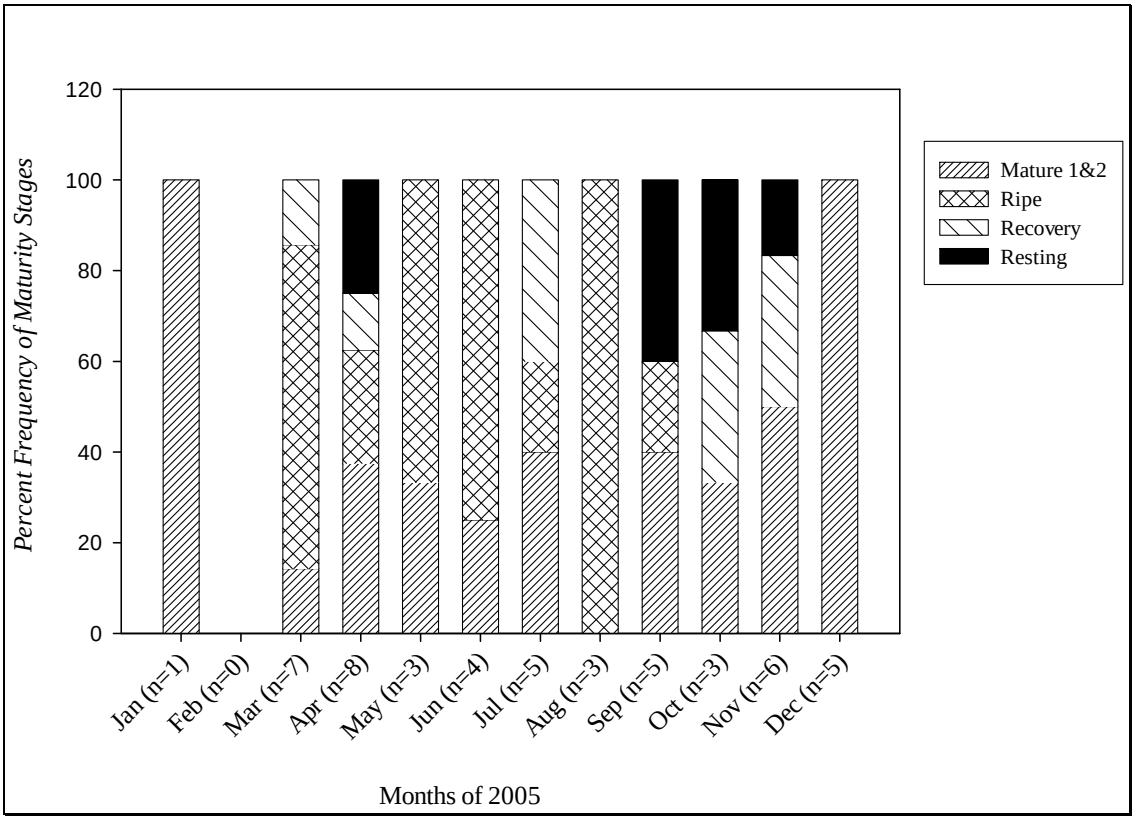

Figure 6. Monthly variation in the percent frequency of 50 *Hypostomus* ovaries assigned to Mature 1 & 2 pooled, Ripe, Recovery, or the Resting state.

Fecundity

Fecundity for the San Marcos *Hypostomus* population was estimated from the number of oocytes found in the 17 ovaries classified as Ripe during the 2005-breeding season. All 12,691 oocytes studied from these Ripe ovaries appeared to be in a mid- to

late-vitellogenic growth phase. The fecundity estimates for these 17 fish varied from 871 to 3,367 oocytes per ovary with a mean of 2,109 per ovary.

Spawning frequency within a breeding season

The spawning frequencies for individual fish were investigated by examining a subset of approximately 100 oocytes from each of the four Mature 2 and 17 Ripe ovaries (actual range 45-106 oocytes per ovary). Oocyte size frequency distributions for each of the 21 fish were plotted in separate graphs (Figure 8).

Foraging Effects on Eggs

Effects of *Hypostomus* foraging on fountain darter eggs

The foraging activities of *Hypostomus* appeared to negatively affect survival rate of fountain darter eggs. The total survival rate of eggs in the eggs-only treatment (two half-rounds with 135 and 24 eggs) was only 2.5 % (1 and 3 eggs, Figure 9). The survival rate of eggs in the eggs-plus-algae treatment (79 and 25 eggs) was 31 % (23 and 9 eggs, Figure 9). When the survival rates of eggs on the four half-rounds were tracked separately, a slightly different but consistent picture emerged. Survival of eggs on the two eggs-only half-rounds was 1% and 13% for a mean of 7%, while the corresponding egg survival rate on the two eggs-plus-algae half-rounds was 36% and 29% for a mean of 33% (

Figure 10).

The egg-loss control procedure showed that few, if any, eggs can be expected to spontaneously detach from half-rounds in a 48-hour period in the absence of *Hypostomus*. Indeed, the total survival rate of eggs on the half-rounds in the two control aquaria (one half-round each, initially with 16 and 10 eggs) was 92% (14 and 10 eggs).

In contrast, egg survival on the half-rounds in the two adjacent aquaria with *Hypostomus* (Figure 2) was only 23% (30 and 43 eggs initially, 5 and 12 eggs after 48 h). Survival rate of eggs on the half-rounds in the two control aquaria was 88% and 100% for a mean of 94%, while the corresponding egg survival rate on the half-rounds in the two adjacent aquaria with *Hypostomus* was 17% and 28% for a mean of 22.5%.

It is important to note that seven eggs were found loose on the bottoms of the two egg-loss control aquaria containing *Hypostomus* (two from one aquarium and five from the other). These seven eggs had apparently been dislodged from the half-rounds by the *Hypostomus*. Although these seven eggs appeared to be intact, they were not counted as having survived the exposure to *Hypostomus* because they would probably have little chance of survival in the wild after having been physically dislodged and left to drift. In contrast, no detached eggs were recovered from the bottom of the two control aquaria.

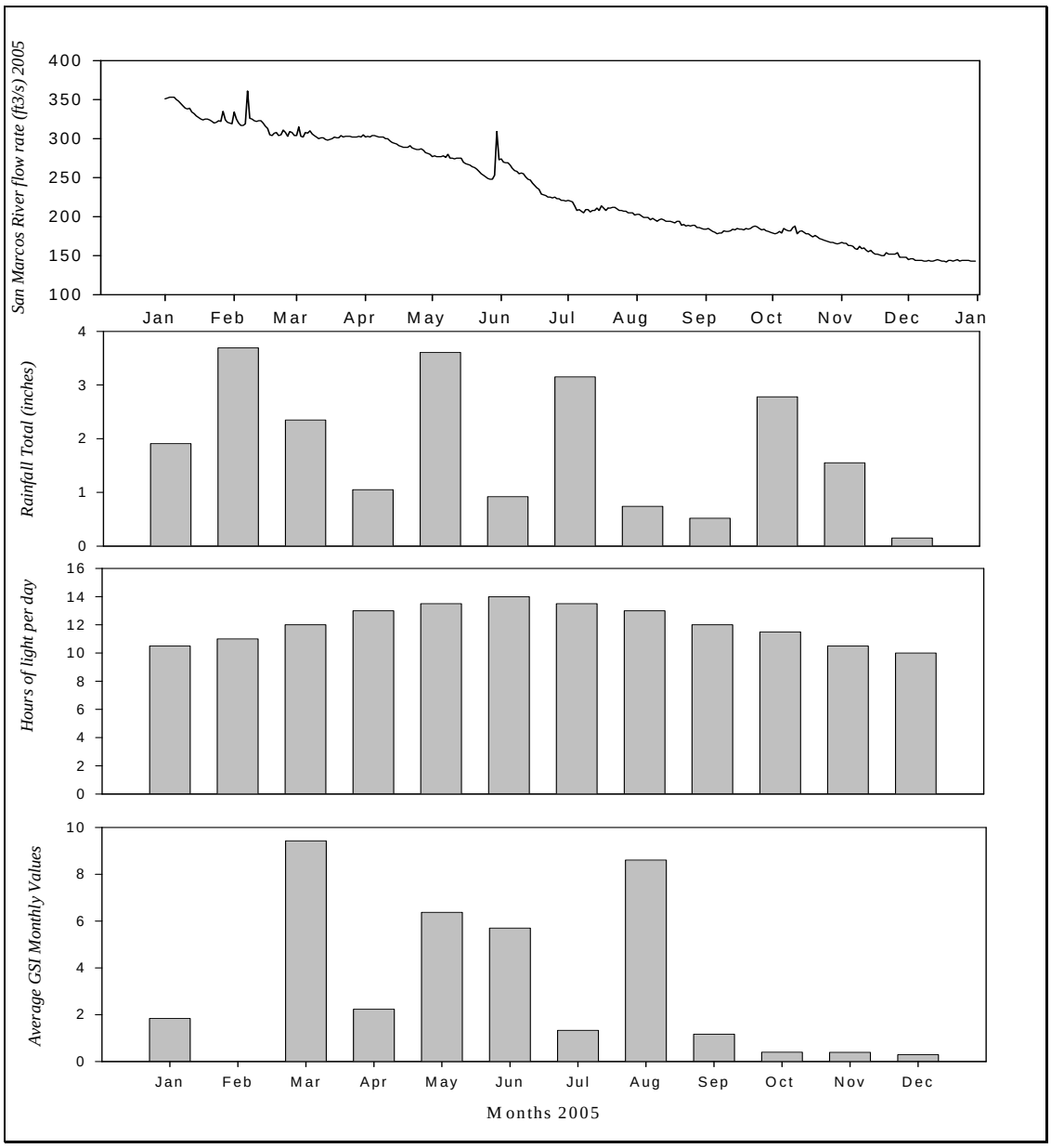

Figure 7. Discharge rates, rainfall totals, and mean monthly hours of daylight in the study site, compared to mean monthly GSI values for the San Marcos River *Hypostomus*.

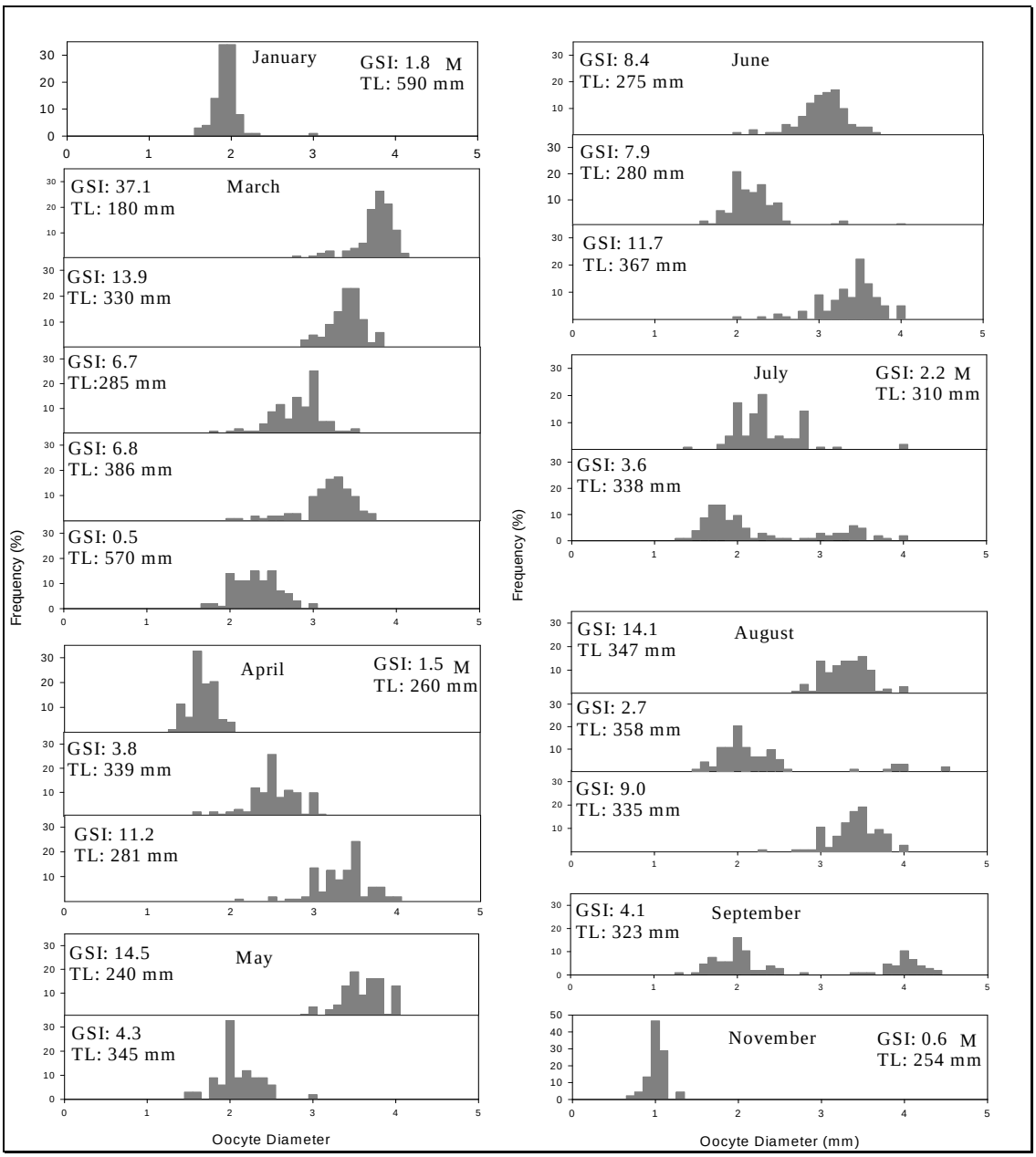

Figure 8. Oocyte size frequency distributions for 21 individual *Hypostomus* (17 Ripe ovaries; 4 Mature 2 ovaries "M") collected from January to December of 2005.

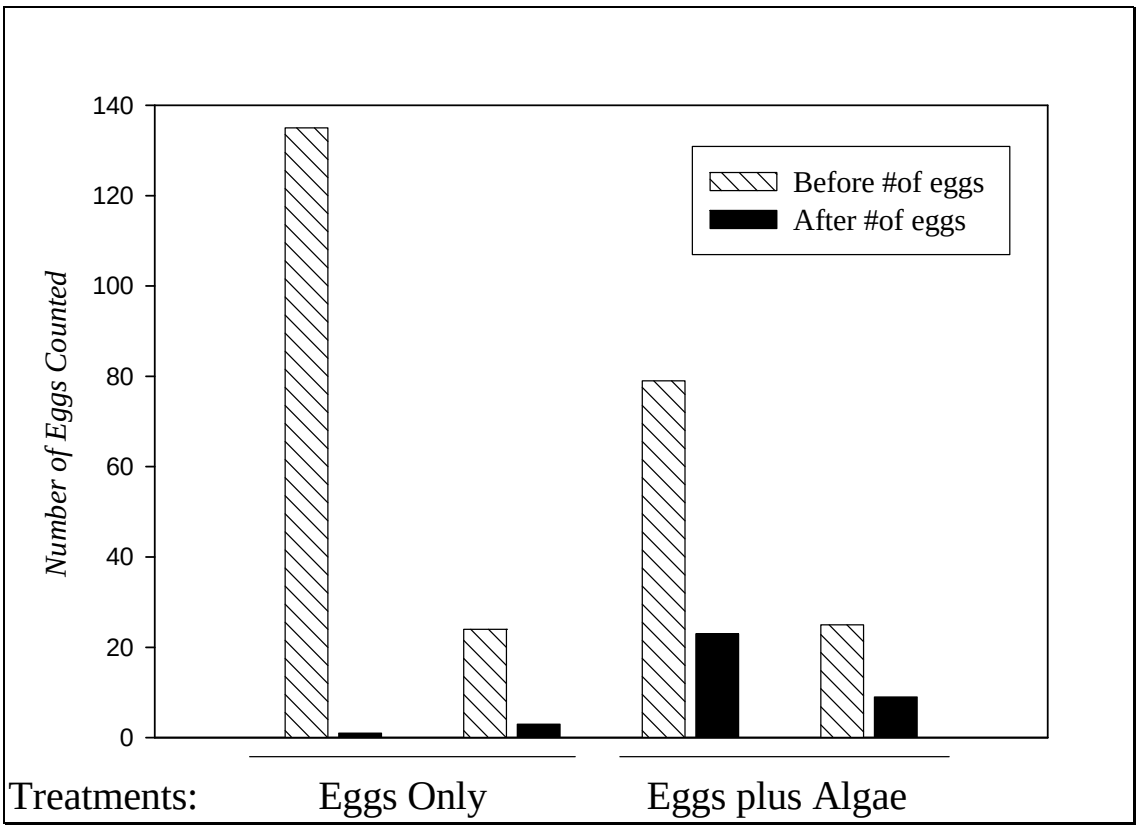

Figure 9. Numbers of fountain darter eggs on individual half-rounds in the eggs-only and eggs-plus-algae treatments before and after exposure to foraging *Hypostomus*.

After the egg-loss control procedure was completed, the two *Hypostomus* were euthanized and dissected. Three intact fountain darter eggs were recovered from the upper intestines of the two *Hypostom*

Error! Objects cannot be created from editing field codes.

Figure 10. The survival rate of fountain darter eggs on the two half-rounds in the eggs-only treatment and the two half-rounds in the eggs-plus-algae treatment after exposure to foraging Hypostomus for 48 h.

Effects of *Hypostomus* foraging on eggs of the Devils River minnow

Foraging *Hypostomus* appeared to also have some deleterious effects on the survival rate of Devils River minnow eggs (Figure 13). Egg survival rate in the eggs-

only treatment was 71 % (one tray; pre-experiment count 185, post-experiment count 132, Figure 14). Egg survival rate in the eggs-plus-algae treatment was 56 % (one tray; pre-experiment count 82, post-experiment count 46, Figure 14). Although egg survival rates in the two treatment trays were slightly lower than in the control tray (81 %; one tray; pre-experiment count 85, post-experiment count 69, Figure 14), the Devils River minnow eggs did not appear to be as threatened by the foraging activities of *Hypostomus* as were eggs of the fountain darter.

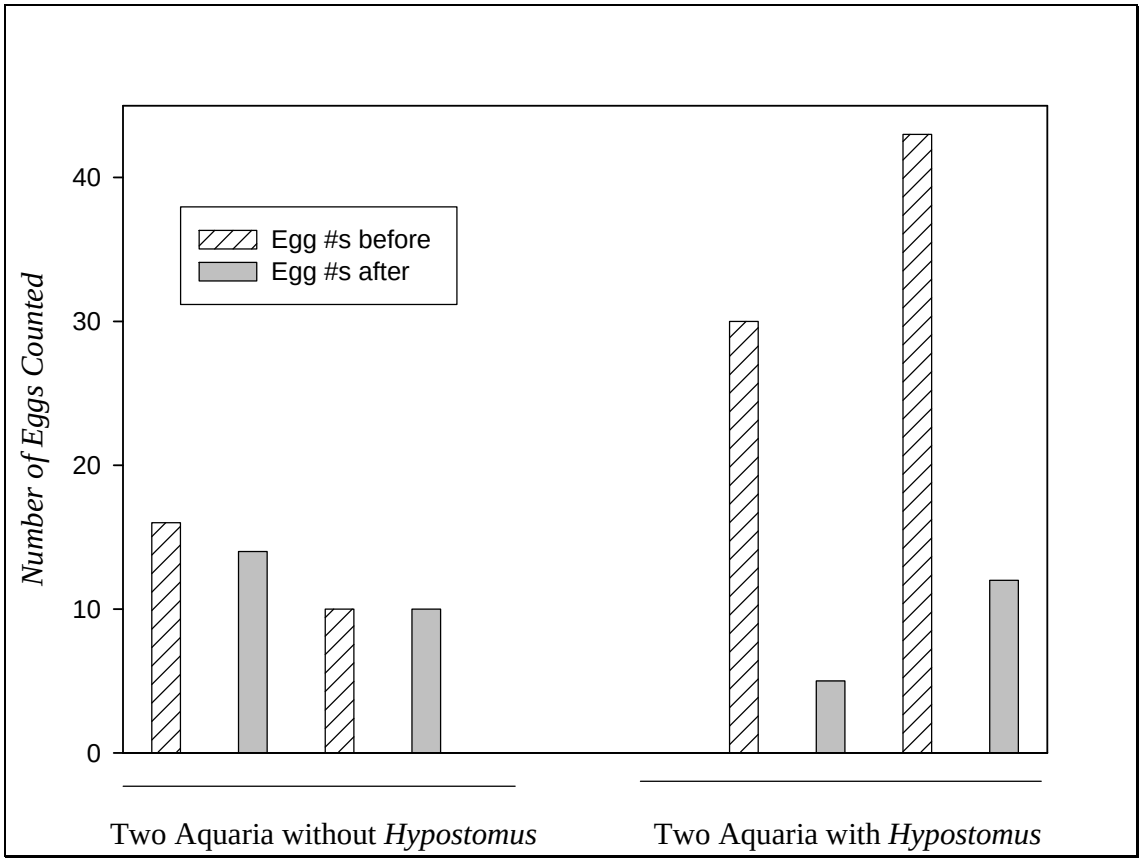

Figure 11. Numbers of eggs on individual half-rounds in the egg-loss control procedure before and after incubation period.

No Devils River minnow eggs were recovered from the digestive tract of the *Hypostomus*, and so there was no evidence of positive trophotaxy by foraging *Hypostomus* towards Devils River minnow eggs

It is important to note that the preparation of all three trays in the Devils River minnow experiment necessitated a great deal of egg handling. This handling could have affected the number of eggs in the final counts because it reduced the adhesiveness of the eggs, which could have allowed eggs to drift out of the trays in which they had been placed. However, such reduction in the counts would not likely result in any bias.

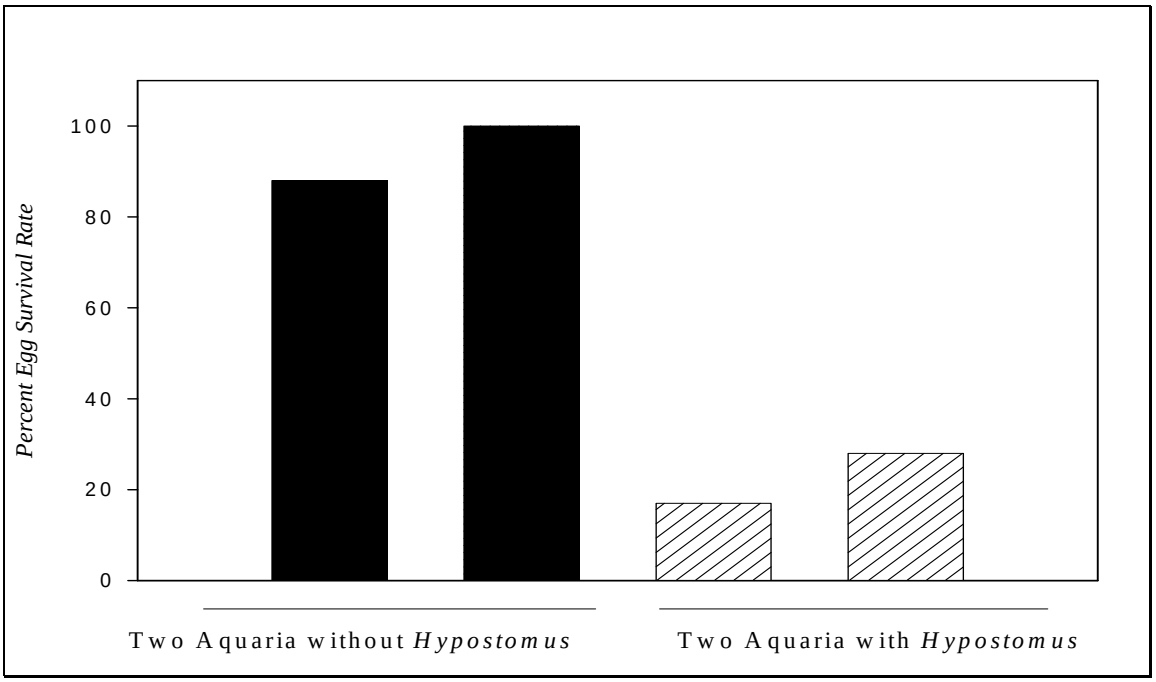

Figure 12. The 48-hour survival rate of fountain darter eggs in the egg-loss control procedure; the half-rounds in the two control aquaria without fish versus survival on the half-rounds in the two aquaria with *Hypostomus*.

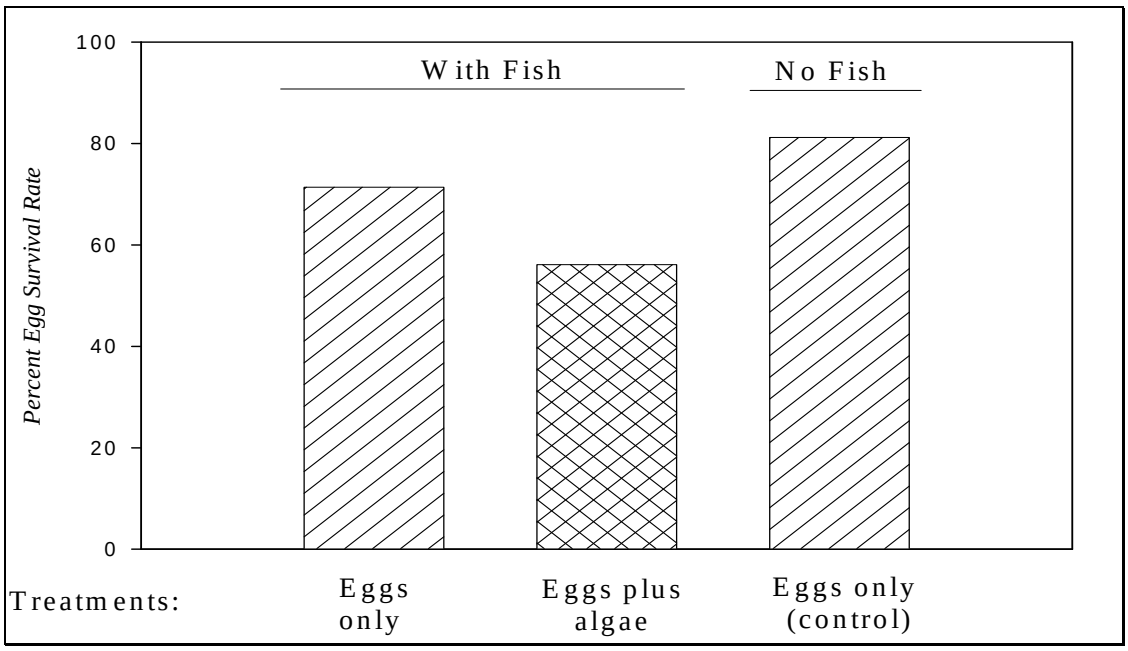

Figure 13. The percent of Devils River minnow eggs remaining in experimental egg trays after exposure to foraging *Hypostomus* for 48 h.

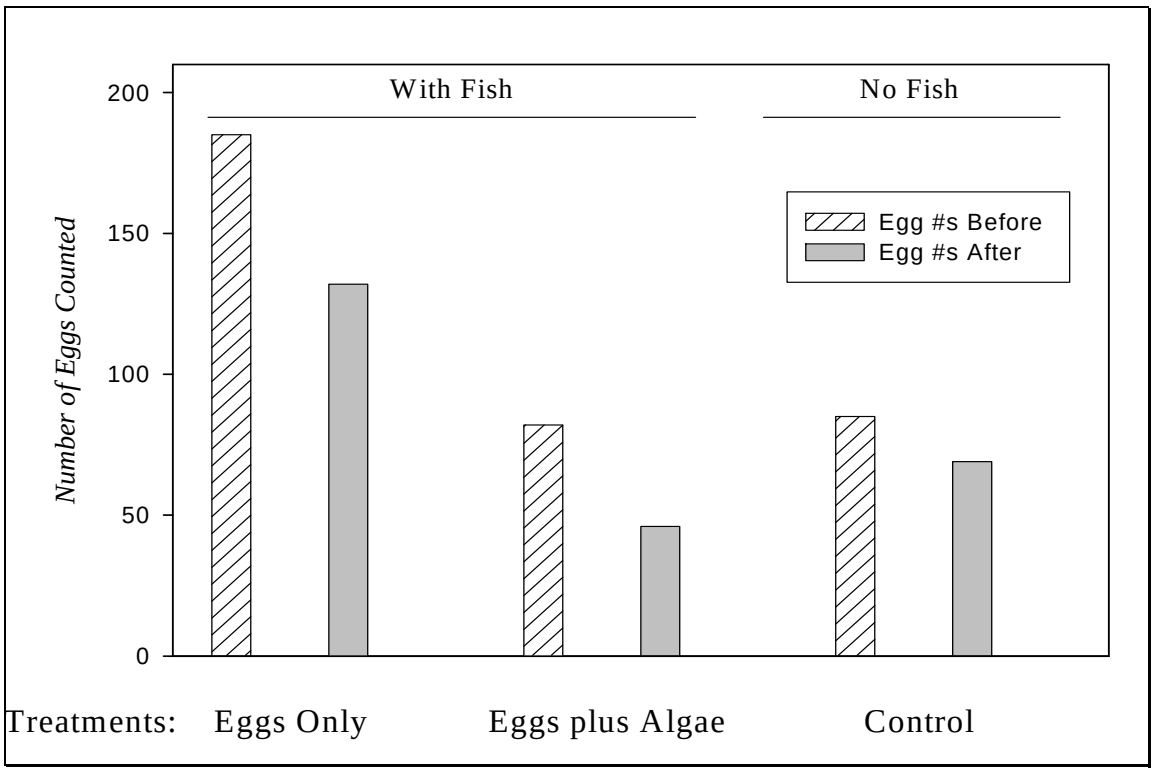

Figure 14. Numbers of Devils River minnow eggs in the eggs-only and eggs-plus-algae trays and the control tray before and after exposure to foraging *Hypostomus* for 48 h.

DISCUSSION

Reproductive Study

Spawning activity of armored catfishes in the native South American habitat peaks during the fall and winter months (Mazzoni and Caramaschi 1997a,b; Duarte and Araújo 2002), and has been linked to fluctuations in water temperature (Mazzoni and Caramaschi 1997a,b), as well as to seasonal peaks in rainfall that result in inundation of the flood plain (Power 1984b).

In contrast, the largest mean GSI values, and the highest frequency of Ripe ovaries for the population of *Hypostomus* living in the San Marcos River were recorded from March through September, suggesting that the spawning activity in the Northern Hemisphere is seasonally opposite to that in the Southern Hemisphere. While some other authors have suggested that rainfall events, river discharge rate, and/or water temperature might influence seasonal spawning patterns of armored catfishes in the Southern Hemisphere (Power 1984b; Mazzoni and Caramaschi 1997a,b; Duarte and Araújo 2002), I could find no evidence that these factors had any influence on seasonal spawning patterns of the San Marcos River *Hypostomus*. Therefore, seasonal variation in photoperiod, which is opposite in the two hemispheres, seems to be the only environmental factor studied thus far that is associated with the seasonally opposite spawning patterns in both hemispheres.

Understanding environmental factors that could influence *Hypostomus* spawning behavior could be key to developing a population management strategy for these exotics. Any program established to control *Hypostomus* populations would also benefit from knowledge of how often individuals of the species spawn in a spawning season, as well as whether or not all individuals of the population spawn at the same time. Although there are many species of fishes that are known to participate in mass spawning events as a group, such as brown surgeon fish (Kiflawi et al. 1998) and *Brycon petrosus* (Kramer 1978), I found no literature reporting such occurrences among *Hypostomus* populations. As was mentioned earlier, *Hypostomus* are known to spawn in a colony in both native and non-native habitats, and it has been reported that, in native habitats, spawning occurs over many months with individuals spawning multiple times during the spawning season (Mazzoni and Caramaschi 1997a,b; Duarte and Araújo 2002).

Although *Hypostomus* in native habitats spawn asynchronously multiple times, it is not clear if this behavior is duplicated in populations introduced into the northern hemisphere. In an attempt to answer this question I considered four basic scenarios of spawning activity that could be applied to any hypothetical population of fish during a breeding season. These scenarios are as follows: (1) synchronous single-batch spawning – individual fish spawn synchronously with conspecifics only once per year during a seasonally restricted breeding season; (2) asynchronous single-batch spawning – individual fish spawn asynchronously with conspecifics once per year, at more or less random times over a seasonally broad breeding season; (3) synchronous multiple-batch spawning – individual fish spawn more than once per year and spawn synchronously with conspecifics, with two or more such synchronous spawning events occurring over a broad

breeding season; (4) asynchronous multiple-batch spawning – individual fish spawn more than once per year, but asynchronously with conspecifics at more or less random times over a broad breeding season. For each of the four spawning scenarios three hypothetical oocyte size frequency distribution graphs representing three typical fish were created (Figure 15). These hypothetical graphs were compared to the oocyte size frequency distribution graphs in Figure 8 and monthly GSI values reported in Figure 5 in order to discern which scenario is best suited to describe the frequency of spawning activity for the San Marcos River *Hypostomus* population.

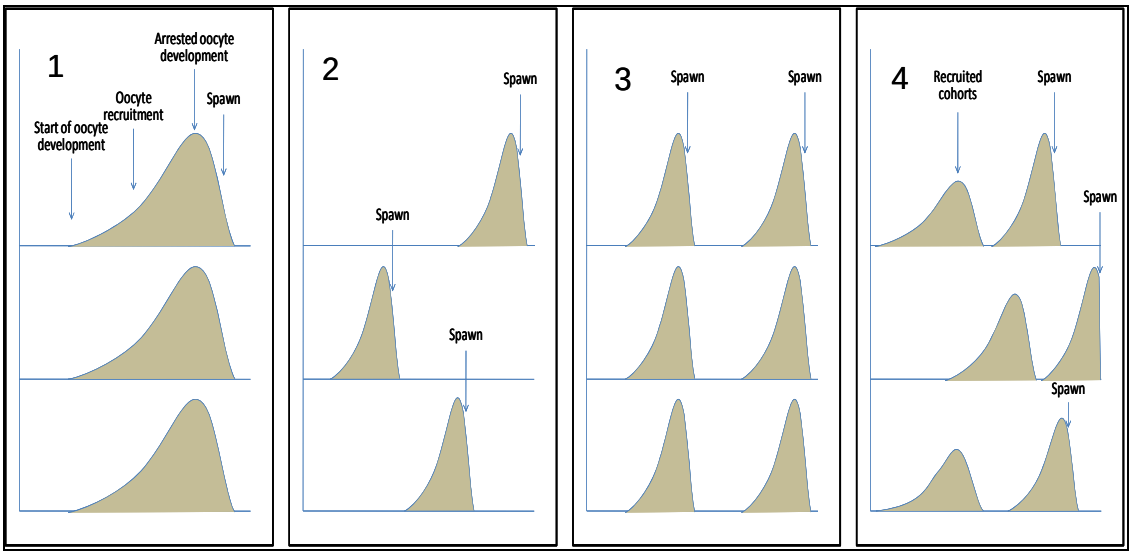

Figure 15. Oocyte size frequency distribution graphs representing three individual fish in four hypothetical spawning scenarios: (1) synchronous single-batch spawning; (2) asynchronous single-batch spawning; (3) synchronous multiple-batch spawning; (4) asynchronous multiple-batch spawning.

Scenario-1 (synchronous single-batch spawning): This pattern would occur over a relatively short breeding season, possibly one or two months, and can be considered unlikely because the breeding season for *Hypostomus* in the San Marcos River appears to occur over seven months from March to September (Figure 5).

Scenario-2 (asynchronous single-batch spawning): This pattern occurs over an extended breeding season of several months, however, individual fish spawn once during a breeding season asynchronously with conspecifics at random. Some, but not all, of the graphs in Figure 8 are very similar to the hypothetical graph for scenario-2. Therefore scenario 2 could be considered as a likely explanation for spawning patterns of the San Marcos River *Hypostomus.*

Scenario-3 (synchronized multiple-batch spawning): This pattern would occur over an extended breeding season of several months, similar to the breeding season of the San Marcos River *Hypostomus*. However, oocyte size frequency distribution graphs in Figure 8 do not the uniformity in the hypothetical graph for scenario-3 in Figure 15 and so this scenario can also be considered unlikely.

Scenario-4 (asynchronous multiple-batch spawning): This pattern is very similar to scenario-2, however individual fish spawn multiple-batches as opposed to single-batches. There are a few graphs in Figure 8 that are very similar to the hypothetical graph for scenario-4 and therefore scenario-4 could also be considered as a likely explanation for spawning patterns of the San Marcos River *Hypostomus*.

Although *Hypostomus* of the San Marcos River are spawning asynchronously, it is not clear how many times individual *Hypostomus* spawn during a single breeding season. In fish that spawn multiple times during a breeding season, individual ovaries will have a continuous distribution of oocytes from primary (perinuclear) to advanced (vitellogenic) growth stages (Macchi and Acha 2000). In the literature, information on oocyte growth stages is paired with oocyte size frequency distribution graphs to determining how often an individual fish will spawn in a breeding season (Wallace and

Selman 1981). The growth stages of oocytes are reported on size frequency graphs in order to identify specific cohorts at different growth stages. Without information on the growth stages of the oocytes, these cohorts would be difficult to detect. The number of times an individual fish will spawn in a breeding season is often determined by the presents of a variety of cohorts in different growth stages. Oocyte size frequency distribution graphs for multiple-batch spawning species often look very different from each other (Zastrow et al. 1991; Rinchard and Kestemont 1996; Plaza et al. 2002; Harada et al. 2003; McBride and Thurman 2003; Dadzie 2007). Therefore, without oocyte growth stage data to compare with oocyte size frequency distribution data, distinguishing patterns of single-batch spawning fishes from multiple-batch spawning fishes can be extremely difficult to do with any degree of certainty.

Growth stages of oocytes for individual *Hypostomus* in the San Marcos River during a single breeding season was not investigated in this study. Also, the oocyte size frequency distribution data for *Hypostomus* in the San Marcos River could be influenced by fish length and seasonality because these variables were not kept orthogonal during this studies collection period. A minimal monthly sample size could possibly further confound the results of this study. Therefore, attempts to assess the number of times individual fish spawn during a single breeding season is severely hindered by these complications. However, similar *Hypostomus* species are reported to spawn multiple times in native habitats (Mazzoni and Caramaschi 1997a,b; Duarte and Araújo 2002), and multiple spawning is considered to be very common among teleosts (Wallace and Selman 1981; Barbieri and Barbieri 1985; Lowe McConnell 1991). Based on the data presented in Figure 5 and Figure 8, the *Hypostomus* of the San Marcos River are spawning

asynchronously with conspecifics, and based on reports from the literature, it is likely that individual *Hypostomus* in the San Marcos River spawn multiple-batches in a single breeding season.

The over all reproductive potential for multiple-batch spawning fishes can be difficult to estimate, however fecundity estimates during a single breeding season were made for the *Hypostomus* in the San Marcos River by counting oocyte numbers in Ripe ovaries. Fecundity estimates for the unknown species of *Hypostomus* in the San Marcos River were compared with estimates made for known *Hypostomus* species in native habitats in order to determine a possible shift from *K* to *r* strategy in the novel habitat. Fecundity in the San Marcos River *Hypostomus* ranged from 871 to 3,367 oocytes per Ripe ovary, with a mean of 2,109. Duarte and Araújo (2002) examined *H. affinis* in the Lajes Reservoir and reported a range of 1,235 to 4,304 oocytes per Ripe ovary, with a mean fecundity of 2,373. Mazzoni and Caramaschi (1995) examined *H. affinis* and *H. luetkeni*, and reported mean fecundities of 1,784 and 845, respectively. Mazzoni and Caramaschi (1997b) later evaluated fecundity for multiple populations of *H. affinis* in the Paraiba do Sul River, southeast Brazil, and found mean fecundities ranging from 1,784 to 2,310 for these populations.

Bagenal (1966) suggested that fecundity could act as a density dependent regulatory mechanism, slowing fish population growth rate at higher densities and promoting a numerical increase of the population at lower densities. While the density of *Hypostomus* in the San Marcos River seems, on a subjective basis, to be high enough to induce crowding effects, the fecundity values for the San Marcos River population are not inconsistent with those of other South American populations reported above.

Consequently, no conclusions regarding crowding effects were drawn using variation in oocyte counts.

When a population is under stress from a density dependent factor, such as crowding, such stress might cause a species to adaptively increase the number of eggs per spawn, which would necessarily result in a decrease in egg size (Tyler and Sumpter 1996). There was a maximum oocyte diameter of 4.5 mm and a mean oocyte diameter of 2.96 mm for Ripe ovaries of the San Marcos River *Hypostomus*. This maximum oocyte diameter is comparable to one South American study, where the maximum oocyte diameters from Ripe ovaries of *H. affinis* and *H. luetkeni* were 4.07 and 5.43 mm (Mazzoni and Caramaschi 1995), respectively. Other studies of *Hypostomus* in the native habitat report a mean oocyte diameter of 3.0 mm for a population of *H. affinis* (Duarte and Araújo 2002), which is very similar to the mean oocyte diameter of Ripe ovaries for *Hypostomus* in the San Marcos River. Consequently, no conclusions regarding crowding effects were drawn using variation in oocyte diameters.

The oocyte diameters and mean fecundity reported for *Hypostomus* in the native habitat were not much different from those reported in the San Marcos River. This could be evidence that, even though *Hypostomus* populations in the San Marcos River appear, subjectively, to be reaching crowding conditions there has not yet been a peak in population density that would initiate a response to such conditions. It is unlikely that natural pressures from the ecosystem will begin to bring the population under control until evidence of crowding, such as increased fecundity or decreased egg size, is detected.

Foraging Study

The survival of fountain darter eggs exposed to foraging *Hypostomus* was substantially lower than in the control. Indeed, three fountain darter eggs were recovered during necropsy of the two *Hypostomus* following the 48-hour egg exposure period, indicating that fountain darter eggs were not just disturbed, but consumed during foraging. This evidence that *Hypostomus* is positively trophotaxic toward eggs of the fountain darter indicates that the exotics are negatively affecting the reproductive success of the already endangered fountain darter in the river. These results are especially significant given that I could find no previous account of whole fish eggs having been recovered from *Hypostomus* gut, and other studies on the diet of *Hypostomus* spp in the native habitat have typically classified fishes of the genus as detritivores or herbivores (Powers 1984a; Cardone et al. 2006; Pouilly et al. 2006).

The survival rate of Devils River minnow eggs was less negatively affected by the presence of foraging *Hypostomus* than was that of fountain darter eggs and no identifiable Devils River minnow eggs were recovered from experimental *Hypostomus*. However, the presence of foraging *Hypostomus* did have some effect on egg survival rate, probably due more to egg disturbance by *Hypostomus* as they forage rather than to incidental ingestion of eggs.

One possible explanation for the different effects *Hypostomus* seems to have on egg survival of these two native species could be related to differences in spawning habits between the two species. The fountain darter is a phytolithophilic spawner, and tends to deposit eggs on exposed surfaces, most typically on macrophytes (Schenck and Whiteside 1977), where they would be accessible to foraging *Hypostomus*. On the other

hand, the Devils River minnow is a lithophilic spawner, and tends to deposit eggs on loose substrate, where many sift down beneath the surface after deposition (Johnston 1999; Gibson et al. 2003) making them less accessible to foraging *Hypostomus*.

There are some possible sources of bias that may have influenced the trophotaxy experiment such as the origin of the experimental *Hypostomus*, which were collected from the San Marcos River. These fish had been exposed to fountain darter eggs in the wild, but not to Devils River minnow eggs. Another possible source of bias would be that the *Hypostomus* used in the foraging experiments were not positively trophotaxic toward the variety of algae cultivated for the treatments. Another factor to consider is the amount of egg handling that occurred in the Devils River minnow foraging study. It is all together possible that egg count numbers were skewed due to eggs drifting out of gravel trays before they could be counted.

Future Management Efforts and Recommendations

Influence of *Hypostomus* on native species management

Evidence from this study strongly suggests that *Hypostomus* foraging behavior can have deleterious effects on the egg survival rate of the fountain darter and Devils River minnow. However, it is also possible that other native fishes not currently listed as species of concern, especially those with phytolithophilic and lithophilic spawning patterns, could be affected by *Hypostomus* foraging activities and become listed as species of concern as a result. Thus, it is imperative that other native fishes restricted to spring runs in Texas should be studied for potential *Hypostomus* effect.

Relevance of reproductive biology to *Hypostomus* population control

If reproducing populations of *Hypostomus* remain in Texas waters, and especially if new introductions of *Hypostomus* occur, then native fishes already affected by these exotics or other factors are likely to become even more imperiled. Although there is much work left to be done, the information collected in this study regarding reproductive biology of *Hypostomus* from the San Marcos River will aid in management efforts designed to control exotic populations of *Hypostomus* in Texas, and perhaps other exotic armored catfishes that might be introduced into similar habitats in the Northern Hemisphere.

Management of Armored Catfishes and Other Exotics

Armored catfishes could potentially be causing a host of other problems that the scientific community has yet to discover. As existing populations of exotics grow unchallenged, and new exotic introductions continue, more research is needed to develop and implement action plans that would effectively limit or reverse these trends. Management efforts developed to deal with armored catfishes might also provide tactics to help deal with other problematic introduced species.

The sources of most exotic fishes introduced into United States waters can be traced to the ornamental pet trade (Howells 1999), and so the pet trade is a major causative factor that must be dealt with before any management efforts can succeed. One positive step in this direction was a request by the United Nations that international organizations associated with promoting responsible pet ownership begin drafting plans for a toolkit of regulatory and non-regulatory measures which would hopefully minimize the introduction of invasive species (Reaser 2004).

LITERATURE CITED

Bagenal, T.B. 1966. The ecological in the geographical aspects of fecundity of the plaice. Journal of the Marine Biological Association of the U.K. 46:161-186.

Barbieri, M.C. and G. Barbieri. 1985. Reproductive biology of the *Gymnotus carapo* (Linnaeus, 1758) on the Wolf dam. Morphology, histology, and seasonal variations in spawning (Teleostei, Gymnotidae). Revista Brasileira de Biologia 45(1/2):3-12.

Barron, J.L. 1964. Reproduction and apparent over-winter survival of the sucker-mouth armored catfish, *Plecostomus* sp., in the headwaters of the San Antonio River. Texas Journal of Science 16:449.

Burdick, Alan. 2005. Out of Eden: an odyssey of ecological invasion. Farrar, Straus, and Giroux Publications (1st Edition), Inc., New York City. NY.

Burgess, W.E. 1989. An atlas of freshwater and marine catfishes: a preliminary survey of the Siluriformes. Tropical Fish Hobbyist Publications, Inc., Neptune City, NJ.

Campbell, E.W. 2004. Brown treesnake fact sheet. U.S. Fish and Wildlife Services. http://www.mesc.usgs.gov/resources/education/bts/invasion/colonize.asp - continental

Cardone, I.B., S.E. Lima, Jr., R. Goitein. 2006. Diet and capture of *Hypostomus strigaticeps* (Siluriformes, Loricariidae) in a small Brazilian stream: relationship with limnological aspects. Brazilian Journal of Biology 66(1A):25-33.

Cohen, K.L, T.H. Bonner, and W.H. Nowlin. 2008. Gut content and stable isotope analysis of exotic suckermouth catfishes (*Hypostomus*) in the San Marcos River, Texas: A concern for spring endemics? Masters Thesis, Texas State University–San Marcos.

Courtenay, W.R., Jr., and J.E. Deacon. 1982. Status of introduced fishes in certain spring systems in southern Nevada. Great Basin Naturalist 42:361-366.

Dadzie, S. 2007. Vitellogenesis, oocyte maturation pattern, spawning rhythm and spawning frequency in *Otolithes rubber* (Schneider, 1801) (Sciaenidae) in the Kuwaiti waters of the Arabian Gulf. Scientia Marina 71(2):239-248.

Diong, C.H. 1982. Population biology and management of the feral pig (*Sus scrofa* L) in Kipahula Valley, Maui. Thesis Dissertation, University of Hawaii.

Duarte, S. and F.G. Araújo. 2002. Fecundity of the *Hypostomus affinis* (Siluriformes, Loricariidae) in the Lajes Reservoir, Rio de Janeiro, Brazil. Revista de Biologia Tropical 50(1):193-197.

Elser, J.J., M.M. Elser, N.A. MacKay, and S.R. Carpenter. 1988. Zooplankton – mediated transitions between N – and P – limited algal growth. Limnology and Oceanography 33(1):1-14.

Flecker, A.S. 1992. Fish trophic guilds and the structure of a tropical stream: weak direct vs. strong indirect effects. Ecology 73(3):927-940.

Gibson, J.R., J.N. Fries, and G.P. Garrett. 2003. Habitat and substrate use in reproduction of captive Devils River minnows. North American Journal of Aquaculture 66(1):42-47.

Harada, T., T. Ozawa, and Y. Masuda. 2003. Maturation and spawning frequency of *Lestrolepis japonica* (Aulopiformes: Paralepididae) in Kagoshima Bay southern Japan. Memoirs of the Faculty of Fisheries, Kagoshima University 52:13-18.

Hood, J.M, M.J. Vanni, and A.S. Flecker. 2005. Nutrient recycling by two phosphorus-rich grazing catfish: the potential for phosphorus-limitation of fish growth. Oecologia 146:247-257.

Hook A.W. and S.D. Porter. 1990. Destruction of harvester ant colonies by invading fire ants in south-central Texas (Hymenoptera: Formicidae). The Southwestern Naturalist 35 (4):477-478.

Hoover, J., K.J. Killgore, and A.F. Cofrancesco. 2004. Suckermouth catfishes: threats to aquatic ecosystems in the United States? Aquatic Nuisance Species Research Program Bulletin 4:1-9.

Howells, R. 1999. Guide to Identification of Harmful and Potentially Harmful Fishes, Shellfishes and Aquatic Plants Prohibited in Texas. Texas Parks and Wildlife, Austin, Texas.

Hubbs, C., T. Lucier, G.P. Garrett, R.J. Edwards, S.M. Dean, E. Marsh, and D. Belk. 1978. Survival and abundance of introduced fishes near San Antonio, Texas. Texas Journal of Science 30:369-376.

Hulbert, J., T.H. Bonner, J.N. Fries, G.P. Garrett, and D.R. Pendergrass. 2007. Early development of the Devils River minnow *Dionda diaboli* (Cyprinidae). The Southwestern Naturalist 52(3):378-385.

Johnston, C. E. 1999. The relationship of spawning mode to conservation of North American minnows (Cyprinidae). Environmental Biology of Fishes 55:21-30.

Kiflawi, M., A.I. Mazeroll, and D. Goulet. 1998. Does mass spawning enhance fertilization in coral reef fish? A case study of the brown surgeonfish. Marine Ecology Progress Series 172:107-114.

Kramer, D.L. 1973. Terrestrial group spawning of *Brycon petrosus* (Pisces: Characidae) in Panama. Copeia 3:536-537.

Leland, J. 1950. Aliens in the backyard: plant and animal imports into America. University of South Carolina Press. Columbia, S.C.

Lopez-Fernandez, H. and K.O. Winemiller. 2005. Status of *Dionda diaboli* and report of established populations of exotic fish species in lower San Felipe Creek, Val Verde County, Texas. The Southwestern Naturalist 50(2):246-251.

Lowe McConnell, R.H. 1991. Ecological studies in tropical fish communities. Cambridge: Cambridge University Press.

Macchi, G.J. and E.M. Acha. 2000. Spawning frequency and batch fecundity of Brazilian menhaden, *Brevoortia aurea*, in the Río de la Plata estuary off Argentina and Uruguay. Fisheries Bulletin 98:283-289.

Mazzoni, R. and E.P. Caramaschi. 1997a. Observations on the reproductive biology of female *Hypostomus luetkeni* Lacepede 1803. Ecology of Freshwater Fish 6:53-56.

Mazzoni, R. and E.P. Caramaschi. 1997b. Spawning season, ovarian development and fecundity of *Hypostomus affinis* (Osteichthyes, Loricariidae). Revista Brasileira de Biologia 57(3):455-462.

McBride, R.S. and P.E. Thurman. 2003. Reproductive biology of *Hemiramphus brasiliensis* and *H. balao* (Hemiramphidae): Maturation, spawning frequency, and fecundity. The Biological Bulletin 204:57-67.

McGregor, E.A. 1922. Observations on the egg yield of Klamath river King Salmon. California fish and Game:161-164.

McMahon, R.F. 1996. The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. American Zoologist 36:339-363.

Naumov, V.M. 1956. The ovogenesis and ecology of the sexual cycle of the murmansk herring *Clupea harengus* L. US Fish and Wildlife Services Special Science Report – Fish 327:203-261.

Nico, L. 2000. Non-indigenous aquatic species fact sheet 766. United States Geological Survey http://nas.ers.usge.gov.queries/SpfactSheet.asp?speciesID=786

Nikolsky, G.V. 1963. The ecology of fishes (translation by I. Birkett). Academic Press, London (1978 Edition), TFH Publications, Inc., Neptune City, NJ.

Plaza, G., G. Claramunt, and G. Herrera. 2002. An intra-annual analysis of intermediate fecundity, batch fecundity and oocyte size of ripening ovaries of Pacific sardine *Sardinops sagax* in northern Chile. Fisheries Science 68:95-103.

Pouilly, M., S. Barrera, and C. Rosalas. 2006. Changes of taxonomic and trophic structure of fish assemblages along an environmental gradient in the Upper Beni watershed (Bolivia). Journal of Fish Biology 68:137-156.

Power, M.E. 1984a. Habitat quality and the distribution of algae – grazing catfish in a Panamanian stream. The Journal of Animal Ecology 53(2):357-374.

Power, M.E. 1984b. Depth Distributions of Armored Catfish: Predator-Induced Resource Avoidance? Ecology 65(2):523-528.

Power, M.E., T.L. Dudley, and S.D. Cooper. 1989. Grazing catfish, fishing birds, and attached algae in a Panamanian stream. Environmental Biology of Fishes 26:285-294.

Power, M.E. 1990. Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. Ecology 71(3):897-904.

Reaser, J. 2004. U.N. seeks PIJAC's help on invasive species. Pet Product News International (July edition):19.

Ricker, W.E., ed. 1968 IBP Handbook no 3: Methods for assessment of fish production in fresh waters. London NW1:Blackwell Scientific Publications.

Rinchard, J. and P. Kestemont. 1996. Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and historical features. Journal of Fish Biology 49:883-894.

Robins, C.R., R.M. Bailey, C.E. Bond, J.R. Brooker, E.A. Lachner, R.N. Lea, and W.B. Scott. 1991. World fishes important to North Americans exclusive of species from the continental waters of the United States and Canada. American Fisheries Society Special Publication 21. American Fisheries Society, Bethesda, MD.

Sax, D.F. 2001. Latitudinal gradients and geographic ranges of exotic species: implications for biogeography. Journal of Biogeography 28:139-150.

Sargent, R.C., P.D. Taylor, and M.R. Gross. 1987. Parental care and the evolution of egg size in fishes. American Naturalist 129(1):32-46.

Schenck, J.R. and B.G. Whiteside. 1976. Distribution, habitat preference and population size estimate of *Etheostoma fonticola*. Copeia 4:697-703.

Schenck, J.R. and B.G. Whiteside. 1977. Reproduction, fecundity, sexual dimorphism and sex ratio of *Etheostoma fonticola* (Osteichthyes: Percidae). The American Midland Naturalist 98(2):365-375.

Strawn, K. 1955. A method of breeding and raising three Texas darters, Part 1. Aquarium Journal 26:408-412.

Terrill, C. 1970. Unnatural landscapes: tracking invasive species. University of Arizona Press. Tucson, Arizona.

Tyler, C.R. and J.P. Sumpter. 1996. Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries 6:287-318.

U.S. Fish and Wildlife Service. 1974. United States list of endangered fauna. U.S. Dept. Int., Fish Wildlife Services.

U.S. Fish and Wildlife Service. 1999. Endangered and threatened wildlife and plants; final rule to list the Devils River minnow as threatened. Federal Register 64:202(20 October 1999):56596-56609.

Vanni, M.J., A.S. Flecker, J.M. Hood and J.L. Headworth. 2002. Stoichiometry of nutrient recycling by vertebrates in a tropical stream: linking species identity and ecosystem processes. Ecology Letters 5:285-293.

Wallace, R.A. and K. Selman. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist 21:325-343.

Whiteside, B.G. and C. Berkhouse. 1992. Some new collection locations for six fish species. Texas Journal of Science 44(4):494.

Westbrooks, R.G. 1953. Invasive plants: changing the landscape of America Washington, D.C. Federal Interagency Committee for the Management of Noxious and Exotic Weeds, 1998.

Zastrow, C.E., E.D. Houde, and L.G. Morin. 1991. Spawning, fecundity, hatch-date frequency and young-of-the-year growth of bay anchovy *Anchoa mitchilli* in mid-Chesapeake Bay. Marine Ecology Progress Series 73:161-171.

VITA

Sandra L. Cook-Hildreth was born in Dallas, Texas on May 31st, 1979, the daughter of Debora Hildreth and Sam Cook. After completing her work at William B. Travis High School, Austin, Texas in 1997, she entered The University of Texas at Austin. She received the degree of Bachelor of Science in Biology from The University of Texas in May 2003. In August 2004, she entered the Graduate College of Texas State University-San Marcos. During the following years she was employed as an intern with the Texas Commission on Environmental Quality in Austin, Texas and became a full-time employee of that agency in January 2008. In May 2008 she became a full-time employee of the Texas Parks and Wildlife Department in Austin, Texas.

Permanent Address: 7104 Lyric Dr

Austin, Texas 78745

This thesis was typed by Sandra L Cook-Hildreth and Dr. David G. Huffman