THE EFFECTS OF HABITAT COMPLEXITY ON THE COGNITIVE PERFORMANCE OF TWO FISH SPECIES AND THEIR HYBRIDS

A Senior Scholars Thesis

by

HEATHER ANNE CHANCE

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designed as

UNDERGRADUATE RESEARCH SCHOLAR

April 2008

Major: Zoology

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ABSTRACT

The Effects of Habitat Complexity on the Cognitive Performance of Two Fish Species and Their Hybrids (April 2008)

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The complexity of an animal's habitat plays a significant role in behavior, morphology as well as other life history traits. Habitat complexity may also play a significant role in an animal's cognitive capabilities such as learning and memory attributes. In the field, I quantified habitat complexity in populations of two species of swordtail fishes – *Xiphophorus birchmanni* and *X. malinche* – as well as two populations of naturally occurring *X. birchmanni*/*X. malinche* hybrids found in the Rio Calnali, Hidalgo, Mexico. Habitat complexity differed between populations with *X. malinche* residing in the most complex and *X. birchmanni* in the least complex. In the laboratory, I tested spatial learning and memory capabilities of *X. birchmanni*, *X. malinche*, and their hybrids; I then investigated the relationship between habitat complexity and cognitive performance as well as the performance of hybrids compared to the parental species. Cognitive performance does not seem effected by habitat complexity. The hybrid populations do not seem to deviate in cognitive performance from that of the parental species.

DEDICATION

This work is dedicated to my parents as well as to all of my friends and family that constantly encourage me not only to accomplish my goals, but to have the courage to take on any challenge that obstructs my path to my goals.

Thank you.

ACKNOWLEDGMENTS

I would like to thank Holly Kindsvater for her help in the field, Dr. Gil G. Rosenthal and Seth W. Coleman for their support, guidance and critique of this manuscript and to the rest of the Rosenthal Behavioral Ecology Lab for their support, encouragement and comments. This work was supported financially by Undergraduate Research Scholars, NSF and NIH.

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CHAPTER I

INTRODUCTION

Learning is defined as the adaptive modification of behavior based on experience (Alcock 2005). Many animals must learn in order to find food and mates and to avoid hazards such as predators within their respective habitats and ecosystems. Spatial learning is employed by those animals allowing for the capability of acquiring both immediate and long-term information about their location as well as their orientation within that environment. A variety of strategies are used in order to find a location in space such as landmarks, global reference systems, path integration, and cognitive mapping (Boysen 2004).

Many species of fish have been shown to be not only capable of spatial learning and memory, but of using multiple methods to form cognitive maps of their environment depending on the type and complexity of the environment (Odling-Smee & Braithwaite 2003; Hughes & Blight 2000; de Perera 2004, 2003; Braithwaite & de Perera 2006). The complexity of the habitat in which an animal lives can exert selection on behavior and underlying neural substrates. Closely related African cichlid species residing in Lake Tanganyika live in diverse habitats that range from sand to large, spindle-shaped rocks. There is great diversity of behavior as well as a range of overall brain size. Fish that live in the more pelagic areas had the smallest brains while those that lived among the large rocks have larger brains (Shumway et al. 2007), which may support the theory

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This thesis follows the style and format of *Animal Behaviour* .

that a larger overall brain size allows for a larger capacity for information processing. Interspecific differences in spatial learning and underlying neuroanatomical substrates suggest that there is a genetic basis to complex behavioral traits, as well as others. Natural hybrid zones between species differing in habitat complexity therefore may provide insight into the evolutionary genetics of spatial learning.

Hybrid zones are defined as narrow regions in which two genetically distinct species meet, mate and are capable of producing offspring hybrids (Barton & Hewitt 1989). It has been assumed that the hybrid offspring of two different species would almost always be unfit in comparison to their parents. In actuality, studies have shown a general pattern of hybrid fitness in plants and animals that is greater than or even equivalent to that of one or both of the parental species (Arnold & Hodges 1995).

A hybrid zone exists between two swordtail species, the highland *Xiphophorus malinche* and the lowland *Xiphophorus birchmanni*, in the Rio Calnali, Hidalgo, Mexico. The secondary sex characteristics of males of the two species are drastically different. *X. malinche* are strictly highland with a pronounced elongation of the lower rays of the caudal fin that are bordered with pigmentation (“sword”), a moderately sized dorsal fin, and an irregular array of oval-shaped flanking vertical bars. *Xiphophorus birchmanni*, on the other hand, lack swords or bear short swords, have a more elongated dorsal fin, and have vertical bars that are expressed in a regular series (Rosenthal et al. 2003).

My thesis quantifies the spatial complexity of *X. birchmanni, X. malinche,* and hybrid habitat in the wild. In the laboratory, I then tested spatial learning abilities of the two species and their hybrids. This allowed me to evaluate (a) whether spatial learning has adapted to habitat complexity and (b) whether spatial learning abilities of hybrids deviate from those of parentals.

CHAPTER II

METHODS

In field

I modeled a rugosity rig from a description by the Shumway Lab of Boston University (Shumway et al. 2007). A string measuring 10 feet was held taught using wooden dowels over the substrate while a light-weight, small link chain of 2.2 cm per link was laid along the contours of the riverbed. At each field site, I took a sampling of topological complexity measurements at intervals conducive to the conditions of the environment. Rugosity is determined by taking the ratio of the length of chain to the length of taught rope. I also recorded maximum depth along the chain.

In lab

Subjects

We captured three populations of swordtail fish using baited minnow traps. Fish were collected from three sites in Calnali, Mexico. *X. malinche* were collected from Chicayotla, Arroyo Xontla (20°55’30” N 98°34’36” W) and hybrids were collected from Calnali-low (20°53’54” N 98°34’37” W) on the Rio Calnali; we collected samples of *X. birchmanni* from the Río Garces (20°56’24” N 98°16’54” W).

Plus-maze

I randomly selected four *X. birchmanni* males and housed them in their own filtered and aerated aquaria (73.5 X 30 cm and 41 cm high). Another four randomly selected males were housed individually in filtered and aerated aquaria (25.5 X 50 cm and 28 cm high) with two randomly selected *X. malinche* females.

Preference-run

I randomly selected six males from the three populations and housed them in filtered and aerated aquaria (73.5 X 30 cm and 41 cm high) with plastic plants as well as six females from each of the same three populations. Water was maintained at 22.3°C and the overhead fluorescent lighting supplied a 12:12h light:dark cycle. Fish were regularly fed a commercial flake food (Tetramin) as well as live brine shrimp nauplii and vinegar eels.

Apparatus

Plus-maze

The plus-maze design (Figure 1) was modified from Brown and Braithwaite (2004) and is comprised of a central compartment (30 X 30 cm and 20 cm high) as well as four adjoining compartments of equal area on each side. The maze was filled with water to a height of 15 cm. Access to each of the four compartments was accomplished by passing through a doorway (3.5 cm wide). Adjacent to each doorway was a tile (3.06 X 2.16 cm) marked by a black shape (rectangle, square, diamond, or circle) each with an area of 22.5 cm. Positioned opposite the doorway of each adjoining compartment, frozen brine

shrimp, *Daphnia*, were hung in screen sachets. Three of the four sachets contained *Daphnia* still in its original packaging while the fourth sachet allowed fish access to the food source by means of the absence of the original packaging. A clear container (8.7 X 8.7 cm and 19.5 cm high) was used to limit the fish to a specific starting area. A brick was initially used to provide cover for the fish inside the starting container that was later replaced by a table (7 X 7 cm) with a height of 2.2 cm. Since fish failed to respond to criterion using this apparatus (see results), we built a new apparatus to evaluate subjects' ability to learn cues associated with shelter.

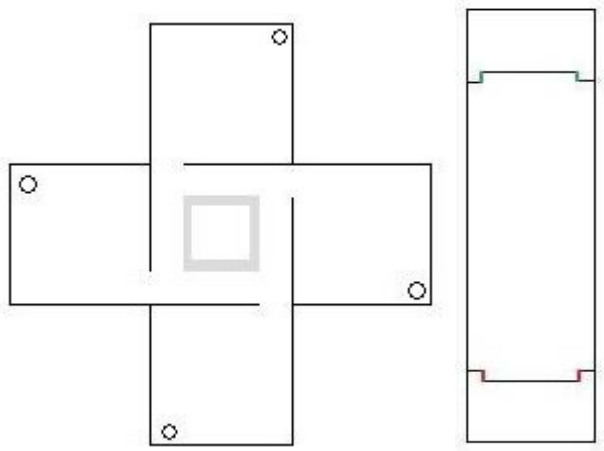

Figure 1. (left) Plus-maze - subjects started in the center – with shelter provided – and given free access to the maze. Each arm was marked with a different shape of equal area. A food reward was located in only one of the arms of the maze. (right) Preference-run: subjects started in the center of the run parallel to the barriers. Barriers were marked with a different shape of equal area. Only one of the two barriers allowed subjects access to the shelter provided by a brick.

Preference-run

The preference-run (19 X 75 cm and 19.5 cm high) contained a brick on each end of similar size and shape (Figure 1). Water was filled to a height of 14cm. In front of each brick, a clear plastic barrier was placed 15 cm into the interior of the run. One of the two barriers had a pair of 2 cm gaps that allowed fish to access the shelter of the bricks and was unseen by the fish facing perpendicular to the barriers. Access to shelter was indicated by a black square (7 X 7 cm), while the other barrier was marked by a black circle of equal surface area.

Procedures

Plus-maze

Shape assignments and food sachets were assigned compartments haphazardly. The rectangle always indicated the compartment with the sachet that allowed for foraging. Each fish was gently transferred from their tanks into the starting chamber and given 2 min to acclimate. Afterwards, the starting chamber was vertically lifted by a simple pulley system and fastened in place. The task of the fish was to find the compartment containing the sachet of *Daphnia* that would allow for foraging. Fish were allowed 10 min to find the correct compartment. Fish were initially not offered food at any other time, but common failure to complete the task required some supplemental feeding of Tetramin in order to sustain health. The position of each fish was monitored and recorded by an overhead camera which input into the Biobserve Viewer system for automated motion tracking.

Preference-run

Each fish was gently transferred to the center of the preference run and released parallel to the barriers. The fish's task was to pass through the barrier marked by the square in order to receive shelter. Fish were given 10 min to complete the task. If unsuccessful, fish were coerced through the proper barrier and left alone for approximately 5 min. Bricks were moved and rotated randomly on a daily basis and barriers were assigned to the ends by flipping a coin. Fish movements were recorded with a Sony Digital Handicam mounted atop a tripod above the run. Videos were scored based on the initial direction traveled, latency to first movement toward a barrier, latency to correct direction if initial direction traveled was away from the reward zone, latency to reach the correct barrier, and latency to pass through the barrier indicated by the square.

CHAPTER III

RESULTS

In field

ANOVA revealed significant among population differences in rugosity ($F_{1,3}$ =3.55, p=0.03; Figure 2). Rugosity – i.e. habitat complexity – was significantly higher at the locale with *X. malinche* compared to the locales with hybrids (p=0.045; p=0.006) and locale with *X. birchmanni* (p=0.009) (Table 1).

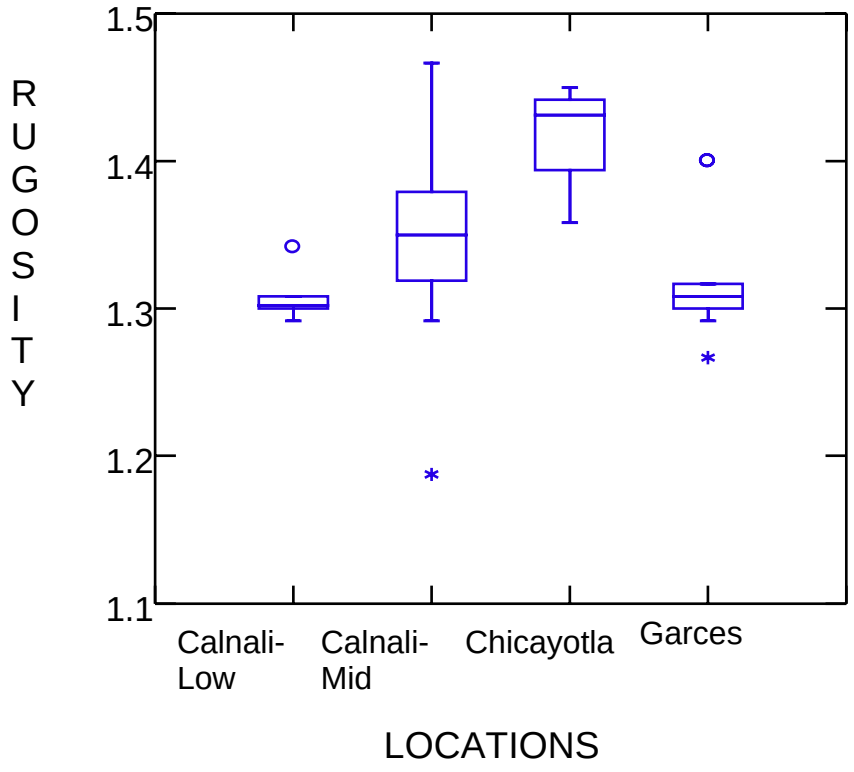

Figure 2. Above is a box plot showing the differences in habitat complexity among the four *Xiphophorus* populations.

Table 1. Fisher's least significant difference test.

SITE$(i)	SITE$(j)	Difference	p-value	95.0% Confidence Interval	
				Lower	Upper
Cal-low	Cal-mid	-0.035	0.270	-0.100	0.029
Cal-low	Chicayotla	-0.110	0.006	-0.185	-0.035
Cal-low	Garces	-0.014	0.637	-0.075	0.047
Cal-mid	Chicayotla	-0.075	0.045	-0.148	-0.002
Cal-mid	Garces	0.021	0.462	-0.037	0.080
Chicayotla	Garces	0.096	0.009	0.026	0.166

In Lab

Plus-maze

In plus-maze trials I found no evidence that the fish learned to associate the stimulus shape with the food reward (Figure 3). Also, I found no differences between the latency to find the food reward – an assay of associative learning – between *X. malinche* from Chicayotla and *X. birchmanni* from Garces (t_{67}=0.09, p=0.92; Figure 4).

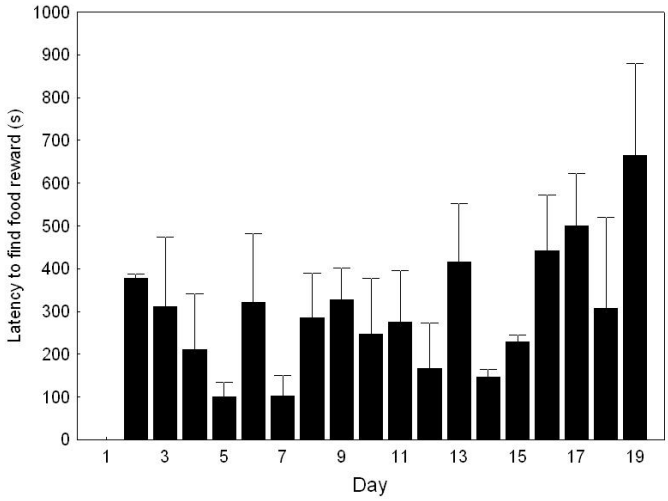

Figure 3. Bar graph showing the mean (±SE) latency to finding the food reward across the trial. The graph indicates that there is no evidence of the subjects learning to associate a particular shape (rectangle) with a food reward.

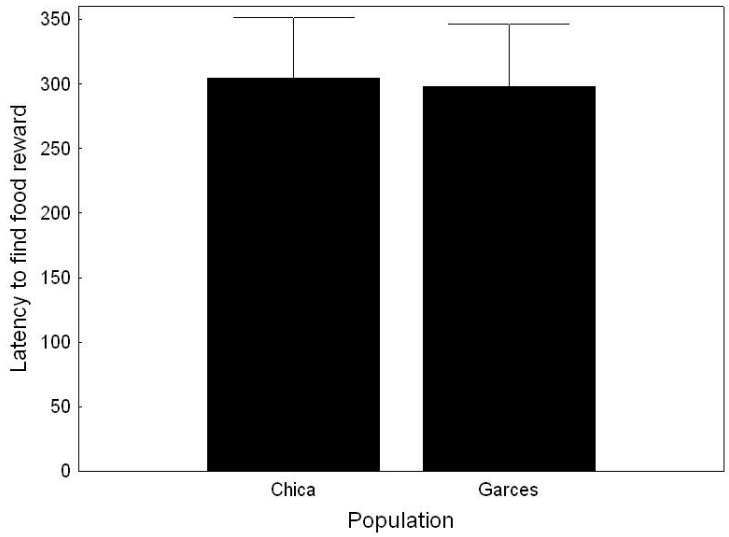

Figure 4. Bar graph showing the mean (±SE) latencies to finding the food reward for *X. malinche* from Chicayotla ("Chica") and *X. birchmanni* from Garces.

Preference-run

In preference-run trials, for all measures of latency – such as latency to moving in correct direction, latency to finding the door to shelter – I found a bimodal effect, with longer latencies early and late in the testing period (Figure 5).

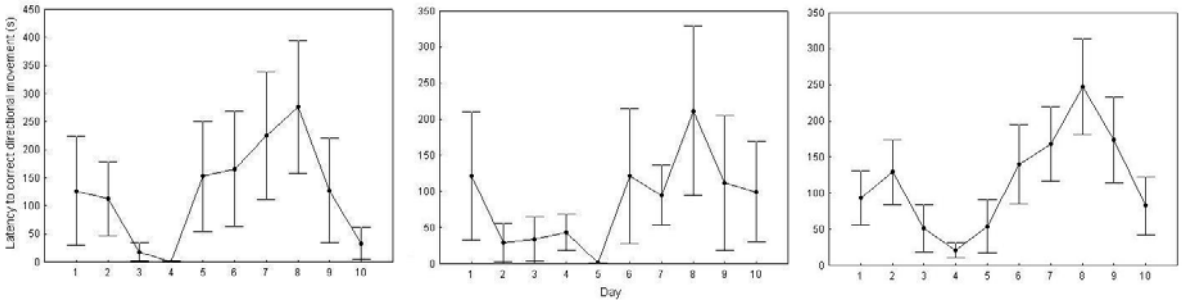

Figure 5. Latency to correct movement for *X. malinche* (left panel), hybrids (middle panel), and *X. birchmanni* (right panel).

CHAPTER IV

CONCLUSIONS

Various ecological factors affect cognitive performance, including habitat complexity and predation pressure. By measuring rugosity among the parental and hybrid populations I found that there are differences between in habitat complexity among *Xiphophorus* populations. In this study, *X. malinche* has a more complex habitat than the other populations. As the locations of the populations are further downstream, the rugosity value – habitat complexity – decreases, with the Calnali-Low hybrid population being of equal complexity to *X. birchmanni*.

There was no evidence of the fish learning to associate the stimulus shape to the food reward in the plus-maze trials. During the trials, the fish were not inclined to search the maze for a food reward. Most of the fish remained hidden under the table provided to them in the starting chamber. The preference-run was then designed to simplify the original plus-maze design and to allow for the acquisition of shelter as a reward.

The fish in the preference-run trials may have learned to associate the stimulus shape with the location of shelter, but displayed a perplexing pattern over the course of the experiment. It is a possibility that only offering shelter as a reward is not enough motivation. Further experiment assays should consider using food as well as shelter or another form of motivation. Using a visual cue to determine visual association may also need to be changed to using another cue such as olfaction.

My tests do not indicate that there are differences in learning abilities between the parental species and their hybrids. The sample size used was not large enough to allow for confident results. Running more assays would allow for a larger sample size and more confidence in results.

The research in the Rosenthal lab continues to explore these questions. We feel that we have identified a good learning assay that can be used to investigate the ecological and evolutionary mechanisms affecting cognitive performance in swordtails, but further refining is necessary.

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