The Biological Constraints of Learning in Fathead Minnows (Pimphales promelas)

Key words: classical conditioning, predator-prey interactions, teleost fish, selection pressures, vision, chemosensory, lateral-line system, biological constraints.

My interest in fish cognition and behavior stems from coursework (Learning Psychology and Sensation & Perception), the intellectually stimulating book, Fish Cognition and Behavior (1), and research in the aquatic animal lab. These experiences taught me how our sensory systems, action systems, and processing systems interact to produce our worldview. Importantly, they also provided the insight that the self-worlds of nonhuman animals differ radically from our own. Surely, Uexkull (5) was correct, to understand the behavior of nonhuman animals, we must view their behavior in light of their self-world, not ours.

In 1966 Garcia and Koelling (6) showed that rats could associate a gustatory response with an electrical stimulus, but not an audiovisual stimulus. The question whether these data showed that animals were constrained from learning certain associations or were in fact prepared through evolution to make certain associations fueled researchers for decades. I will explore the latter view by examining anti-predatory behavior in fathead minnows (*Pimphales promelas*), which are subject to multiple selection pressures (i.e., aerial, aquatic). Aerial attacks come from above and are accompanied by the sudden appearance of a dark shape and pressure waves from the diving bird. Aquatic attack strategies often comprise either ambush, or pursuit. In an ambush attack the predator sits and waits, until prey swim within attacking distance, at which point a sudden attack is launched. Pursuit predators typically initiate chase when prey are sighted.

It is proposed that these attack strategies may be more readily associated with certain stimuli than others. For example, an aerial attack is preceded by a visual stimulus (VS) and the pressure wave generated by the attack which would be detected by the fish's lateral line system. There is little by way of a chemosensory stimulus (ChemS) associated with the attack. It is possible then that minnows could more readily associate VS or lateral line stimuli (LLS) with an aerial attack than these fish could associate a ChemS with an aerial attack. Three experiments are proposed to test this idea. In Exp 1 an aerial attack will be preceded by either a VS, or ChemS or LLS. It is predicted that VS and LLS will be more easily associated with an aerial attack than will ChemS. Exp 2 involves simulated attacks by an ambush hunter. For the following reasons, it is predicted that LLS and ChemS will be more easily associated than VS. Ambush hunters are often more round and blunt faced than pursuit hunters and probably produce pressure waves in front of them as they attack. In addition, ambush hunters are virtually motionless which could strengthen ChemS. For aquatic pursuit predators, it is predicted that VS and ChemS will be more easily associated than LLS. By virtue of their attack style and shape, pursuit hunters likely generate minimal pressure waves. However, detection of their ChemS can warn of predators in the area and VS can provide information about the level of threat.

<u>Subjects.</u> Fathead minnows inhabit fresh water, have a well developed octavolateralis system, well developed eyes producing excellent color vision and an olfactory system that allows them to detect chemosensory cues at extremely low concentrations (2,7).

<u>Materials.</u> Large tanks will be required for housing and clear acrylic tanks will be used for testing. Filtration can be either through a closed-water or open-water system. The conditioned stimuli will consist of a VS (a blue flashing light), ChemS (a neutral stimulus: grinding highly aromatic flowers and placing in solution with freshwater) and a LLS (a Lubel underwater speaker set at 50 Hz with a square wave).The three different attack styles will be simulated using an aerial model, an ambush hunter model (flounder), or a pursuit hunter model (Pike; 1; 7; see previous research essay).

Procedure. In each of three experiments, groups of 30 minnows will be transferred from the housing tanks to the experimental tank and allowed to acclimate. Each day, for five days, a group of minnows will receive 4-30 sec presentations of the conditioned stimulus (CS). Each group will receive only one CS type. For groups in the experimental condition, at 20 sec into the CS presentation a simulated attack by a predator will occur. After 10 sec, the predator will be removed from the tank and the CS will offset. The ITI will be approximately 120 min. For groups in the control condition, minnows will receive unpaired presentations of the CS and predator attack (3). Control groups will receive two trial types in which the 30 sec CS either precedes the 10 sec predator attack by 15 min, or follows the 10 sec predator attack by 15 min. A total of 8 replications of each experimental condition for each experiment will be conducted.
Analysis. As was shown in my honors thesis, response measures will likely differ depending on the predator. Thus, it is expected with the aerial predator that minnows will startle and descend in the water column. With the ambush predator, it is expected that minnows will startle and then school more tightly. A similar response is expected for the pursuit predator, but I will need to conduct this study to determine antipredatory response. Parametric tests will be used when the data meet the appropriate assumptions; nonparametric equivalents will otherwise be substituted. For each experiment, a repeated measures ANOVA with two between groups factors CS Sensory Modality (octavolateralis system, vision, chemosensory reception) and Group (paired, unpaired) and one within groups factor (trials) will be used to analyze the data. Significant main effects will be further analyzed with post hoc tests (e.g., Neuman-Keuls test). Significant interactions between the three factors will be further analyzed using simple main effects tests(4).
Expected Findings. If these predictions are confirmed, they will extend the biological constraint literature to a different behavioral system, fish predation. It other words in fish, being able to readily associate a lateral-line stimulus with an aerial attack carries more biological significance than not being able to associate a chemical stimulus with an aerial attack. The latter is not of importance to the animal, because that is not how its' self-world works. Additionally, the study provides converging evidence about the conditional boundaries of such genetic organization (8). That is, the amount of experience necessary for maintenance and complete elaboration of the response as well as its degree of conditionability by arbitrary reinforcers provides information about the boundary conditions of such genetic organization. The results will also shed light on predator-prey interactions and lead scientists to test other species for similar results. This could in turn aid in species conservation. For example, for rapidly declining salmon populations, if predation is a significant threat to the population, then learning about the system could help us deduce other ways to protect the species. Particularly if predation is coming from a novel source (i.e., one the species did not evolve with; e.g., fishing by humans). As a recent graduate and woman of color I understand the importance of research experiences, in an undergrad education role models and mentors; thus, I can capitalize on this by involving women and people of color in my work. Further, this proposed research will help build a foundation of research skills and publications to allow me attract external funding for my center of excellence. To my knowledge these experiments have yet to be done, and represent an original contribution to science.
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The motto for Southwestern University reads `It is not who you know but what you know.¨ I firmly stand by this aphorism and I know that I love animals and that I have loved animals as long as I can remember. As a child I would accompany my father to the military veterinary clinic and spend hours watching him work with domestic pets and exotics. As I got older, I assisted with animal husbandry and often assisted with minor surgeries. However, as I grew intellectually and physically, my interests diverged from veterinary medicine to a research career in animal behavior, most probably through academia. In addition, I hope to someday make a strong impact on the field of animal behavior and serve as an important role model for women and men of color who look to mentors for the strength and courage to pursue their passions. Realization of these dreams will require a doctoral degree from a high quality animal behavior program and it is for that reason that I am applying for a NSF Graduate Research Fellowship.

Since those days of working with my father, numerous experiences have affirmed and enhanced my qualifications for this fellowship. At the end of ninth grade, I was accepted to the Texas Academy of Mathematics and Science (TAMS) a statewide competitive application is required to secure one of the 240 slots available annually. During the summer of 2004, I worked in Dr. William Rowland's lab under the auspices of NSF's REU program in animal behavior. What made this summer so interesting and so exciting was that I was able to independently conduct my own experiments and for the first time I was a member of a real research team. The opportunity left me well grounded in basic laboratory techniques and introduced me to an animal model, which I previously thought was served best fried with chips.

The following year, I was accepted to Rice University's AGEP program, with the promise of conducting research with tigers. Working in a zoological setting taught me that the broader impacts of scientific research are as important as the impact of the knowledge on the scientific community. Being socially awkward, the need to communicate the purpose, methodology and findings to lay people came as a great challenge. I stood in front of the tiger enclosure for more than 350 hrs documenting their behavior with my computer software program. As a consequence of their popularity, I encountered hundreds of people asking me a range of questions about the tigers and my study. I learned to effectively communicate to a wide audience general knowledge of tigers, the problems associated with stereotypic behavior in captive animals, and the promise of behavioral enrichment to make more natural the captive animal's behavior.

My two-year joint fellowship between the United Negro College Fund and Merck provided an opportunity to work in the state of the art Merck Research Laboratories, where I was immersed in the fast paced world of pharmaceutical research. The experience gave me tremendous insight into industry sponsored research and informed a life-long goal of mine to establish a center for the study of behavioral systems in nonhuman animals.

At Southwestern University (SU), I was an active member of the SU Animal Behavior Society (SUABS), a student organization. In my sophomore year, I initiated, organized and led the first SU Wild Animal Behavior Day. Over 30 elementary students were introduced to the college's animal research programs, provided information about how to greet a dog and recognize the signals that indicate the dog is safe to approach, informed about the different careers that are possible in the field of animal behavior, and showed the kids what could be accomplished if they applied themselves to their academic work. In my junior year, I was elected as the Animal Behavior Student Representative. In this role, I communicated the thoughts and concerns of the animal behavior majors to program faculty, and for the majors and interested students provided academic advice and information about internships and fellowships. In addition, I played an important role as a local arrangements coordinator for the Brown

Symposium, the premier academic program at SU. The Symposium's topic concerned Jakob von Uexkull's ideas on the "umwelt" and involved four keynote speakers who discussed the self-worlds of great whales, intelligent machines, the "right-brains" of humans, and Weddell seals. I organized students who made animal-themed snacks for Symposium attendants, found ushers and speaker chaperones, and generally helped to make sure the Symposium ran smoothly.

As an alumnus of a liberal arts institution that focuses more on developing and implementing sound teaching pedagogies my professors, as well as my mentors have cultivated in me the desire to give back to the human kindness that I have benefited from. Through mentoring future scientists I hope to assist them in realizing their passion for science, so that one day they can answer the question 'why study science?', in a manner similar to the beautifully articulated statement of Henri Poincare, " …he studies it [science] because he delights in it, and he delights in it because it is beautiful. If nature were not beautiful, it would not be worth knowing, and if nature were not worth knowing, life would not be worth living." As a means to achieve this end, I became a science teacher for underserved, at risk youth at Sam Houston Math, Science and Technology Center, a Title 1 high school; where 98% qualify for free and reduced lunch and the primary language spoken at home is not English. I draw upon my experience of learning a foreign language in both Costa Rica and Ghana, and research experiences, to develop inquiry based lesson plans that require these young scholars to think critically and challenges them to ask questions that not only pertain to classroom but their everyday activities and worlds. I nurture the scholars' understanding and appreciation for the relationship between mathematics, science and civic engagement as well as encouraged youth participation in organized math competitions and the development of extensive well designed, research projects for science fair events. I use my academic network to bring in speakers from different academic settings, so that students can encounter positive role models so that bridges can be built and horizons broadened. For my efforts I was awarded a 2009 Project Grad Innovative grant. The funds will be used provide the students with the resources to develop robotic shark models similar to the one used in my King Creativity Grant funded project.

Additionally, the NSF Fellowship serves to advance the achievement of my other academic and professional career goals. The academic and career goals that I have are to complete a doctorate in animal behavior and a fellowship in neuralethology. I then plan to secure a tenure track position at a research intensive institution where I will conduct solid empirically based research to contribute to scholarship and develop an international reputation as a competent and respected research scientist. Meanwhile, I will establish partnerships to secure adequate funding and resources to establish a center of excellence in behavioral studies replete with a board of directors and advisors from an array of backgrounds. This center would prepare other scientists and researchers from diverse disciplines to pursue and develop innovative research in the field in a variety of settings-on-site and artificial. Through this center tours and experientially based summer camps are provided to allow students, faculty and teachers of all ages and and backgrounds to gain awareness and exposure so that they may learn about and experience the intellectual thralls of science. Through the program I hope to target and heavily recruit quality underrepresented minorities and individuals that share in the desire to expand the landscape of opportunity to people with whom I share a similar background. It is my dream that this center be like Antarctica where there is a consensus that no one person or country owned the continent and that all science coming out of there would be made freely available to anyone who expressed an interest. In essence, a politically free center, where individuals of diverse backgrounds and common interests can meet and collaborate to solve problems of mutual concern.

My active involvement in research began in the summer 2004, where I worked in the aquatic laboratory of Dr. William Rowland, through NSF's REU program. Since 2004, I have completed seven research internships in five different settings and immersed myself in the research endeavors of the Aquatic Animal Behavior laboratory at Southwestern University. In Dr. Rowland's lab, under the mentorship of his graduate student, Teresa Dzieweczynski, we examined the effects of an audience on aggressive behavior in male Siamese fighting fish (*Betta splendens*). Dummy fish served to control audience behavior. We demonstrated that mere presence of a conspecific did not affect the aggressive behavior of interacting males. Because previous studies in this species have found audience effects when using a live conspecific, we concluded that a fundamental difference exists between studying the audience effect with live vs. dummy audiences. That is, differences can be attributed to whether the audience was a passive or active participant in the interaction. Furthermore, it is difficult to determine when a subject transitions from being an audience to being one of the interacting parties. These findings and conclusions may require a redefinition of an audience. During the course of the experiment, I had full responsibility for keeping laboratory logbooks, maintaining the equipment and seeing to the welfare of the fish. Also, I learned to utilize software to record behavioral observations from the control and experimental conditions. As a consequence of conducting all experimental trials, scoring, and analyzing the data, I was named third author on the paper that was published in 2006 in the journal Ethology.

In the second internship opportunity, I worked in a zoo setting with Indo-Chinese tigers (*Tigris corbetti*) on a project designed to reduce stereotypic behaviors. Attempts to reduce stereotypic behavior in captive animals often involve environmental and behavior enrichment procedures designed to simulate more natural conditions. Such procedures are widely implemented but often with moderate success and short-term improvement. In fact, with large carnivores, critical assessment of enrichment procedures, and the animal's behavioral needs associated with feeding, are often neglected. Over two summers, as a Rice-AGEP fellow, we tested the effects of a feeding schedule with fasting and a double feeding (twice the daily ration) incorporated as enrichment, and additional enrichment conditions on stereotypic behavior in Indo-Chinese tigers. Interestingly, double-feeding followed by a day fasting and scent conditioning manipulations increased stereotypic behavior, whereas the provisioning of food, bones, and tactile objects that simulate prey behavior elicited natural behaviors and decreased stereotypical behavior. The results were presented at the Rice-AGEP 2006 Annual Banquet, the Southwestern University Undergraduate Research and Creative Works Symposium, and the 2006 Animal Behavior Society Meeting. The latter presentation was made possible when I was awarded a Charles H. Turner Travel Grant sponsored by ABS. Furthermore, this study served to aid the Houston Zoo Inc., and Association of Zoo`s and Aquariums in their future development of the Indo-Chinese Tiger species survival plan. Moreover, it functions to assist Zoo personnel in modifying existing enrichment plans and implement similar feeding schedules for their other large cats.

In 2007, I submitted a successful application for one of fourteen national fellowships sponsored jointly by the United Negro College Fund and Merck pharmaceutical company. As a UNCF-Merck Fellow, over two successive summers, I worked in the departments of Alzheimer's Behavioral Pharmacology and Alzheimer's Neural Science Drug Discovery. I collaborated with Greg Dillon and co-workers to determine the physiological basis of attention deficits in mice (recently submitted for publication). The project first required development of a method to assess attention. To that end, we modified the 5-CSRT attention task to eliminate confounds commonly associated with that task. With the new procedure, we found that C57Bl/6 mice could acquire a novel two-choice visual discrimination task with 80% accuracy within 8-10 days. Then, attention performance could be assessed within a single test session during which four different stimulus durations (0.5, 1, 2, or 10 sec) were randomly presented. During attention testing, the percentage of correct responses decreased as a function of stimulus duration, indicating that performance decrement paralleled the increasing attention demands of the task. Attention performance was also shown to be sensitive to mPFC damage and pretreatment with the muscarinic receptor antagonist scopolamine. The results underscored the utility of our revised task to assess sustained attention in mice in a relatively high-throughput manner. With further testing, this task might provide a more appropriate tool for the characterization of animal models and for testing new pharmacological agents.

At Southwestern University, I served as a research associate in the Aquatic Animal Behavior Laboratory. In my first project, I recruited a student in Physics and another in Biology to apply for an internal grant to support student research. We proposed to test whether a fish could learn to associate a cue with an imminent attack. We argued that predator fish might provide cues (movement, dorsal fin rise, shape change, etc) just prior to attacking their prey. If prey could learn about those cues, they might be able to save energy by taking evasive action only in the presence of these pre-attack cues. The proposal was funded and we used our diverse backgrounds to develop a robotic shark that presented a pre-attack cue, filmed the attack, and could be controlled remotely. Though the shark could swim, its eyes could be lit on command, and it recorded video images, its slow and cumbersome swimming movement negated its effectiveness as a predator.

In my undergraduate thesis I abandoned the robotic shark and simplified my approach. Three experiments determined whether mummichogs (*Fundulus heteroclitus*) could associate a cue with an impending predator attack. In the first two experiments, a light was presented to 30 fish in a large tank for 20 sec followed by a simulated flounder attack (Exp 1) or an attack by a pelican (Exp 2). Mummichogs in the control group received unpaired presentations of the light and the predator. Mummichogs responded strongly to the conditioned stimulus (CS) that predicted either an aerial or aquatic predatory attack. Interestingly, in response to CS Flounder mummichogs startled, and then schooled tightly. In response to CS Pelican, mummichogs responded startled, and then descended in the water column. Schooling was not observed. In both experiments, the conditioned responses (CR) were predator specific and predator appropriate and would have improved the mummichogs chances of surviving an attack. In Ex. 3, mummichogs learned about both predators. A yellow light signaled a pelican attack and a purple light signaled a flounder attack. Surprisingly, the responses to the conditioned stimuli differed from those observed in Exps 1 and 2. Mummichogs responded to CS Pelican by descending slightly in the water column and by schooling. In response to CS Flounder mummichogs descended, but to a lesser degree than observed in Exp 2. Overall, it appeared that the conditioned defensive behaviors were being generalized in that they contained elements of both conditioned responses. In addition, it appeared that the previous trial (pelican or flounder) affected the form of the CR to the current CS. Two conclusions were reached. First, fish could learn to associate a stimulus with an imminent attack and the resulting conditioned response was predator specific and appropriate. Second, it was possible, that learning could also play a role in the development of a generalized defensive strategy. That is, in those instances where prey are attacked by multiple predators their defensive strategies become more generalized and less predator specific.