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ARTICLE
Laboratory Class Project: Using a Cichlid Fish Display Tank to Teach Students about Complex Behavioral Systems

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Laboratory activities serve several important functions in undergraduate science education. For neuroscience majors, an important and sometimes underemphasized tool is the use of behavioral observations to help inform us about the consequences of changes that are occurring on a neuronal level. To help address this concern, the following laboratory exercise is presented.

The current project tested the prediction that the most dominant fish in a tank of cichlids will have gained the most benefits of its position resulting in the greatest growth and hence, become the largest fish. More specifically: (1) is there evidence that a social hierarchy exists among the fish in our tank based on the number of aggressive acts among the four largest fish; (2) if so, does the apparent rank correspond to the size of the fish as predicted by previous studies? Focal sampling and behavior sampling of aggressive acts between fish were utilized in the data collection. Collectively, the data suggest a social dominance hierarchy may be in place with the following rank order from highest to lowest: Fish A > Fish B > Fish D > Fish C. While the largest (Fish A) seems to be at the top, Fish C ended up being ranked lower than Fish D despite the fact that Fish C is larger.

Overall, the project was considered a success by the instructor and students. The students offered several suggestions that could improve future versions of this type of project, in particular concerning the process of constructing a poster about the project. The implications of the data and student learning outcomes are discussed.

Key words: cichlid; dominance hierarchy; behavioral analysis; poster presentation; laboratory group project

Performing behavioral observation, like many skills, is something that must be practiced and developed to be done well. A laboratory class is an ideal setting to introduce students to the use of behavioral observation. Engaging in “hands-on” activities where students collect behavioral data provides the opportunity to engage students in discussions about the fundamentals of good observation. This type of skill is something that will serve students well in their scientific pursuits.

Cichlid fish as a model for studying agonistic behavior
Generally speaking, within a group of conspecifics in a particular region, individuals compete for resources. When there are asymmetries in size, strength, or fighting ability, dominance relationships tend to develop (Clement et al., 2004). There are examples from numerous species where a linear hierarchy is formed both in nature as well as in a laboratory setting, including cichlid fish (Barlow, 2000; Chase et al., 2002). For cichlids there are several physiological consequences of having higher social status, including increased growth rate of the body and the growth of new neurons (Hofmann et al., 1999; Hofmann and Fernald, 2000). Furthermore, social rank has been found to influence monoamine activity (Winberg et al., 1997) as well as expression of genes that influence coloration and behavior (Burmeister et al., 2005).

Cichlid fish have been a valuable model system for understanding the behavioral dynamics and physiological substrates responsible for these dominant and subordinate behaviors (Clement et al., 2004). The cichlid’s complex behavior is known to support a sophisticated social system that provides the opportunity to analyze the interaction between social rank and behavioral coping strategies (Clement et al., 2004).

Some cichlid fish have been shown to have a social system in which a fraction of the males dominate food and nesting sites, and therefore, have access to females (Clement et al., 2004). These dominant males are brightly colored (blue or yellow) and vigorously defend territories, exchange threat displays with territorial neighbors, chase subordinates, and court females (Clement et al., 2004).

In aggressive encounters between cichlid fish, physical attacks occur most often when both participants are closely matched in ability and body size is a key determining factor in the outcome (Coleman et al., 1999). Larger bodied fish have greater defense mechanisms against attacks by other fish due to their thicker body parts, greater energy reserves, and larger jaws (Coleman et al., 1999).

Designing the class activity
This project can be reproduced in a wide variety of settings (different departments or different class contexts) with only minimal cost and upkeep. It can also be readily accomplished by undergraduate students. Furthermore, this behavioral exercise can be used early in the semester and form a starting point for neurobiological analyses later in the semester. The current version was conducted by undergraduate biology majors in an animal behavior course at Creighton University. There were several sections in the current course (all taught by the same instructor) and this activity allowed all the students to collaborate and pool their data for a more thorough

analysis.

The use of a cichlid display tank provides several advantages. Cichlids are relatively easy to obtain and maintain in captivity. Observations of the fishes' behavior can be made with minimal intrusion into the fishes' environment. Cichlids regularly engage in interactive behaviors that are readily observable and relatively easy to operationally define. Among the most obvious behaviors is a biting or lunging behavior of one fish towards another fish. Finally, cichlids are already known to establish dominance relationships in captivity that can be observed via their social interactions (Clement et al., 2004).

In the current context, it made sense to construct a poster version of the results of this project. This poster would work in conjunction with a poster that already existed describing the fish themselves, but without discussion of the fishes' behavior. Thus, it was decided that the students could work in conjunction to produce a poster that describes the fishes' behavior. This would not only benefit the students themselves, but would enhance the appreciation of these fish by any who see this poster.

The current project examined two main questions: (1) is there evidence that a social hierarchy exists among the fish in this tank based on the number of aggressive acts among the four largest fish; (2) if so, does the apparent rank correspond to the size of the fish. The null hypothesis of this experiment is that there is no social hierarchy among the cichlid fish meaning that there should not be any differences in the aggressive acts of one fish towards other fish. This hypothesis was suggested to the class by the instructor. The possibility of examining other facets of fish behavior was discussed and rejected by the students.

Figure 1. Picture of the tank used during the observations. The final product poster produced by this project is seen on the wall to the lower left of the fish tank.

MATERIALS AND METHODS

The Environment:
The cichlids in this study were part of a display tank (65-gallons), which was artificially lit and filtered, containing four larger cichlids, four medium cichlids, six smaller cichlids, and four plecostomid fish. The tank was constructed of glass on all sides. Large coral formations were present that created caves and crevices in which fish could hide. The tank was located in the hallway on the fourth floor of the Hixson-Lied Science Building at Creighton University (see Figure 1).

The Subjects:
The four largest cichlids were chosen as focal individuals for this study. The relative size of the fish was determined by qualitative visual inspection. These four were easier to identify and were hypothesized to be more active in establishing and maintaining a dominance hierarchy. The four focal fish were lettered for identification (see Figure 2): A, the largest blue striped fish; B, medium blue/yellow striped; C, medium blue, no stripes; D, smaller blue, black dorsal fin). Categories were designated for the other fish in the tank. Category E contained four medium sized yellow fish and category F contained all other fish in the tank. Only fish A, B, C & D were individually observed and scored.

Figure 2. The fish observed are pictured above. From left to right, the top two panels show fish "A" and fish "B," the middle two panels show fish "C" and fish "D" and the lower two panels show examples of category "E" and category "F" fish.

Data Recording:
Focal sampling (observing only one individual animal at a time), and behavior sampling (only recording when a particular behavior occurred) were utilized in conjunction for the data collection (see Martin and Batson, 2005 for more detailed descriptions of data collection techniques). Each student was randomly assigned one of the four focal fish to observe for 30 minutes. Each focal fish was observed by six students netting three total hours of observation per fish and 12 total hours of observation of the tank. Focal sampling should decrease the chances of missing a behavioral event by allowing each student to focus on an assigned fish instead of trying to monitor every fish simultaneously. Additionally, it was decided that in the

current context the best approach was to determine how each of the focal fish related to the entire community of fish (thus the creation of category E and F fish) as well as examining the relationship between the individual focal fish.

Behavior sampling was selected to allow students to focus on particular behaviors which could be clearly defined. To address this hypothesis, an agonistic behavior was selected because establishing dominance should involve some form of aggressive action towards other fish. A behavior, dubbed an "attack" for this project, was selected and was operationally defined as any nipping or biting motion towards another fish.

During the observations, the number of attacks made by or against the focal fish was recorded during each 30-minute session. In each case the identity (or category) of the other fish was noted and was indicated as the "attacker" or "defender" with respect to the focal fish. All observations were made during a one-week period in the semester.

Data Analysis:
Data for each fish were compiled and analyzed using a Chi-squared (**$\chi 2$**) analysis. The **$\chi 2$** analysis tests the distribution of a data set versus the assumption there will be equal distribution among the possibilities. In the current context, if there is no hierarchical organization, then the attacker and defender events should be occurring at equal frequency across all fish. Also, when each fish is examined individually, there should be an even distribution of attacker and defender events.

Constructing a Poster:
Once the data had been obtained, groups of students were assigned to work on different sections of a poster. Groups were made of two to three students each. Three groups of students were asked to submit an introduction section; two groups submitted a methods section; two groups submitted graphs or summaries of the data; and two groups submitted conclusions sections. The instructor then evaluated the submissions and synthesized the submitted material into a cohesive poster.

RESULTS

The data collected demonstrate an uneven distribution of the number of attacker versus defender events. The largest fish, Fish A, while often being the aggressor fish, stands out because it was the least often attacked, resulting in the greatest aggressor to defender ratio (74:5 or 14.8). The second largest fish, Fish B, had the highest raw score for number of attacks, but was attacked by other fish more than Fish A, resulting in a moderate aggressor to defender ratio (109:42 or 2.6). Fish C, although the third largest fish, was by far the most often attacked. Also, Fish C was least often the attacker, resulting in the worst aggressor to defender ratio (27:103 or 0.26). Finally, Fish D, the smallest of the four focal fish, had a greater number of attacks than Fish C and was attacked less often than Fish C resulting in an aggressor to defender ratio similar to Fish B (74:32 or 2.3). These data are shown in Figures 3, 4 and 5.

	Observed	Expected
A-difference score	39.0	19.0
B-difference score	14.0	19.0
C-difference score	-76.0	19.0
D-difference score	23.0	19.0
$\chi 2 = 755.68$, $p < 0.01$		

Table 1. **$\chi 2$** analysis for the difference scores (attacker – defender) for the four focal fish versus each other. Encounters with category E and F fish were not included in this analysis.

	Observed	Expected
A-aggressor	74.0	39.5
A-defender	5.0	39.5
$\chi 2 = 60.27$, $p < 0.01$		
B-aggressor	109.0	75.5
B-defender	42.0	75.5
$\chi 2 = 29.73$, $p < 0.01$		
C-aggressor	27.0	65.0
C-defender	103.0	65.0
$\chi 2 = 44.43$, $p < 0.01$		
D-aggressor	74.0	53.0
D-defender	32.0	53.0
$\chi 2 = 16.64$, $p < 0.01$		

Table 2. **$\chi 2$** analysis for the difference scores (attacker – defender) for each of the four focal fish examined individually versus the entire community of fish (includes category E and F fish).

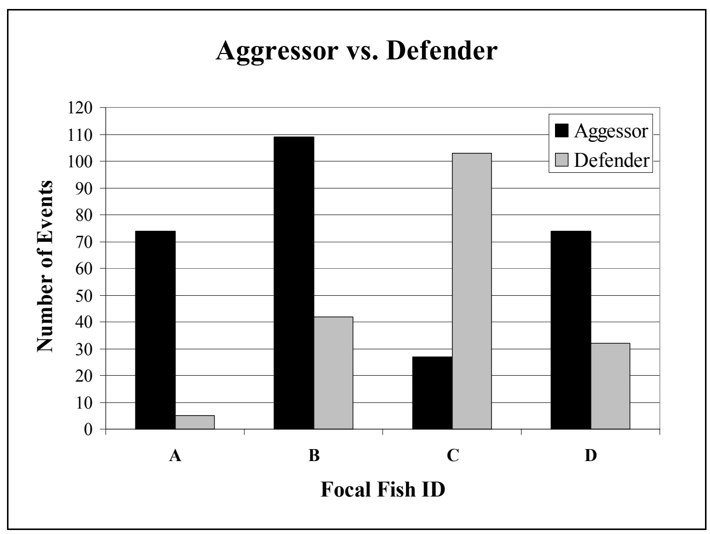

Figure 3. The bar graph indicates the number of times that each fish was the attacker (black) versus the number of times that each fish was attacked (grey) by any fish in the tank for each of the four focal fish.

Statistical Analysis:
The data collected was subjected to two different statistical analyses using the Chi-squared (**$\chi 2$)** test. For the first test, the difference between number of attacks and number of defenses was calculated for each fish and compared to the null hypothesis of an even distribution across all four fish.

The **χ2** analysis revealed a significant result (**χ2** = 755.68, p < 0.01). Table 2 shows the **χ2** analysis when broken down for each individual fish. These numbers include all events including encounters with categories E and F fish. For each fish there was a significant difference between attacker and defender events (Fish A, **χ2** = 60.27, p < 0.01; Fish B, **χ2** = 29.73, p < 0.01; Fish C, **χ2** = 44.43, p < 0.01; Fish D, **χ2** = 16.64, p < 0.01).

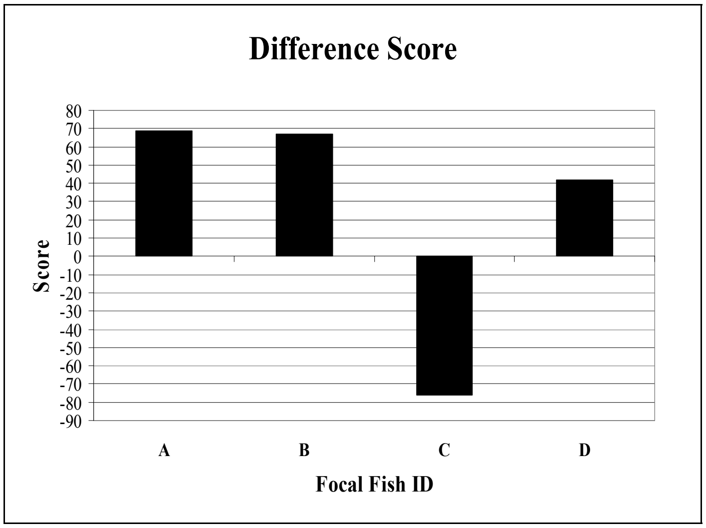

Figure 4. The bar graph indicates the difference in number of times that each fish was the attacker versus the number of times that each fish was attacked by any fish in the tank.

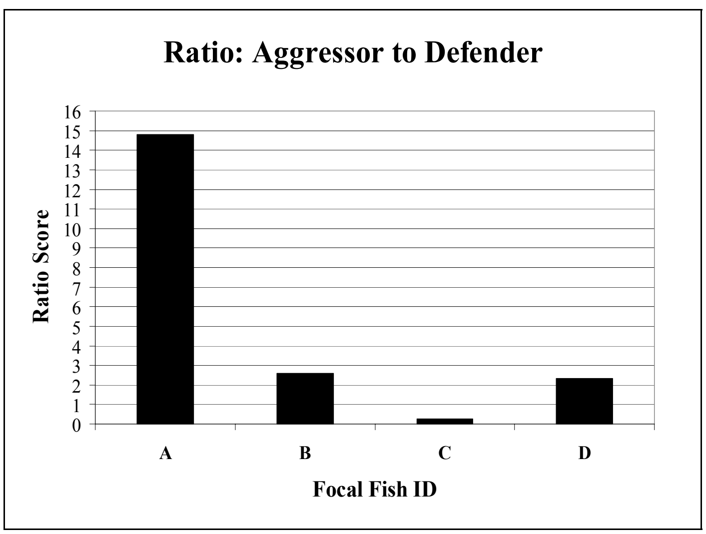

Figure 5. The bar graph indicates the ratio score for the difference in number of times that each fish was the attacker versus the number of times that each fish was attacked by any fish in the tank.

DISCUSSION

The null hypothesis in this study was that there would be no difference in agonistic behavior with respect to the four focal fish. Based on the results of the **χ2** analysis seen in Tables 1 and 2, the null hypothesis was rejected. The current data support the idea there is a complex social structure present in this fish tank. Among the four focal fish: Fish A was the least often attacked; Fish B was the most frequent attacker; Fish C was attacked most frequently. The data collectively suggest that a dominance hierarchy may be in place with the following rank order from highest to lowest: Fish A > Fish B > Fish D > Fish C. However, it should be noted that the data in this study does not constitute sufficient justification for labeling the social structure as a “linear” hierarchy. As Appleby (1983) eloquently points out, it is possible to see what appears to be a linear, or near-linear, hierarchy due to chance factors. In order to explicitly test for linearity, the relationship between each pair of individuals needs to be determined. A linear hierarchy should be transitive in nature (if A is dominant to B, and B is dominant to C, then A is also dominant to C, etc.). It can not be assumed that the rankings are transitive and a failure of this test means that the social hierarchy is not linear.

The current data are in partial accordance with previous studies that suggest that a dominance hierarchy will be consistent with the size of the animal. It should be noted that current data do not allow for a determination of the directionality of causation. Does a fish grow larger because it is dominant or does it become dominant because it is larger? Here, Fish A (the largest) seems to be at the top; however, Fish C ended up being ranked lower than Fish D despite the fact that Fish C is larger. This suggests that it would not be as simple as saying that size determines the social rank. Obviously, there are multiple factors in place and further tests would be necessary to more fully appreciate the richness of the social behaviors of these fish (see Barlow, 2000 for additional information about the richness of the world of cichlids).

Some of these additional factors were noted during the course of this investigation. Future attempts at this type of exercise should strive to standardize these factors or otherwise better account for them. First and foremost, not all of the fish in the tank were of the same species of cichlid. There is a very rich diversity of cichlid species that have been identified (Barlow, 2000) and there can be behavioral differences from one species to another. Second, no attempt was made to determine the gender of these fish, thus not allowing for a distinction between agonistic behaviors and mating behaviors. Third, the size of the fish was not directly measured; only a qualitative visual inspection was used. A quantitative measure of the size of the fish (length, mass, etc.) would be useful for a more detailed analysis. Fourth, the coloration pattern was not consistent across the four focal fish. Some of these differences are the result of being of different species; however, as Burmeister et al. (2005) points out, the color of the cichlid can change depending on the social rank. Fish A and Fish B have coloration patterns that are associated with fish of greater social rank. A note about coloration that several students noticed was a bright red patch on one of the ventral fins of Fish B that disappeared after the observations were completed. This sort of color change has been associated with mating behavior. Fifth, the fish were not always visible during the observation period. During the data collection, students kept track of the amount of time that their focal fish was visible. It was frequently noted that the fish would spend time "hiding" in cave-like crevices in the coral formation. It was suggested that this is a defensive behavior since a fish that spends more time "hiding in a cave" is less likely to be attacked by other fish. The fish that engaged in this behavior most frequently was Fish D (data not shown). Engaging in this behavior, or having the opportunity to avoid encounters with other fish, can have an obvious impact on the number of agonistic encounters. All of these factors may have contributed to the observed behaviors and attempts should be made to control for these factors in future iterations of this type of exercise.

After this project was completed, the students were asked to think of ways that this study could be improved. Several worthwhile suggestions were brought forward. It was noted by several students that a number of the fish, in particular Fish D (as noted above), spent significant amounts of time "hiding in coral caves," thus decreasing the opportunities directly interact with the other fish. This may be a strategy used by the fish to avoid a potential physical encounter. This lack of interactive behavior was not directly measured or taken into account as a type of defensive behavior. Several students argued that the lack of interaction should be considered a type of interaction behavior. It was also noted by some students that the location of this tank in a public hallway provided the possibility that the fish could be reacting to the presence of humans passing by the tank (especially when a class has recently been let out). It was thus suggested that the tank be more isolated for the purposes of these observations to limit possible distractions from outside the tank. Another concern that was raised involves the number of student participants. It was felt that there may be problems of consistency in defining what constitutes an attack by a fish. This could be addressed by limiting the number of observers, instituting more rigorous training procedures for doing this observation, and being extremely precise about defining what constitutes an "attack" by another fish. This could also be a perfect opportunity to introduce some inter-rater reliability measures as part of the data analysis. This could be accomplished by having two students observing simultaneously with the explicit instructions not to communicate with others during the observation (to avoid biasing each other's interpretation). This would allow the students to directly compare measures and assess the magnitude of inter-rater consistency. A final concern raised by the students was that some of the interactions might have been mating behaviors and not agonistic behaviors. Most students expressed a desire to know the sex of the fish to aid in the interpretation of the data.

The students were also asked about their experience with the project, in particular, the process of making a collaborative poster of the data collected. Overall the sentiment amongst the students is that the project helped them to better appreciate what is involved with performing behavioral observations and analyzing the outcome. However, there were several issues raised that could have made the project run more smoothly. First, the students were asked to write each of their sections (introduction, methods, results, or conclusion) and turn them in at the same time. This did not facilitate good communication between groups. For example, several of the people writing the conclusions sections found it difficult to write without knowing what the people doing the results sections had done. Second, some of the students were more familiar with analysis methodology. The greatest help from the instructor was needed by the students trying to analyze the data. An alternative method for this portion of the exercise would be to have each group of students construct an entire poster; then, have students critique the posters of the other groups. This would provide the opportunity for students to get feedback on their work and to learn from how others chose to present the same data set.

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