Thesis

Chronic Effects of Intermittent Sound Disturbance of Buenos Aires Tetra

Submitted by

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Abstract

The hearing-specialist *Hyphessobrycon anisitsi* was subjected to two, independent, five-week trials to determine the differences between growth and behavior in intermittently disturbed and quiet environments. One experimental group was subjected to intermittent anthropogenic boating noise (135±3 dB rel: V/μPa) twice per day for one hour. The other only experienced ambient noise (85±3 dB rel: V/ μPa). Growth in length, weight, and Fulton's condition factor were compared between groups using repeated-measures ANOVA. Frequency of observation for five types of behavior was compared with two-tail *t* tests. Linear regression was used to examine evidence for habituation or sensitization. We found no difference in growth between trials. However, strong differences between three of the five behaviors were observed, showing sensitization (i.e. increased display of stress behaviors) over one-hour periods of noise exposure. Also, sensitization to the onset of noise was evident from increased darting over the course of the five week trials. Finally, subjects showed decreased behavioral responses to the initial stimulus in darting and normal swimming. These results suggest that *H. anisitsi* is not stressed enough by sound to show changes in growth, but that sound does induce behavioral changes.

Introduction

Some animals affected by noise disturbances experience negative fitness consequences (Wright et al. 2007) such as reduced growth, reduced foraging success, increased vulnerability to predators, auditory sensitivity shifts, reduced reproduction via disrupted mate signaling, auditory tissue damage, and death by intolerable stress hormone levels (Popper & Hastings 2009). Different fish species habituate to noises to varying degrees depending on their sensitivity to the noise generated and the duration of the disturbance. Fishes have a wide array of adaptations to detect sound. For example, some fishes have simplistic auditory anatomy and detect a limited range of frequencies and decibel levels (Smith et al. 2004b), whereas others have Weberian ossicles that amplify vibrations in gas bladders to increase detection sensitivities (Fay & Popper 2000). With such a varying degree of hearing in fishes, more data from many different species are needed to fully comprehend fish auditory capabilities. In addition, understanding the consequences of noise disturbance on fish species' auditory capabilities has become more important due to the increase of anthropogenic noise in aquatic environments and high human dependency on fish for food (Popper & Hastings 2009).

Hearing specialists, such as species with Weberian ossicles, are more sensitive to noise disturbances than hearing-generalists because they detect sounds across wider frequency ranges and can discern relatively weak sounds (Amoser & Ladich 2005). Most behavioral studies on hearing specialists have focused on mating vocalization and long-range movement behaviors, post-disturbance, which are long-term effects of disturbance (Wardle et al. 2001; Morley et al. 2014). There are relatively few data on immediate stress responses during disturbance periods.

The frequency and duration of a sound can affect fish species differently (Popper & Hastings 2009). Three main types of anthropogenic noise impact aquatic systems. First, constant noises are moderate increases in background noise for long periods of time, such as heavy boat traffic or aquaculture-related noises from pumps (Slabbekoorn et al. 2010). Second, short, powerful sounds last a few seconds, but with high intensity or high decibel levels, such as sonar or mining explosions (Popper & Hastings 2009). Third, intermittent noises raise background noise levels, but occur at shorter intervals than constant noises, but longer than short, powerful sounds (Smith et al. 2004a). Unfortunately, intermittent noise is less studied, but is the more likely scenario in the wild (Smith et al. 2004a).

Intermittent-noise environments may not allow hearing specialists to physiologically or behaviorally habituate as easily as the other categories of noise and may induce a cycle of recurring stress with alternating periods of disturbance (noisy periods) and physiological recuperation (quiet periods). This cycle could put the fish in a constant cycle of injury and healing, which may chronically alter their behavior or growth.

We performed lab experiments on the hearing-specialist fish Buenos Aires tetra (*Hyphessobrycon anisitsi*) in an intermittent-noise environment to determine chronic and acute behavioral changes (i.e., growth and health differences). We hypothesized that *H. anisitsi* would show increased stress behaviors and reduced growth due to inability to habituate. If our hypothesis is correct, intermittent-noise disturbance should cause: (1) a decrease in non-stressed behavior, (2) an increase in stressed behaviors chronically and throughout noise disturbance times, and (3) reduced growth.

Materials and Methods

Husbandry/ experimental set-up

Two sets of trials were performed: a quiet control trial (85±3 dB rel: 1 V/μPa) and an experimental noisy trial (135±3 dB rel: V/μPa). The duration of each trial was 5 weeks. Specimens were purchased from Petco® and brought to the lab within 20 minutes of purchase. Males and females were randomly assorted into tanks to eliminate effects of sexual dimorphism in growth. Prior to each trial start date, fish had 24 hours to acclimate to their tanks. Noisy and quiet trial runs were conducted using 30 specimens divided equally among five tanks. After the first 30 control specimens had finished the control trial, 30 different fish were purchased for the experimental trial and, again, divided equally among tanks.

Five, 20-gallon tanks were set for a 12:12 hour day/night cycle with fluorescent lighting lasting from 0700 to 1900. All tanks were kept at 25-27^{o} C with a submerged 50 watt tank heater. Tanks were filtered and pumped using an Aqeon Quietflow 10 system to reduce noise and clean the tanks. Filters were changed on the first day of week 3 for both trials. Each tank had gravel substrate and was visually blocked on three sides to reduce outside stimulation. In addition, each tank had an individual speaker set to the back side for sound projection. Though the quiet trial had no sound projected through it, a speaker was still placed in the same manner as the noisy trial as a control.

Specimens were observed twice daily for behaviors. Two tanks were observed daily, from 0700-0800 and 1700-1800 respectively. Commercial flake food was provided 20 to 15 minutes prior to observation times in 0.15 ± 0.001 g portions per tank. The reason for providing food at this time was to reduce the occurrence of aggressive or stress behaviors associated with feeding. For behavioral analysis, one random tank was selected for observation each observational period with a random number generator (www.random.org). During each observational period, the following behaviors were examined at two-minute intervals (Altmann 1974): normal swimming, darting, nipping, freezing, and air gulping (Schreck et al. 1997) (Table 1).

Once per week, mass and fork length (FL) of each fish were measured in all five tanks, after anesthetizing fish in a clove oil solution (Mitjana et al. 2014). All 30 specimens were weighed and measured on day 7 of each week after the 1700-1800 observation periods. Mean growth of individuals from all tanks combined was calculated for each week for each treatment. Fulton condition factor was used to determine average condition of individuals each week (Fulton 1902; Ricker 1975).

Sound projection and measurement

The audio recording used for the simulation of boating noise was a 1 hour digital recording of a QSLA9 Cummings diesel engine idling on a hydrophone in Lake Conroe, TX. These sounds were downloaded and stored in a computer, then projected through a 5.1 speaker system (VM Audio EXMS581W 1000 Watt system) for 1 hour during noisy trial observation periods. Speakers were set centered on the back side of each tank 5 cm away from the glass to reduce potential of vibration on the tank itself. Each speaker was capable of producing frequency ranges from 35Hz-20 KHz, with a total power output of 370 RMS watts for the entire system.

Sound measurements were made prior to introduction of fish into tanks using the H1a hydrophone (Aquarian Audio Products, Inc., Anacortes, WA, sensitivity= -190dB re: 1V/µPa frequency range= 1 Hz to 100 KHz). Average dB level was determined after measuring all eight corners of the tank and the center. Measurements and calculations were made according to broadband spectrum analysis procedures (Davidson 2007).

Statistical analyses

Analyses of behavioral observations were conducted using a two-sample, independent *t*-test to compare mean percentage of each behavioral category documented at each two minute interval. Simple linear regressions were used for all behavior categories to examine the influence of time in minutes within the observational period, on the percentage that the behavior was displayed.

ANOVA with repeated measures (week as a covariate) was used to compare FL, weight and Fulton condition factor between treatments. Simple linear regressions of regression slope versus the trial number and of the y-intercept of within-trial frequency versus time were used to assess habituation/sensitization within and among trials, respectively, over the trial period. All statistical analyses were performed in the SPSS statistics program. All *p*-values were based on two-tailed tests with an alpha level of 0.05.

Results

Survival

Both trials had several mortalities. Three of the thirty specimens in quiet trials died after infection with *Ichthyophthirius multifiliis,* a common aquarium parasite, within a single tank. In noisy trials, three tanks had mortalities. Within the three tanks, four individuals had their caudal fins nipped off. All fish corpses were found prior to morning observations and removed immediately after discovery.

Behavior frequencies

Mean frequency of observation varied between treatments for three of the five behaviors: nipping, darting, normal swimming, freezing, and air gulping (Table 2). Nipping and darting were more frequent in noisy trials and increased linearly in frequency during the time exposed, whereas there was very little nipping in quiet trials (Figure 1). Accordingly, normal swimming was lower and decreased during observational times in noisy trials; whereas in quiet trials, normal swimming was the predominant behavior at all times (Figure 1). Air gulping and freezing behaviors did not show any significant difference between the noisy and control trial during each observational period (Table 2).

Behavior habituation

Increases in the steepness of positive within-trial relations of the frequencies of nipping and darting behaviors over time, with concurrent decreases in frequencies of normal swimming, suggested individuals were increasingly sensitized to the noise disturbance (Figure 2). The y-intercepts regressions showed a decrease of initial behavioral stimulus to normal swimming and darting behaviors, suggesting habituation, with no significant change to nipping behavior suggesting maintenance of stimulus levels throughout the study (Figure 3).

Length, weight and Fulton's condition factor

Fork length differed between quiet (mean = 4.1 ± 0.44 SD cm) and noisy (3.6 ± 0.33 cm) trials, but week had no effect on this comparison (Table 3). Similarly, weight differed between quiet (1.3 ± 0.35 g) and noisy (1.0 ± 0.22 g) trials, but week had no effect (Table 3). Fulton condition factor did not differ between quiet (2.0 ± 0.21) and noisy (2.0 ± 0.22) trials (Table 3).

Discussion

Comparisons of length, weight, and Fulton condition factors between treatments indicated that the intermediate-noise disturbance did not affect *H. anisitsi* growth (Table 3). Differences of FL and weight can be attributed to initial differences in fish size between trials. The lack of effect may reflect ability to heal sound damage between disturbances. Alternatively, decibel levels may not have been high enough to induce physiological damage. In this situation, the typical level of a single diesel engine's boating noise (135 dB rel 1V/µPa) may not be powerful enough to damage the hearing tissue (Wysocki & Ladich 2005). However, the Parana River, which *H. anisitsi* is native to, may see multiple engine boats or larger boats with more powerful engines, which creates higher decibel noise projections (Paini et al. 2009) . In its native environment *H. anisitsi* may still be susceptible to tissue damage. In addition to tissue damage, *H. anisitsi* may be affected by increased stress effects exacerbated by louder noise. When subjected to more stress by louder noise, metabolic processes may be affected. If metabolic processes are further affected by louder noise, then resources used for growth may be used to resist noise effects and not utilized for growth, but not in this case. Lastly, the feeding behavior of *H. anisitsi* may have played a role in growth of the specimens (Selye 1946). Our fish were fed *ad lib* and may have compensated for metabolic stress affects by eating more food. However, outside of the lab fish are not necessarily able to eat enough to compensate for stress. In the wild if the specimen cannot forage enough to compensate for stress, they would likely show the growth consequences of noise.

Another potential reason for the lack of growth consequences may be the sound frequencies projected by the diesel engine. Our study used a broad spectrum analysis of many frequencies to determine decibel levels. Broad spectrum sound detection takes all frequencies within the hydrophone's frequency range to determine the decibel level of the noise. However, individual frequencies can have individual decibel levels, which may not harm *H. anisitsi* at the decibel levels created by boats (Wysocki & Ladich 2005). To further understand this aspect of hearing in *H. anisitsi*, future research should seek to describe hearing frequency ranges and narrow spectrum decibel levels of tests (Kastelein et al. 2008).

Behavioral data showed dramatic response to noise and suggested increased sensitivity over the five-week period. However, initial stimulus levels of behavior to normal swimming and darting showed a habituation with a decrease of initial darting as the experiment progressed. However, there was no change in nipping, which shows that as the experiment progressed, the initial level of nipping at the onset of noise remained strong while the increase in nipping throughout each trial was increasingly severe. In other words, fishes appeared to be increasingly sensitive to the intermittent noise, causing increased aggression (i.e. nipping). The increase of nipping over the single hour is particularly important when considering mortalities. Mortalities in the quiet group appeared to be due to an *I. multifiliis* infection in a single tank. This common aquarium parasite can cause death in healthy specimens (Matthews 2005). In contrast, mortalities in the noisy group appeared to be associated with increased, noise-induced nipping behavior because the caudal fins were nipped down to the peduncle, which may have hindered feeding and increased social inferiority, while increasing stress to these individuals.

Contrary to lab specimens, wild individuals may have the option to move away from stressful noises. Moving away from the noise disturbance may mitigate the effects of noise stress. However, if optimal habitats are restricted to noise-disturbed waters, fish will be forced to either deal with stress or occupy sub-optimal habitats.

Evidence for sensitization over time supports our initial hypothesis that intermittent noise may not allow for habituation. There may be several possibilities to explain reasons for the increase of stressed behavior over time. Fish may not be able to habituate to their environment because they do not have enough time between intermittent disturbances to do so. Additionally, the aggressive nipping behavior may increase stress levels. Some fish within the school would be the victim of nipping, which would increase their stress. While the increase of nipping in our study did not affect growth rates, perhaps over longer time periods, growth and fitness would have been compromised. Or, perhaps death occurs prior to onset of growth effects.

Perhaps the most puzzling question derived from our study is the apparent change in behavior without effects on growth. This is especially perplexing considering the intricate cause and effect relationships of physiology and behavior (Schreck 1997). One explanation may be Hans Selye's General Adaptation Syndrome (GAS) (Selye 1946). If the body stays in the alarm stage of Selye's GAS before returning to homeostasis, this would result in a physiological response which may change behavior but not alter growth rates. The alarm stage is characterized by releasing hormones such as adrenaline, noradrenaline and cortisol. These hormones affect behavior in the short term, but can be removed from the body fairly quickly, from minutes to hours (Schreck 1997). If the noise disturbance is only long enough to push *H. anisitsi* into the alarm stage, not to resistance or exhaustion stages, it would not be expected to affect growth.

If the GAS is the reason for strong behavioral and little growth consequences of our studies, the ability of *H. anisitsi* to handle stress hormones becomes increasingly important. Since our study shows an increasing sensitivity of stress behavior over time, this would mean that the stress hormone levels would have to increase over time also. Future studies should seek to evaluate hormone levels to see if they correlate with the behavioral responses.

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Table 1. Description of behaviors observed in Buenos Aires tetras (*Hyphessobrycon anisitsi*) every two minutes during 60-minute observation periods.

Behavior	**Description**
Normal Swimming	All fish swim < 3 body lengths · s^{-1} for five seconds or more
Agitated Swimming	Fish swim > 3 body lengths · s^{-1} for > 1 s, but not at another fish
Nipping	Fish accelerate toward and nudge or nip each other
Freezing	Fish shows no operculum or fin movement for > 1 s
Air Gulping	Fish move within 1 cm of water surface with repetitious mouth-sucking motion for > 1 s

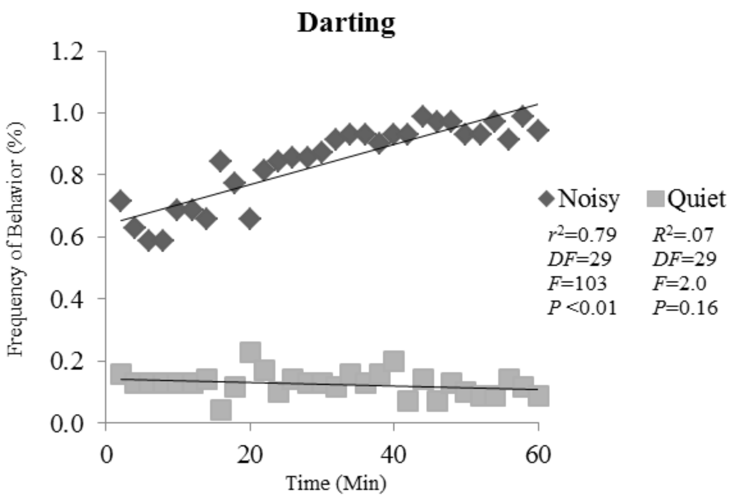
Table 2. Results of two-sample independent *T*-tests for mean frequency and standard of deviation of five observed behaviors measured at two-minute intervals during quiet versus noisy treatments.

Behavior	**Mean ± SD**	***DF***	***P***	***T***
Quiet Normal Swimming	69 ± 0.5	58	< 0.001	12.0
Noisy Normal Swimming	46 ± 10.0			
Quiet Darting	9 ± 2.7	58	< 0.001	-29.0
Noisy Darting	59 ± 9.0			
Quiet Nipping	6 ± 2.2	58	< 0.001	-15.0
Noisy Nipping	50 ± 15.0			
Quiet Air Gulping	1 ± 1.0	58	0.260	-1.1
Noisy Air Gulping	2 ± 0.8			
Quiet Freezing	1 ± 1.9	58	0.050	-2.0
Noisy Freezing	2 ± 1.3			

Table 3. Results of ANOVA with repeated measures of fork length (FL), weight, and Fulton's condition factor (FCF) of *Hyphessobrycon anisitsi* subjected to quiet versus noisy environments over a five-week period. Measurements were conducted weekly on each individual fish (30 fish per treatment).

Source	***DF***	***MS***	***F***	***p***
FL	1	1.00	17	<0.001
FL X Week	1	< 0.01	< 0.01	0.979
Between-subject error	28	0.12		
Within-subject error	28	0.06		
Weight	1	0.74	17	<0.001
Weight X Week	1	< 0.01	0.02	0.896
Between-subject error	28	0.10		
Within-subject error	28	0.05		
FCF	1	< 0.01	0.24	0.631
FCF X Week	1	0.01	0.34	0.567
Between-subject error	28	0.05		
Within-subject error	28	0.03		

Figure 1 – Cumulative (all samples combined) frequency of three behaviors (nipping, darting, normal swimming) observed in Buenos Aires tetras (*Hyphessobrycon anisitsi*) every two minutes during 60-minute observation periods during noisy and quiet treatments. Results of simple linear regressions are provided.



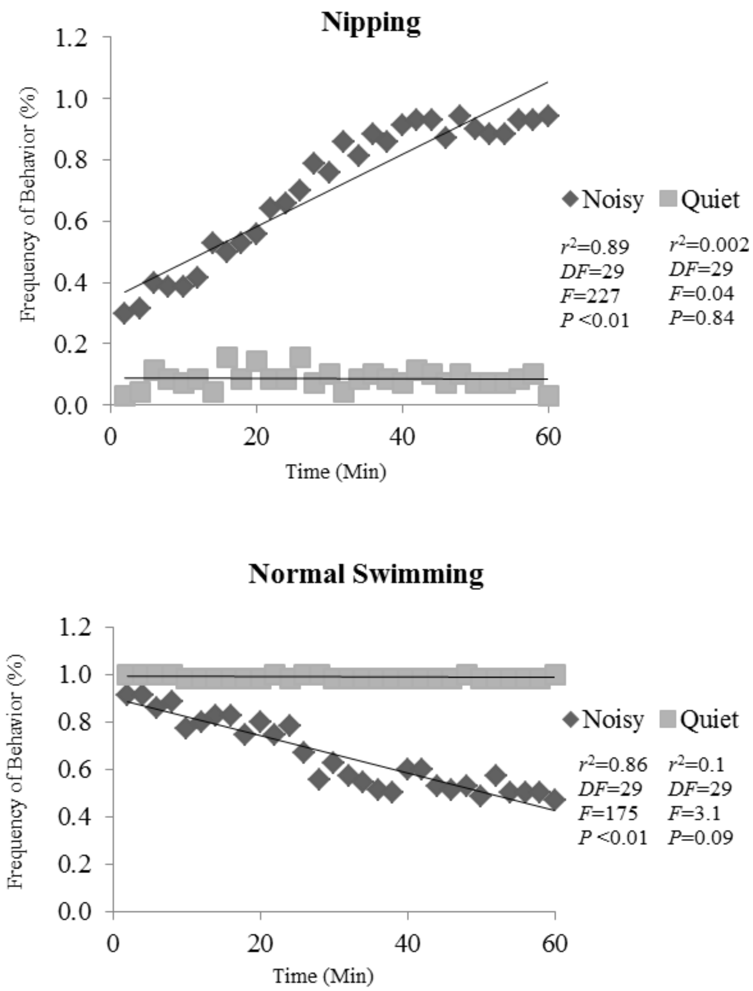


Figure 2 - Slope of regression line values of only individual noisy trials throughout the five-week study showing three behaviors of Buenos Aires tetras (*Hyphessobrycon anisitsi*): nipping (A), darting (B), and normal swimming (C). Results of simple linear regression are provided.

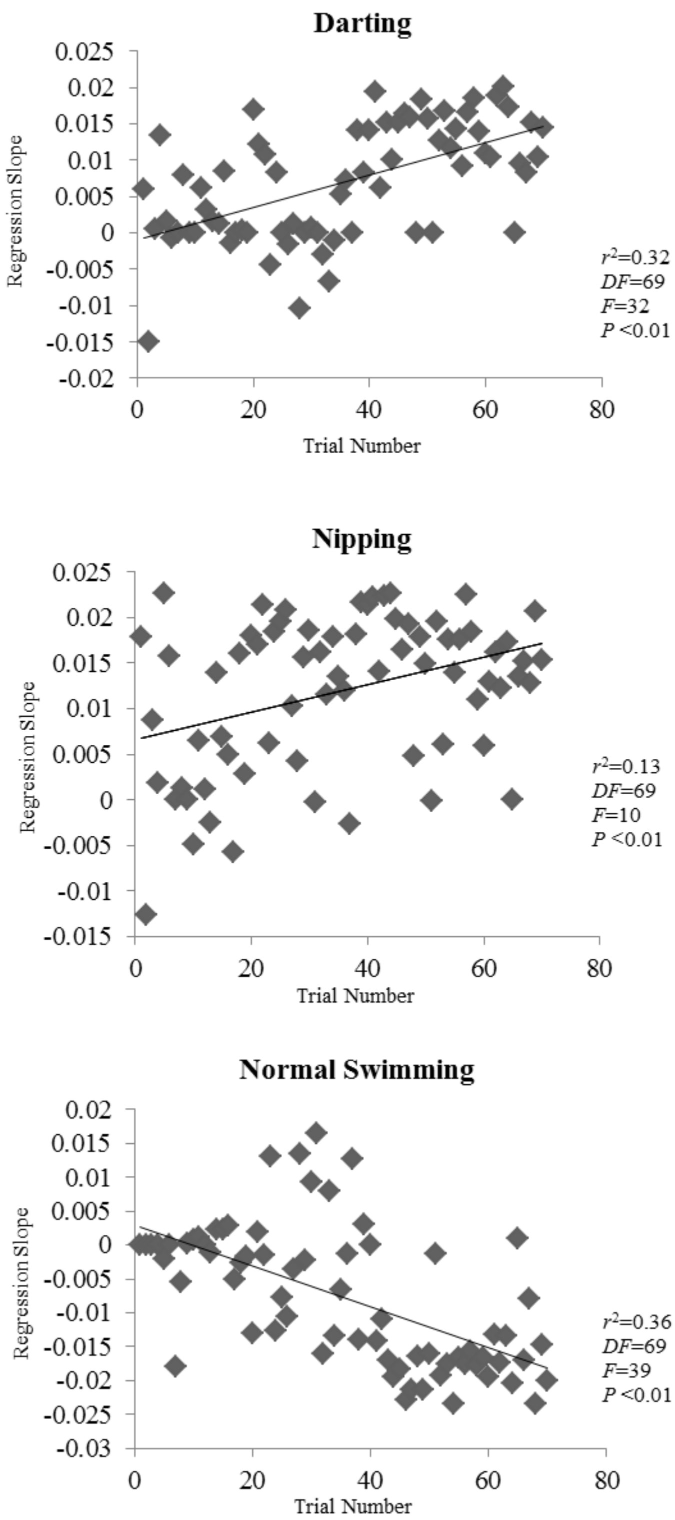
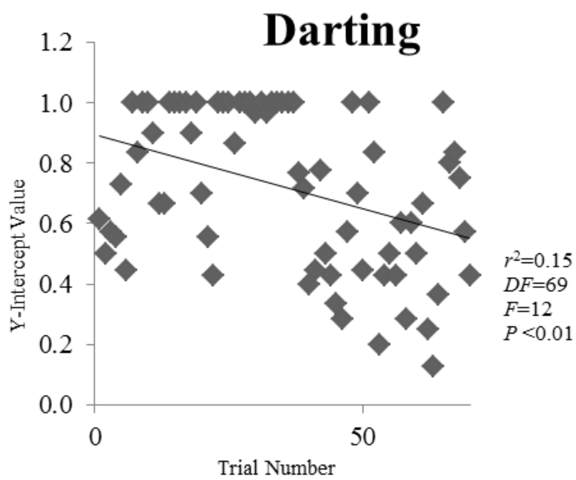
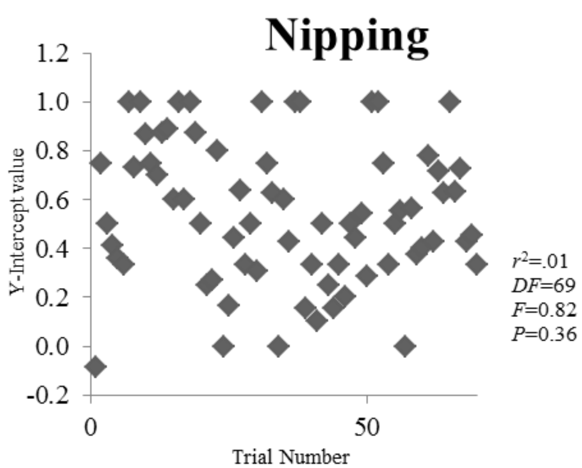
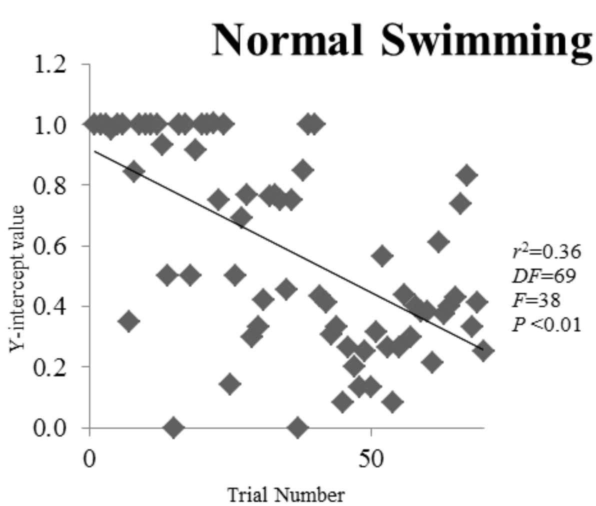


Figure 3 - Y-intercept values of each trials regression line over the entirety of the five-week noisy trials showing three behaviors of Buenos Aires tetras (*Hyphessobrycon anisitsi*): nipping (A), darting (B), and normal swimming (C). Results of simple linear regression are provided.


Darting
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Y-Intercept Value
0
50
Trial Number
r²=0.15
DF=69
F=12
P <0.01


Nipping
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
Y-Intercept value
0
50
Trial Number
r²=.01
DF=69
F=0.82
P=0.36


Normal Swimming
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Y-intercept value
0
50
Trial Number
r²=0.36
DF=69
F=38
P <0.01