

4-2014

Predation Avoidance Mechanisms of Juvenile Arapaima spp.: Significance of Synchronized Breathing and Sound Production

Jesse Eric Burle Olsen

Follow this and additional works at: <http://digitalcommons.esf.edu/honors>



Part of the [Aquaculture and Fisheries Commons](#)

Recommended Citation

Olsen, Jesse Eric Burle, "Predation Avoidance Mechanisms of Juvenile Arapaima spp.: Significance of Synchronized Breathing and Sound Production" (2014). *Honors Theses*. Paper 32.

Predation Avoidance Mechanisms of Juvenile *Arapaima* spp.:
Significance of Synchronized Breathing and Sound Production

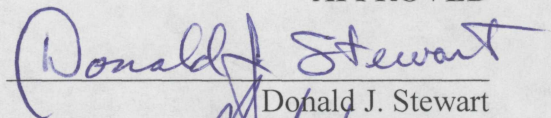
by

Jesse Eric Burle Olsen
Candidate for Bachelor of Aquatic Biology and Fisheries Science
Department of Environmental and Forest Biology
With Honors

April 2014

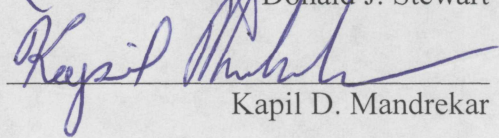
APPROVED

Thesis Project Advisor:



Donald J. Stewart

Second Reader:

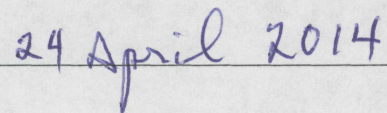


Kapil D. Mandrekar

Honors Director:

William M. Shields, Ph.D.

Date:



© 2014
Copyright
J. E. B. Olsen
All rights reserved

ABSTRACT

Arapaima spp. are large, commercially important but poorly studied freshwater fishes endemic primarily to the Amazon and Essequibo rivers of South America. Arapaima are obligate air breathers with a modified lung-like swim bladder. Surfacing to respire exposes arapaima to a heightened predation risk, especially the small juveniles. Surfacing in synchrony could reduce predation risk of individual arapaima. Synchronous breathing has been observed with many air-breathing fish, but it has not been documented with arapaima. Many fishes also are known to produce characteristic sounds to startle predators when attacked, but such sounds have not been reported for arapaima. An investigation of predation defenses of juvenile arapaima was conducted by quantifying breathing frequency patterns over a diel cycle. Also, the ability to produce startle sounds was evaluated by simulating predation (i.e. grasping the fish). Young arapaima exhibited synchronized breathing throughout the day; mean number of arapaima per breathing event was significantly higher ($p < 0.01$) during daylight than after dark. More pronounced synchronized breathing during daytime suggests a response to greater predation risk from visual predators like birds. A characteristic sound was recorded during respiration events. No disturbance sounds were observed when simulating predation on smaller arapaima (25-45 cm TL); however, sounds were recorded with larger arapaima (75-125 cm TL), both in and out of the water. This first demonstration of sound production ability in arapaima raises the possibility that these sounds could be used in various social contexts. Further investigation of these sounds is needed to understand their functions throughout the life of the arapaima.

TABLE OF CONTENTS

<u>ACKNOWLEDGMENTS</u>	iii
<u>LIST OF FIGURES</u>	iv
<u>LIST OF TABLES</u>	vi
<u>INTRODUCTION</u>	1
<u>METHODS</u>	5
<u>RESULTS</u>	11
<u>DISCUSSION</u>	14
<u>CONCLUSIONS</u>	17
<u>LITERATURE CITED</u>	19

ACKNOWLEDGMENTS

I wish to extend my thanks to the following people, organizations, and institutions for helping with my research. I would first like to thank Donald Stewart, my academic and research advisor, for excellent advice throughout my college career, for the extensive help I received, and for the opportunity to pursue research on arapaima. I would also like to thank my graduate mentor Kapil Mandrekar for all of the advice I have received and for pushing me to pursue research topics. I would like to thank the SUNY-ESF Honors Program and donors to the Honors Program for the funding and support that made my research possible. I wish to extend thanks to those who helped me in Brazil including the SEPAq Aquaculture facility staff, the Santa Rosa community, and the UFOPA community, specifically Lenise Silva and her students. Lastly I would like to thank the SUNY-ESF community, friends, and family for the support I have received.

LIST OF FIGURES

Figure 1. Mean submerged interval per arapaima (morph-1) and 95% confidence intervals of the mean over all sample periods at different times of day. The mean number of breaths per minute of all fish was calculated and divided by the number of fish in the tank to get mean number of breaths per fish per minute. This number was inversed to get the mean submerged interval per fish. Time periods marked with an asterisk are trials where breathing frequency was significantly clumped. The mean submerged interval for each fish was compared against all observational periods using a one-way ANOVA and Tukey's test for multiple comparisons. Means that do not share a letter in the matrix above are significantly different from each other.

Figure 2. Frequency of number of arapaima (morph-1) per breathing event for day and night trials. Mean number of arapaima per synchronous breathing event over all observation periods was significantly higher ($p < 0.01$) during the day ($n = 600$ breathing events) than at night ($n = 1069$). Breathing involving an individual fish (i.e. without a conspecific response) occurred more frequently at night.

Figure 3. Comparison of mean number of arapaima (morph-1) per synchronous breathing event of observational periods on the same day ($n = 19$ fish). Error bars represent 95% confidence interval of the mean. Means during the daytime on both 12/19 and 12/20 were significantly different (t-test, $p < 0.001$) from corresponding means during nighttime.

LIST OF FIGURES (CONTINUED)

Figure 4. Out-of-water exhale sound waveform (A) and spectrogram (B) of an arapaima (morph-2) during a simulated predation event.

Figure 5. In-water exhale sound waveform (A) and spectrogram (B) of an arapaima (morph-2) during a simulated predation event.

Figure 6. Relation between mean submerged interval and body mass for juvenile arapaima (morph-3) held in 950 L laboratory tanks. Based on a linear regression analysis, the slope was significantly different than zero ($p = 0.023$).

Figure 7. Mean submerged interval ($\text{min} \pm \text{S.E.}$) for each arapaima (morph-1) over trial periods. Arapaima number three had the highest mean submerged interval (trial 1, 8.40 ± 2.93), while arapaima number ten has the lowest mean submerged interval (trial 3, 2.88 ± 0.37).

LIST OF TABLES

Table 1. Mean submerged interval per arapaima (morph-1) per min. Breathing was significantly clumped during the day during all trials and during the night after trials starting at 2050.

Table 2. Mean submerged interval (min) of the different arapaima (morph-3) observed. Means were calculated using observational trial period breathing intervals.

INTRODUCTION

Most fishes are constantly at risk of being preyed upon, and to avoid becoming prey, many fish have developed anti-predator tactics. These tactics include structural adaptations such as long spines and behavioral patterns ultimately to discourage a predator or disrupt a predation event. Young fish are extremely vulnerable to predation due to their small size. Many behavioral mechanisms for predation avoidance have arisen, including schooling and startle sounds. Fish in a school have a lower probability of being captured than a solitary fish (Brock & Riffenberg 1960; Godin 1986; Landeau and Terborgh 1986). If a predator can only process or consume one prey fish at a time, the chance of any individual being eaten decreases with increasing group size. Startle sounds are sounds produced by the prey fish to momentarily startle the predator, allowing escape (Kaatz and Stewart 2012; Ladich 1997). Such sounds can also be used to inform other conspecifics of the presence of a predator. Most fish use a combination of these anti-predator tactics to avoid being consumed (Godin 1997).

All fish are faced with the problem of gaining enough oxygen from the water to support their metabolic needs. Many adaptations have arisen among fishes for attaining oxygen in aquatic environments that frequently undergo hypoxia. Aerial respiration has evolved at least 67 times in many groups of fish using different respiration techniques and organs (Graham 1997). Of these air breathers, obligate air breathers require aerial respiration constantly and risk drowning if constrained underwater, even in water with normal oxygen saturation (Graham 1997). The need to surface frequently puts obligate air breathers at a relatively higher risk of predation from both large aquatic and terrestrial predators, including birds.

Synchronized air breathing, characterized by many fish surfacing to respire at the same time or in a quick succession, has been documented in many fishes (Hill 1972; Shlaifer and Breder 1940; Sloman et al. 2009) and is thought to be an anti-predation mechanism (Kramer and Graham 1976). Surfacing as a group in a short period of time reduces the individual predation risk of each fish while also causing surface agitation that could disrupt the line of sight to the prey. It is known that fish school to reduce predation risk (Partridge 1982) and the development of synchronized breathing could have been an extension of that behavior. The phenomena of synchronized breathing has developed independently among different groups of fish, which suggests that it may be more associated with the behavior of aerial respiration than the evolutionary lineage of any particular fish group (Kramer and Graham 1976).

The arapaima is a large tropical freshwater fish primarily endemic to the Amazon and Essequibo river drainages; they can reach sizes of up to 3 m and 200 kg. Arapaima spend most of their time near the surface of the water because as obligate air breathers they must inhale air into a highly specialized lung-like swimbladder in order to respire. Juvenile arapaima hatch with gills that can perform O₂ uptake from water and are initially guarded in the nests as eggs and non-swimming larvae (Castello 2008b). After about nine days however, the juveniles have made the transition to obligate air breathing (Brauner et. al 2004, Graham 1997). After this time juveniles follow the male into the flooded forests (Castello 2008a,b) and have been observed to surface to breathe in groups in synchronized breathing events (D. Stewart, pers. obs.). Rising to the surface to breathe exposes juvenile arapaima to fish-eating birds and other predators, so it is thought that these events reduce the risk of predation of the individual arapaima. Juvenile arapaima of

sizes up to 50-70 cm total length (TL) have been found being guarded by adults and, after that size, they tend to become solitary (D. Stewart, pers. comm.). However, in artificial laboratory conditions where larger arapaima are housed in groups, I have observed schooling behavior and synchronized breathing events in arapaima up to 85 cm.

Despite the arapaima's importance in Neotropical floodplain ecosystems (Castello et al. 2011), they have never been examined for behavioral predation defense mechanisms. Arapaima spend most of their time in relatively shallow water (i.e. <4 m), and juveniles are especially vulnerable to predation due to their small size and need to be near the surface. Synchronized air breathing has been documented in other air breathing fishes that live in similar environments (Lima Filho et al. 2012; Sloman et al. 2009). While synchronized breathing with arapaima has been observed, it has never been thoroughly studied or quantified. The proximate cause of synchronous breathing events is also unknown, and it was of interest to determine if surfacing to breathe was associated with hearing conspecifics surfacing to respire. Hearing conspecifics respire at the surface could signal to others that it is safe for other arapaima to respire as well. If synchronized breathing events could be documented, it was also of interest to examine any patterns between synchronized breathing and time of day. A change in synchronized breathing frequency could indicate a possible response to the perceived amount of risk to the arapaima.

Arapaima also have never been evaluated for possible communication mechanisms. Startle sound production has never been documented with arapaima and it was of interest to investigate if the air breathing organ of the arapaima could be used to produce startle sounds. The unique respiratory system possessed by the arapaima could

be a pre-adaptation for sound production, and while I have observed that breathing events by arapaima do make a characteristic noise, the function of those sounds as a possible communication mechanism remains unknown.

The objectives of this study were: 1) to investigate the ability of arapaima to produce sounds; 2) to gather evidence to determine if arapaima exhibit synchronized breathing; and if they do, 3) examine relationships between synchronized breathing and time of day. I tested the following three hypotheses:

1) H_0 : arapaima cannot produce startle sounds.

H_A : arapaima produce startle sounds.

2) H_0 : breathing frequency is random.

H_A : breathing frequency is significantly clumped, providing evidence of synchrony.

3) H_0 : there is no correlation between synchronized breathing events and time of day.

H_A : the frequency of synchronized breathing events is correlated with time of day.

Given that the taxonomic status of fish the genus *Arapaima* is currently in review (Stewart 2013a,b), it was difficult to know what species of arapaima I had at the time of this study. For this reason, I consider this a study of fishes in the genus *Arapaima* as a whole. The fishes that I used in various experimental settings were of different origins, and so they will be referred to as morph-1, -2, and -3.

METHODS

Synchrony Among Grouped *Arapaima*

These experimental trials were carried out on the Secretaria de Estado de Pesca e Aquicultura (SEPAq) Aquaculture facility outside of Santarém, Brazil, in December, 2013. Behavioral observations were conducted on groups of 29, 19, and 18 arapaima housed in a 1500 L tank (163 cm diameter, 75 cm deep). The fish ranged from 25 cm to 45 cm total length (TL) and were less than one year in age. These fish had come from an aquaculture facility in Pixuna, a village in the Amazon várzea near Santarém (arapaima morph-1). All arapaima were housed in one tank and were recorded as a group. The arapaima also were kept with an abundance of small prey fish (*Oreochromis niloticus*) to serve as nutrition during the trials. The presence of breathing event sounds was investigated using a hydrophone (Aquarian Audio H2a-XLR-15) specialized for low amplitude sounds. The hydrophone was placed on the bottom of the tank in the same location each time (+/- 5 cm). Breathing sounds were recorded using an H1 Zoom portable audio recorder. Breathing event sounds and timing were recorded during ten 1-h observational periods over the course of five days. These observational periods were conducted throughout the day and night to investigate any changes in breathing patterns related to time of day. During each trial the hydrophone was placed in the tank to record breathing sounds while the arapaima were observed as a group using a headlamp. Each breathing event would be recorded by marking the time it occurred and if other arapaima surfaced to breathe within 5 s of the previous breathing event. Grouped breathing events were defined as one or more arapaima surfacing to breathe within 5 s after another conspecific breathing.

The mean number of arapaima per synchronous breathing event was compared between observational periods during daytime and nighttime using a t-test. This comparison was made to investigate possible trends between synchronous breathing and the time of day. Synchrony of breathing events was also analyzed by quantifying the temporal frequency of such events. Observational periods were divided into 60-s segments. The distribution of breathing events within each interval was analyzed for clumping by calculating the Coefficient of Dispersion (CD, variance/mean ratio, Sokal and Rohlf 1981). The CD will be greater than 1 for clumped distributions, will equal 1 for random distributions, and will be less than 1 for even distributions. The significance of the observed CD was then compared to the expected value for a random distribution (i.e. $CD = 1$) using a t-test. A significantly clumped distribution of breaths provides good evidence for synchrony (Kramer and Graham 1976, Chapman and Chapman 1994).

Startle Sound Production

These observations also were conducted in December of 2013. Arapaima housed at the SEPAq Aquaculture facility were recorded using a microphone in the air and a hydrophone in the water as they were being transferred from one pond to another. These arapaima also originated from Pixuna but were a different brood than the arapaima previously mentioned (hence, morph-2). These arapaima ranged from 75 cm to 125 cm TL and were approximately two years of age. One H1 Zoom recorder was used to record the sounds produced by arapaima out of the water, while another H1 Zoom recorder was used with an Aquarian Audio hydrophone model H2a-XLR-15 to record any noises that the arapaima made in the water. All arapaima were corralled into a pen on the side of the culture pond, and then held by hand in the water for approximately one minute before

they were brought on land to be measured and weighed. The hydrophone or microphone was held as close to the operculum as possible to record any noises made during an exhaling event. The sounds were analyzed for peak frequencies and waveform characteristics using the program Audacity 2.0.5.

Synchrony Among Visually Isolated *Arapaima*

Behavioral observations were conducted on ten individual arapaima during December, 2013, over a period of two days. Each arapaima was housed in a 1 m³ cage made of poly-vinyl-chloride tubes and plastic mesh. The cages were placed in an 8 m by 20 m pond at the SEPAq facility and were partially raised above the surface of the water to allow atmospheric respiration by the arapaima. The fish ranged from 25 to 45 cm TL and were less than one year in age (morph-1). These arapaima were also housed with an abundance of small prey fish (*Oreochromis niloticus*) in their cages to provide food. In an attempt to elicit a breathing response from the arapaima, the water was splashed every 3 min. This interval was chosen based on the estimated submerged interval of arapaima of this size class, but was changed to every 4 min after the first trial when it was observed that the mean submerged interval was closer to 4 min. The arapaima were observed from shore (about 2 m distance) and were recorded when they surfaced to breathe. Grouped breathing events were defined as one or more arapaima surfacing to breathe within 5 s of another conspecific breathing. These data were collected to determine if arapaima would exhibit synchronized breathing when visually isolated but not audibly isolated.

Synchrony of breathing events was analyzed by quantifying the temporal frequency of such events. Observational periods were divided into 60-s segments. The distribution of breathing events within each interval was analyzed for clumping by

calculating the Coefficient of Dispersion (CD, variance/mean ratio, Sokal and Rohlf 1981), and analyzed as described previously in the Synchrony Among Grouped *Arapaima* section.

Breathing Responses to Sound Playback (Morph-1, in Brazil)

These experiments also were carried out at the SEPAq Aquaculture facility outside of Santarém, Brazil, in December, 2013. Behavioral observations were conducted on a group of 19 arapaima housed in a 1500 L tank (163 cm diameter, 75 cm deep). The fish ranged from 25 to 45 cm TL and were less than one year in age (morph-1). All arapaima were housed in one tank with live prey fishes (juvenile *Oreochromis niloticus*) for food and were recorded as a group. A breathing sound from this group of arapaima was recorded using an Aquarian Audio hydrophone model H2a-XLR-15 connected to an H1 Zoom portable recorder. A breathing sound was isolated from the recording and played using an underwater speaker system (Lubell Labs UW-30PA), playing sounds from a laptop computer. The speaker was placed on a bucket near the surface of the water in the same location each time (+/- 5 cm). The speaker was attached to a 30 watt amplifier powered by a 12V battery and sounds were played using VLC Media Player running on a laptop computer. The speaker was placed in the tank and 5 min passed before any observations were made. This period served to allow the fish to become acclimated to the presence of the speaker in their environment. The breathing sound was played every 3 min in an attempt to initiate a synchronized breathing event. The use of a 3 min breathing interval was used due to observational data from these arapaima that showed that the mean submerged interval of these arapaima during the day was approximately 3 min. If any arapaima surfaced to breathe within 5 s of the sound

playback, it was deemed a response. A 5 s period was used to show correlation between the sound playback and arapaima surfacing to breathe due to the approximate time it takes for an arapaima to respond to the breathing sound by surfacing. If any arapaima surfaced during the recording or within the 5 s period following the end of the sound, it was deemed as a response to the recording. Grouped breathing events were recorded if either during or after the sound was played one or more arapaima surfaced to breathe.

Breathing Responses to Sound Playback (Morph-3, in Syracuse)

Seven juvenile arapaima were used to investigate a response to pre-recorded breathing event sounds. The fish ranged from 65 cm to 85 cm TL and were approximately three years in age. These fish had originated from an aquaculture facility in Peru (morph-3). The experiments took place at the Lafayette Field Station on the SUNY-ESF campus in Syracuse, NY, between the hours of 2100 and 2300 during late April to early May, 2013. The fish were separated and housed in two 950 L tanks with three fish in one tank and four fish in the other. The speaker and heater were left on to maintain normal conditions that might otherwise affect arapaima behavior if varied during an experimental period. The presence of a behavioral response to breathing sound events was investigated using an underwater speaker system (Lubell Labs UW-30PA). The speaker was placed on the bottom of the tank in the same location each time (+/- 5 cm). The speaker was attached to a 30 watt amplifier powered by a 12V battery, and sounds were played using VLC Media Player running on a laptop computer.

Trials were conducted on the two tanks in a sequential manner. Before the fish were subjected to any breathing event sounds, each tank was observed for 15 min and

breathing events were recorded. Each arapaima could be identified by pigmentation patterns on its body, and attention was paid to which individual arapaima was surfacing at each time. These data were collected to determine if there were any trends in grouped breathing events specific to each arapaima or day without the presence of an artificial stimulus. Grouped breathing events were defined as one or more arapaima surfacing to breathe within 5 s of another conspecific breathing. Once one tank had been observed, the process was repeated with the other tank.

To illicit a behavioral response to breathing event sounds, breathing events recorded a year earlier with these same arapaima were played at 2 min intervals to investigate any possible correlation between hearing breathing events and traveling to the surface to breathe. The use of a 2 min breathing interval was used due to observations by Mandrekar et al. (MS In Prep.), which showed that juvenile arapaima surface to breathe approximately every 2 min in this laboratory setting. The speaker was placed in the tank and 5 min passed before any observations were made. This period served to allow the fish to become acclimated to the presence of the speaker in their environment. A 5 s response period and playback response criteria mentioned in the previous section were used to evaluate response to sound playback. All breathing events were recorded on an individual basis during sound playback trials in order to monitor when the arapaima were surfacing to breathe and if any other trends could be observed. Once one tank had been tested, the process was repeated with the other tank.

RESULTS

Synchrony Among Grouped *Arapaima*

Observations using three different group sizes of arapaima were collected (Figure 1). Arapaima engaged in synchronized breathing events throughout the day; however, the degree of synchrony was different between daytime and nighttime (Table 1). Arapaima were more likely to school and surface in close proximity to conspecifics and appeared to surface at a greater angle to the water surface during the daytime compared to during the nighttime. The mean number of arapaima per synchronous breathing event was significantly higher ($p < 0.01$) during the daytime (3.38 fish/event, $n=600$ breathing events) than during the nighttime (2.29 fish/event, $n=1069$ breathing events). The maximum number of arapaima during any synchronous breathing event was 13, and that was observed during the day. There was a higher frequency of events with greater numbers of arapaima per breathing event during daytime than during night (Figure 2). When the same group of arapaima was observed during a single 24 hour period (encompassing both daytime and nighttime observational periods), the mean number of arapaima per synchronous breathing event was significantly higher during daytime than at night (Figure 3). Upon analysis for clumped breathing events using a Coefficient of Dispersion, breathing frequency was found to be significantly clumped in 11 of the 16 trials (Table 1). Of the trials where breathing was not significantly clumped, all were at night between the start times of 1800 and 2045. Arapaima were only observed to pursue and consume the prey fish at night. A one way ANOVA of mean breaths per fish per minute over the sample days showed that some of the means were significantly different

from each other (Figure 1). No trend was observed between mean submerged interval per fish and time of day.

Startle Sound Production

Of the 37 arapaima hand-held to simulate a predation event, 13 arapaima produced exhale noises above the water and four produced noises below the water. Of the 13 arapaima that produced exhale noises in the air, only two clear sounds could be isolated from the recordings due to interference from background noises. The exhale sounds produced above the water had peaks on the spectrogram at 65 Hz (-28 dB), 1234 Hz (-55 dB), and 2055 Hz (-54 dB) with a frequency at highest intensity at 65 Hz (-28 dB) (Figure 4). Of the four arapaima that produced sounds below the water, only two clear recordings could be isolated. The sounds produced underwater had peaks on the spectrogram at 62 Hz (-29 dB) and 923 Hz (-45 dB) with a frequency at highest intensity at 62 Hz (-29.0 dB) (Figure 5).

Synchrony Among Visually Isolated *Arapaima*

These arapaima were observed during the daytime on three observational periods for a total of 3.25 h. Respiration events showed some variation in the amount of disturbance they created at the surface of the water. Arapaima number four would frequently create a large splash when surfacing to breathe. Arapaima number three was the most elusive and surfaced to breathe without creating many sounds or splashes at all. All arapaima exhibited very little response to splashing trials and responded by surfacing to breathe after only five of the 48 splashes (10.4%). Mean submerged intervals from the arapaima were calculated and ranged from 3.1 min to 8.4 min, with an overall mean of

4.45 min (Figure 7). Analysis of the CD showed that the breathing events were not significantly clumped during any of the trials. Arapaima were observed to surface to breathe within 5 s after a conspecific, however, on many occasions. A mean of 18% of all breaths taken by all arapaima were within 5 s of another arapaima breathing. It rained during two of the three observational periods. The number of total breaths per minute appeared to increase slightly during the rain, but this increase was not significant.

Breathing Responses to Sound Playback (Morph-1, in Brazil)

Four sound playback trials in the tank containing 18 fish were made. An arapaima breathing sound recorded from this group of arapaima was played every 3 min during an observational period of 30 min. Arapaima showed very little response to breathing sound playback. They surfaced to breathe within 5 s of sound playback only three out of the 36 times the sound was played (8.3%). It is unclear whether these responses were a coincidence or correlated to the breathing sound playback.

Breathing Responses to Sound Playback (Morph-3, in Syracuse)

Six trials on the two tanks were made with 6 h of observations and 3 h of sound response trials. During the observational periods, the mean submerged interval was 2.50 min with the largest fish (2A) having the highest mean submerged interval of 3.02 ± 0.17 min and the smallest fish (1A) having the shortest mean interval of 1.88 ± 0.12 min (Table 2). Larger fish generally had a longer average submerged interval than smaller fish (Figure 6).

During the sound playback trials, two different single breathing sounds were played with varying success. The first sound had at least one arapaima respond at a rate

of 9% (for n = 32 playbacks) and the arapaima did not seem to directly respond to the sound but rather happened to be breathing when the recording was playing. The second sound had at least one arapaima respond at a rate of 34% (for n = 32 playbacks) and on some occasions the arapaima were observed directly responding to the sound by surfacing to breathe.

DISCUSSION

Breathing Synchrony

The finding that arapaima participate in synchronized breathing events led me to reject the null hypothesis that breathing frequency is random. Grouped arapaima exhibited strong synchrony during the day and less so at night. This latter finding led me to reject the null hypothesis that there is no correlation between synchronized breathing events and time of day. The finding that there was a significantly higher mean number of arapaima per synchronous breathing event overall during the daytime versus at night and with same-day trials suggests that synchronized breathing is more important as a predation defense during the day than at night. The lack of significantly clumped breathing between the hours of 1800 and 2045 could have been a response to a perceived lower predation risk from visual predators. During the day, arapaima could be seen much easier by a predator as they surfaced to breathe. The finding of a greater number of arapaima per breathing event during the day also supports the possibility of a greater perceived danger of being preyed upon.

The lack of synchrony at night, specifically right after sundown, could have been a result of the arapaima hunting for the small prey fishes in their tank during those times. Arapaima are suspected to be nocturnal predators (Watson et al. 2013) and the arapaima in my experimental tank (morph-1, in Brazil) were observed to only hunt at night. Pursuing a prey fish during the day could be energetically costly and could increase the risk of the arapaima being seen and preyed upon. During the day the arapaima would tightly school in the shaded areas of the tank and were not observed to pursue prey fish. Although the arapaima were observed to pursue prey fish during all nighttime observational periods, the lack of synchrony early in the evening followed by significantly clumped breathing later in the night (Table 1) may have been an artifact of a greater focus on hunting until the arapaima had been satiated. Following this period, the arapaima could once again focus on schooling and synchrony behavior.

The finding that arapaima housed individually in outdoor cages did not significantly respond to artificial splashing suggests that sounds associated with arapaima breathing events might not have a communicative purpose, so I fail to reject that null hypothesis. This lack of response, however, could have been due to the wrong sound quality being produced by my splashing. The lack of significant synchrony in the trials involving these visually isolated arapaima in cages is interesting. The breathing observed in these arapaima were not significantly clumped; however, breathing events of one arapaima were sometimes followed by breathing of adjacent or distant arapaima in quick succession. Overall, 18% of all breathes were within 5 s after another arapaima surfaced, but it is unclear whether this was a response to those breaths or a coincidence.

Breathing Responses to Sound Playback

The finding that the arapaima did not significantly respond to breathing sound playback leads me to accept the null hypothesis that sounds associated with arapaima breathing events do not have a communicative purpose. The small response to sound playback trials using two different groups of fish suggests that sound is not as important of a motivator for synchronized breathing as sight, or perhaps, lateral line stimuli. It does, however, raise the possibility that the wrong sound was being played. The lack of a splash accompanying the sound playback also could have confused the arapaima.

Sound Production

I have demonstrated for the first time that arapaima have the ability to produce sounds. The sounds produced above and below the water both have very low frequencies at highest intensities (~60 Hz), which would travel well underwater and have the potential for communication. The above-water exhale sound produced by the arapaima had a broad range of frequencies from 60 Hz to 21000 Hz. This sound is very close to the overall hearing range of most terrestrial mammals. One main predator of small arapaima is the giant river otter (*Pteronura brasiliensis*). While the giant river otter has not been analyzed for its auditory threshold, the North American river otter (*Lontra canadensis*) has been evaluated; its auditory threshold is 450-35000 Hz with a peak sensitivity of 16000 Hz (Gunn 1988). The exhale sound produced by the arapaima above-water spans the auditory threshold of the giant river otter's North American relative, and thus, potentially could be targeted towards startling such predators.

Although arapaima can produce sounds, the sounds that I recorded were not typical of a startle sound, which leads me to accept the null hypothesis that arapaima do

not produce startle sounds. These sounds were produced under stressful circumstances but did not have the characteristics of a startle sound. Most startle sounds are pulsed many times in an attempt to ward off a predator (Kaatz and Stewart 2012). Only 11 of the 32 fish sampled produced sounds above water, and of those fish, most only made one sound that was not pulsed or repeated. While we now know that arapaima have the ability to produce sounds, we do not know the context that such sounds might be used by the arapaima. Further investigation is needed to understand how these sounds are used and what they might communicate.

CONCLUSIONS

Juvenile arapaima are at risk of predation due to their shallow-water habitat and physiology. We now know that arapaima possess anti-predatory, synchronous breathing behavior that could reduce individual predation risk. The finding that juvenile arapaima synchronize their breathing events is consistent with observations on other air-breathing fishes (Kramer and Graham 1976; Chapman and Chapman 1994). My findings show that synchrony is an important method of potentially reducing predation risk that is more prevalent during the day when juvenile arapaima are most vulnerable. Sight and lateral line stimuli are likely the most important senses in organizing and initiating these breathing events, with sound perhaps playing some lesser role.

Arapaima do possess the capability to produce sounds during breathing events as well as on other occasions. While we do know the context of breathing sound production, the other sounds produced are not characteristic of startle noises. These sounds may actually be a startle noise unique to the arapaima; however, further investigation is needed to better understand the context of these sounds and when they are

produced. Further studies of sound production could show if the trait is more pronounced or reduced in different populations. An investigation of sound production in wild arapaima during breeding season could determine if sound production is used in courtship behavior. An investigation using an isolated control fish as well as visually isolated fish could determine if the sound of breathing events has an impact on breathing behavior. Investigations of these topics could allow us to further understand when sounds are produced and their importance in the behavior of arapaima. More studies are needed to better understand the behavior of these interesting and important fishes.

LITERATURE CITED

- Brauner, C. J., Matey, V., Wilson, J. M., Bernier, N. J., & Val, A. L. (2004). Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *Journal of Experimental Biology*, **207**(9), 1433-1438.
- Brock, V. E., & Riffenburgh, R. H. (1960). Fish schooling: a possible factor in reducing predation. *Journal du Conseil*, **25**(3), 307-317.
- Castello, L. (2008a). Lateral migration of *Arapaima gigas* in floodplains of the Amazon. *Ecology of Freshwater Fish*, **17**(1), 38-46.
- Castello, L. (2008b). Nesting habitat of *Arapaima gigas* (Schinz) in Amazonian floodplains. *Journal of Fish Biology*, **72**(6), 1520-1528.
- Castello, L., Stewart, D. J., & Arantes, C. C. (2011). Modeling population dynamics and conservation of arapaima in the Amazon. *Reviews in Fish Biology and Fisheries*, **21**(3), 623-640.
- Chapman, L. J., & Chapman, C. A. (1994). Observations on synchronous air breathing in *Clarias liocephalus*. *Copeia*, **1994**, 246-249.
- Godin, J. J. (1986). Antipredator function of shoaling in teleost fishes: a selective review. *Le Naturaliste Canadien*, **113**, 241-250.
- Godin, J. J. (1997). Evading predators, pp. 191-236. In: Godin, J. J. (ed.). *Behavioural ecology of teleost fishes*. Oxford, Oxford University Press.
- Graham, J. B. (ed.). (1997). *Air-breathing fishes: evolution, diversity, and adaptation*. New York, Academic Press.

- Gunn, L. M. (1988). A Behavioral audiogram of the North American river otter (*Lutra Canadensis*). Doctoral dissertation, San Diego State University.
- Helfman, G., Collette, B. B., Facey, D. E., & Bowen, B. W. (2009). *The diversity of fishes: biology, evolution, and ecology*. New York, John Wiley & Sons.
- Hill, L. G. (1972). Social aspects of aerial respiration of young gars (*Lepisosteus*). *The Southwestern Naturalist*, **16** (3&4), 239-247.
- Kaatz, I. M., & Stewart, D. J. (2012). Bioacoustic variation of swimbladder disturbance sounds in Neotropical doradoid catfishes (Siluriformes: Doradidae, Auchenipteridae): Potential morphological correlates. *Current Zoology*, **58**(1), 171-188.
- Kramer, D. L., & Graham, J.B. (1976). Synchronous air breathing, a social component of respiration in fishes. *Copeia*, **1976**, 689-697.
- Ladich, F. (1997). Agonistic behaviour and significance of sounds in vocalizing fish. *Marine and Freshwater Behaviour and Physiology*, **29**(1-4), 87-108.
- Landeau, L., & Terborgh, J. (1986). Oddity and the 'confusion effect' in predation. *Animal Behaviour*, **34**(5), 1372-1380.
- Lima Filho, J. A., Martins, J., Arruda, R., & Carvalho, L. N. (2012). Air-breathing behavior of the jeju fish *Hoplerythrinus unitaeniatus* in Amazonian streams. *Biotropica*, **44**(4), 512-520.
- Mandrekar, K.M., Powers, C., & Stewart, D. J. (MS In Preparation) Respiratory behavior and diurnal activity in juvenile *Arapaima* spp.
- Partridge, B. L. (1982). The structure and function of fish schools. *Scientific American*, **246**(6), 114-123.

- Shlaifer, A., & Breder, C.M. (1940). Social and respiratory behavior of small tarpon. *Zoologica*, **25**, 493-512
- Sloman, K. A., Sloman, R.D., De Boeck, G., Scott, G.R., Iftikar, F.I., Wood, C.M., Almeida-Val, V.M.F., Val, A. L. (2009). The role of size in synchronous air breathing of *Hoplosternum littorale*. *Physiological and Biochemical Zoology*, **82**(6), 625-634.
- Sokal, R. R., & Rohlf, F. J. (1981). Biometry: the principles and practice of statistics in biological research. San Francisco, W.H. Freeman and Co.
- Stewart, D. J. (2013a). Re-description of *Arapaima agassizii* (Valenciennes), a rare fish from Brazil (Osteoglossomorpha: Osteoglossidae). *Copeia*, **2013**(1), 38-51.
- Stewart, D. J. (2013b). A new species of *Arapaima* (Osteoglossomorpha: Osteoglossidae) from the Solimões River, Amazonas State, Brazil. *Copeia*, **2013**(3), 470-476.
- Watson, L. C., Stewart, D. J., & Teece, M. A. (2013). Trophic ecology of *Arapaima* in Guyana: giant omnivores in Neotropical floodplains. *Neotropical Ichthyology*, **11**(2), 341-349.

A						A	A	A	A	A	A			
B					B		B	B	B	B	B			
C		C	C	C	C		C		C	C	C			
	D	D	D	D	D		D		D	D	D		D	
	E	E	E	E	E		E		E	E			E	E
	F	F	F	F	F				F			F	F	F

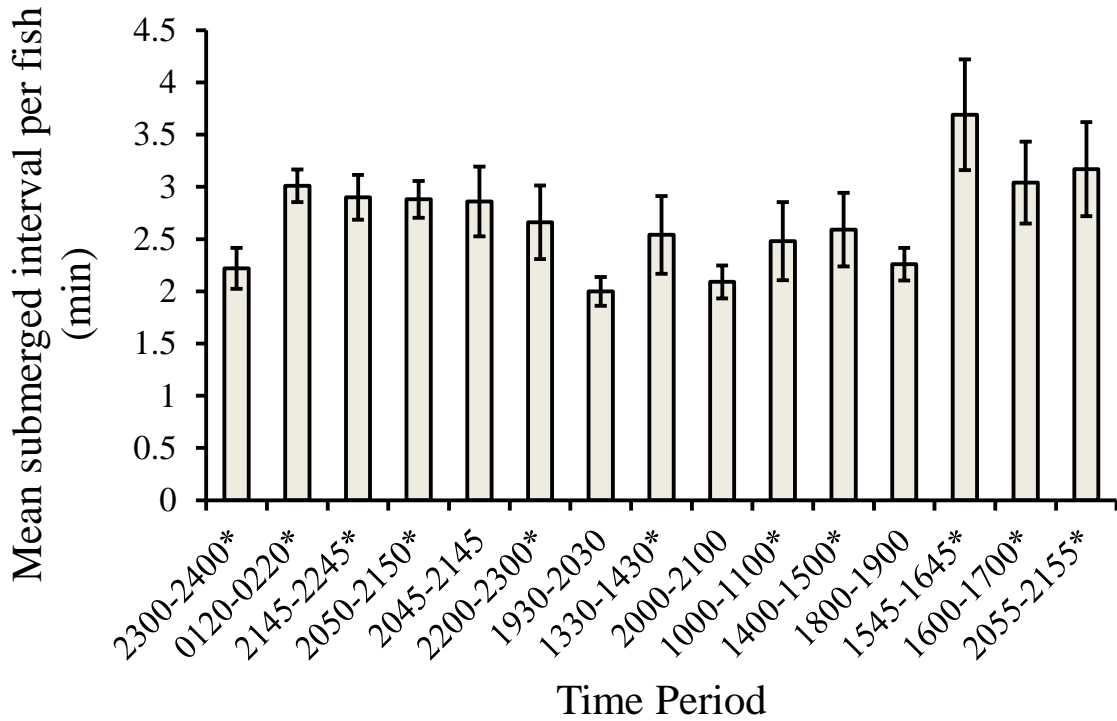


Figure 1. Mean submerged interval per arapaima (morph-1) and 95% confidence intervals of the mean over all sample periods at different times of day. The mean number of breaths per minute of all fish was calculated and divided by the number of fish in the tank to get mean number of breaths per fish per minute. This number was inversed to get the mean submerged interval per fish. Time periods marked with an asterisk are trials where breathing frequency was significantly clumped. The mean submerged interval for each fish was compared against all observational periods using a one-way ANOVA and Tukey's test for multiple comparisons. Means that do not share a letter in the matrix above are significantly different from each other.

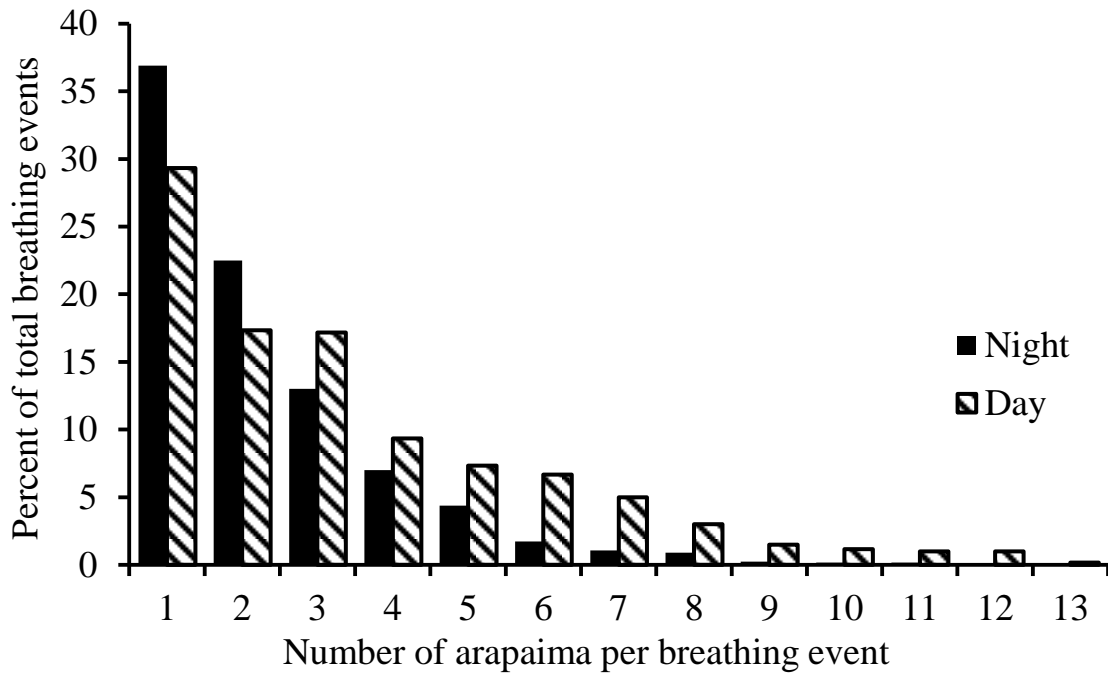


Figure 2. Frequency of number of arapaima (morph-1) per breathing event for day and night trials. Mean number of arapaima per synchronous breathing event over all observation periods was significantly higher ($p < 0.01$) during the day ($n = 600$ breathing events) than at night ($n = 1069$). Breathing involving an individual fish (i.e. without a conspecific response) occurred more frequently at night.

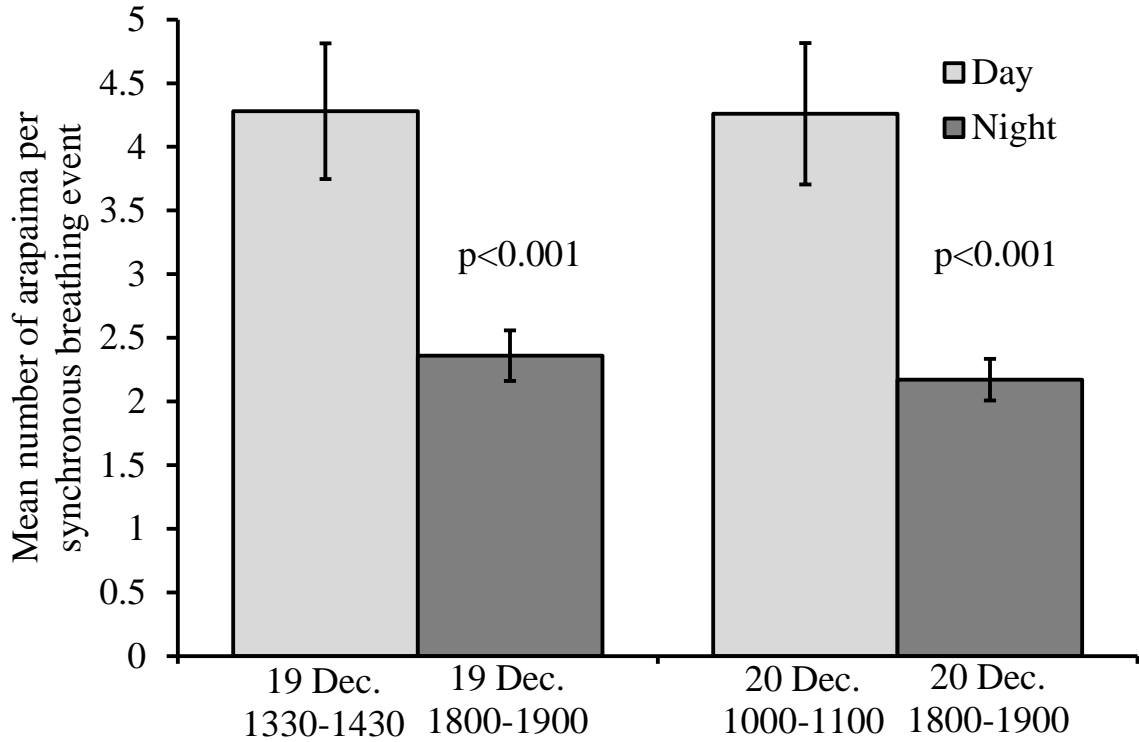


Figure 3. Comparison of mean number of arapaima (morph-1) per synchronous breathing event of observational periods on the same day (n = 19 fish). Error bars represent 95% confidence interval of the mean. Means during the daytime on both 12/19 and 12/20 were significantly different (t-test, $p < 0.001$) from corresponding means during nighttime.

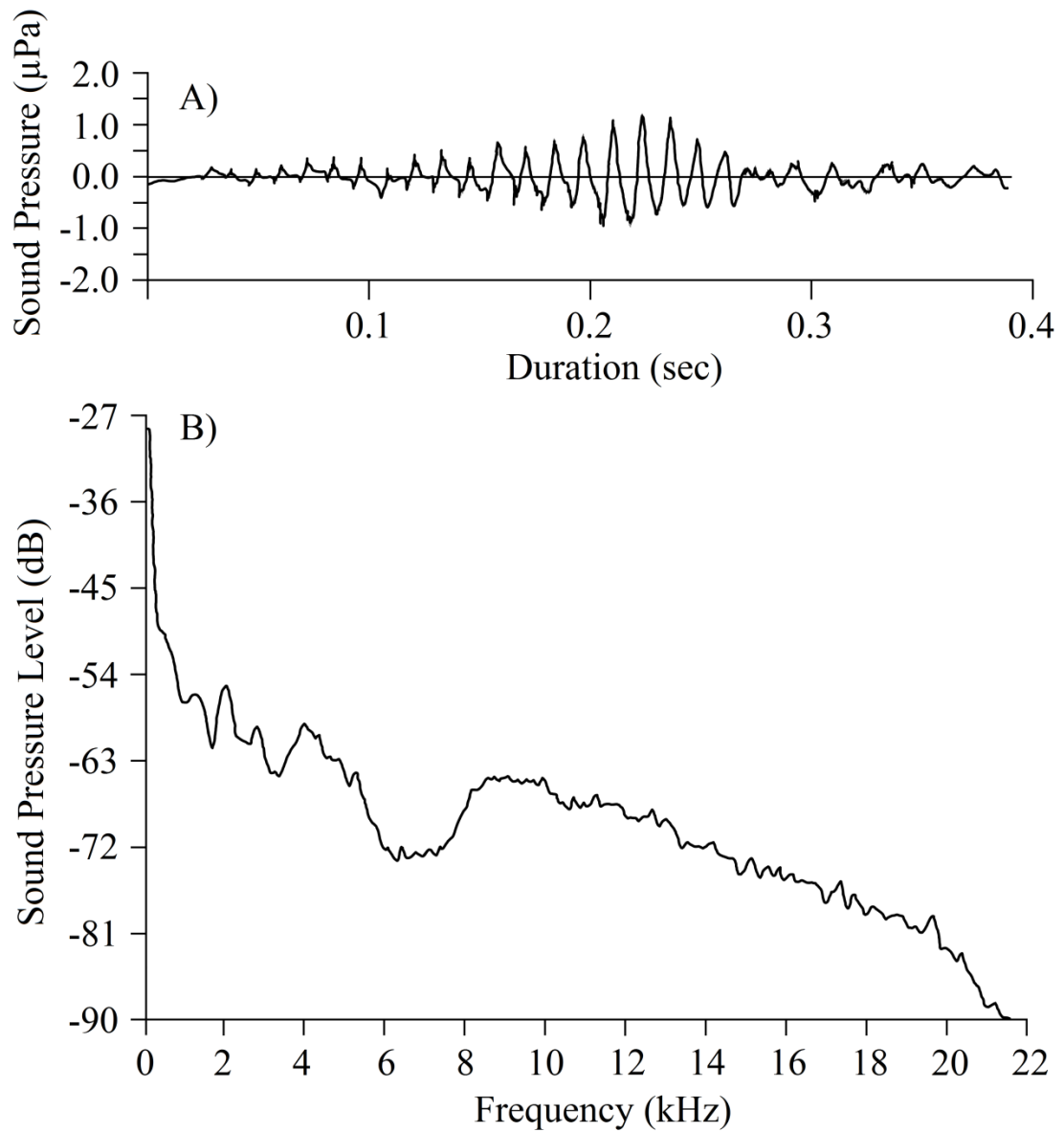


Figure 4. Out-of-water exhale sound waveform (A) and spectrogram (B) of an arapaima (morph-2) during a simulated predation event.

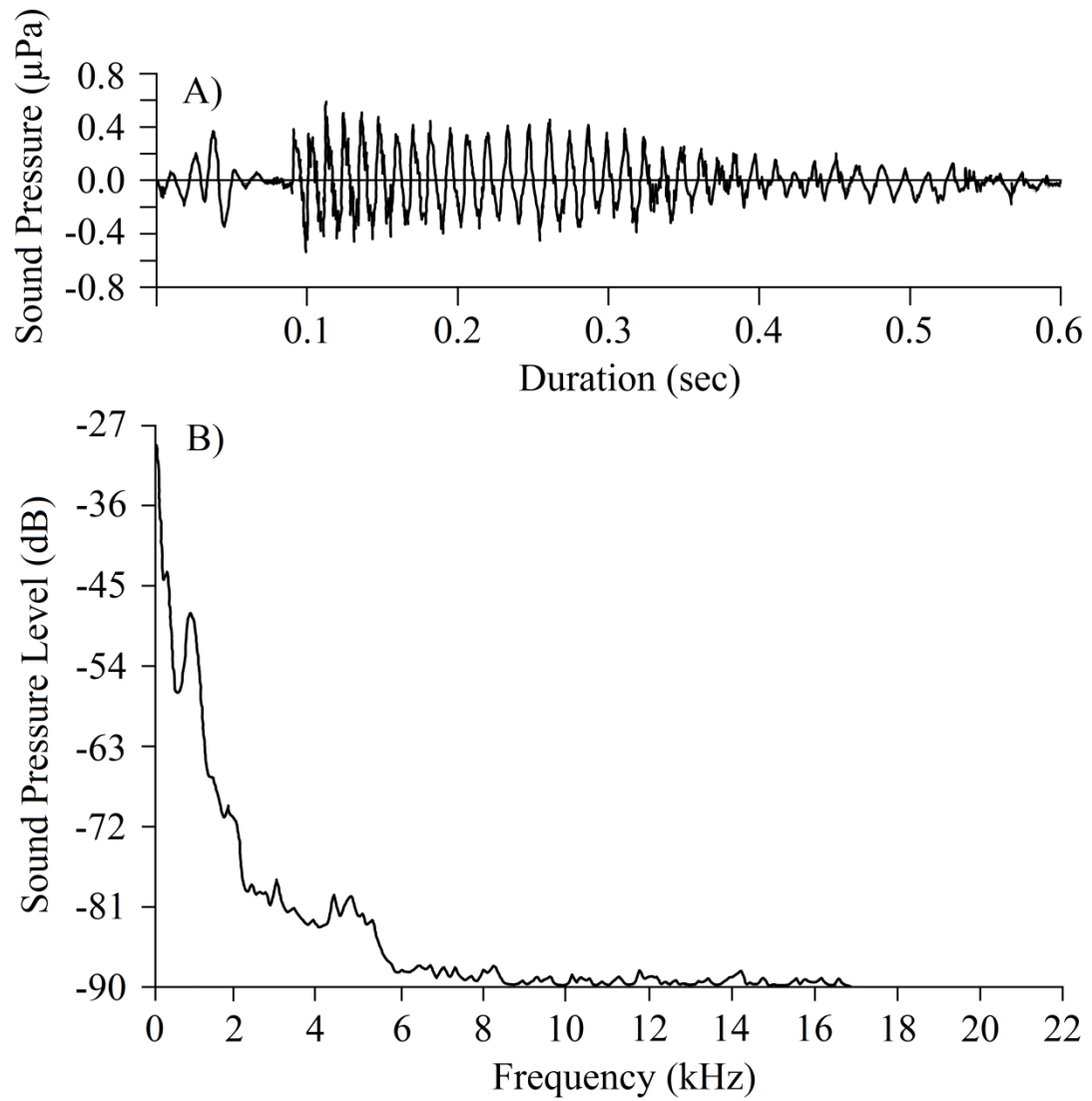


Figure 5. In-water exhale sound waveform (A) and spectrogram (B) of an arapaima (morph-2) during a simulated predation event.

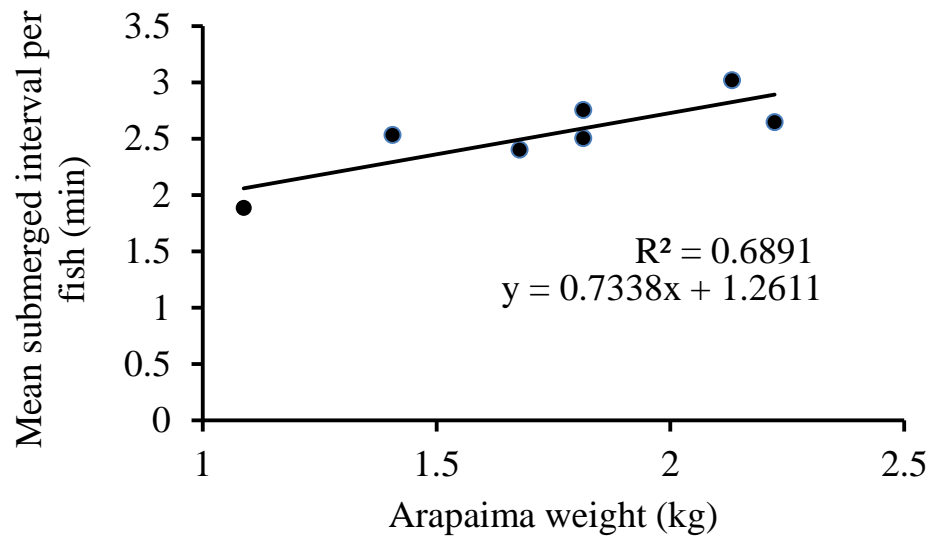


Figure 6. Relation between mean submerged interval and body mass for juvenile arapaima (morph-3) held in 950 L laboratory tanks. Based on a linear regression analysis, the slope was significantly different than zero ($p = 0.023$).

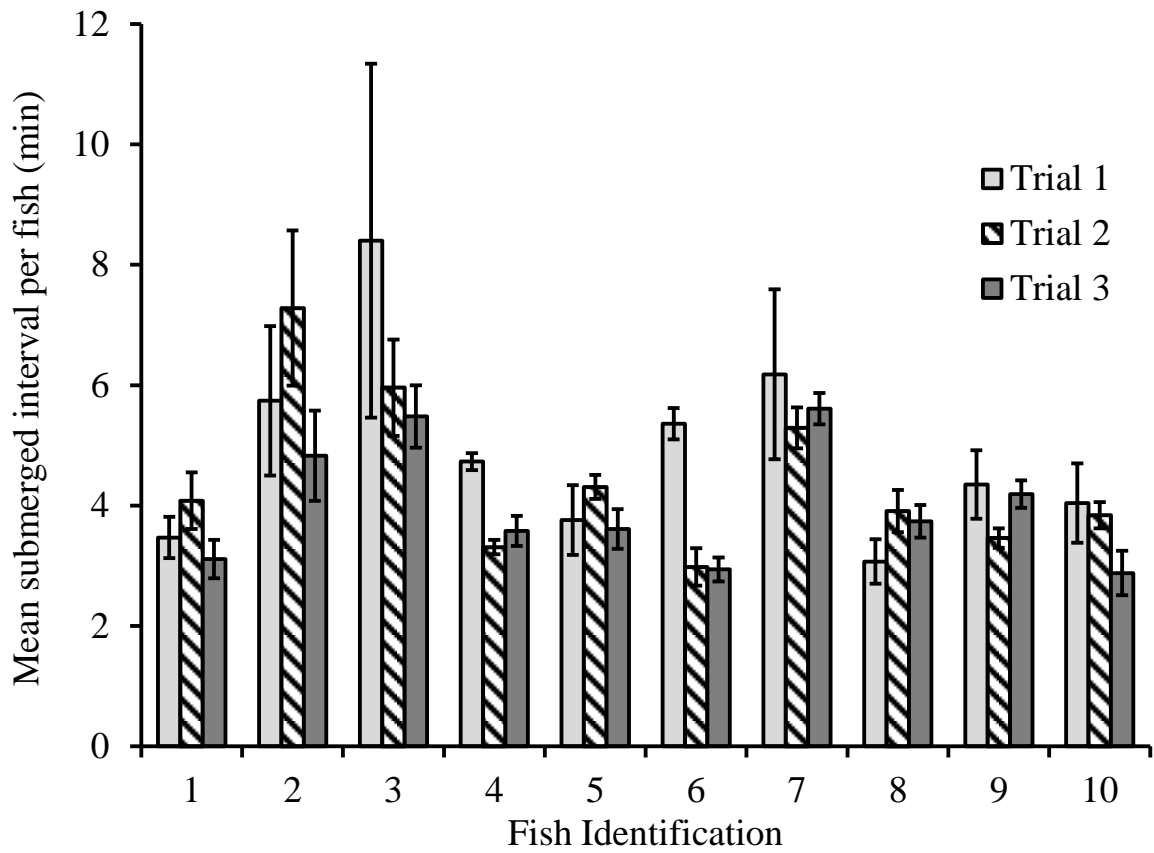


Figure 7. Mean submerged interval (min \pm S.E.) for each arapaima (morph-1) over trial periods. Arapaima number three had the highest mean submerged interval (trial 1, 8.40 ± 2.93), while arapaima number ten had the lowest mean submerged interval (trial 3, 2.88 ± 0.37).

Table 1. Mean submerged interval per arapaima (morph-1) per min. Breathing was significantly clumped during the day during all trials and during the night after trials starting at 2050.

	Start time	Number of fish	Mean submerged interval/fish	Coefficient of dispersion	Clumped breathing?
Day	1000	19	2:29	3.15	p<0.001
	1330	19	2:32	3.10	p<0.001
	1400	19	2:35	2.36	p<0.006
	1545	19	3:41	1.87	p<0.015
	1600	19	3:02	1.75	p<0.040
Night	1800	19	2:15	0.68	p=0.843
	1930	19	2:00	0.87	p=0.633
	2000	19	2:05	0.80	p=0.712
	2045	19	2:52	1.53	p=0.098
	2050	29	2:53	2.58	p<0.009
	2055	18	3:10	2.10	p<0.007
	2145	29	2:54	3.93	p<0.001
	2200	19	2:40	2.15	p<0.012
	2300	29	2:13	2.32	p<0.032
	0120	29	3:01	3.00	p<0.002

Table 2. Mean submerged interval (min) of the different arapaima (morph-3) observed.

Means were calculated using observational trial period breathing intervals.

Arapaima identification	1A	1B	1C	2A	2B	2C	2D
Number of breathes	38	33	30	26	27	29	27
Mean submerged interval	1.88	2.40	2.50	3.02	2.75	2.52	2.63
Standard deviation	0.75	0.98	0.95	0.88	0.85	0.72	0.88