Fall 1-17-2018

Morphological Correlates and Behavioral Functions of Sound Production in Loricariid Catfish, with a Focus on *Pterygoplichthys pardalis* (Castelnau, 1855)

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Recommended Citation

Slusher, Monique Renee, "Morphological Correlates and Behavioral Functions of Sound Production in Loricariid Catfish, with a Focus on *Pterygoplichthys pardalis* (Castelnau, 1855)" (2018). *Dissertations and Theses.* Paper 4155.
https://doi.org/10.15760/etd.6043

Morphological Correlates and Behavioral Functions of Sound Production in Loricariid Catfish, With a Focus on *Pterygoplichthys pardalis* (Castelnau, 1855)

by

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A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biology

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Portland State University
2017

Abstract

The Neotropical catfish *Pterygoplichthys pardalis* produces a harsh stridulation sound upon manual capture. This stridulation sound is made on the abduction of the pectoral fin spine, and is accomplished by friction of a ridged dorsal condyle against a rough spinal fossa of the cleithrum in the pectoral girdle. The sound produced has an average frequency of 121 Hz, and is used with other anti-predator adaptations such as bony subdermal armor and defensive fin-spreading. *Pterygoplichthys pardalis* does not display behavioral modification in response to conspecific stridulation sound, and therefore it is likely that stridulation sound in *P. pardalis* is being used as a predator deterrent.

ACKNOWLEDGEMENTS

I thank my advisor Randy Zelick for all his help and guidance over the course of my master's degree. I also thank my lab mates Nikko Merrell and Whitney Gayer for their assistance and companionship. Finally, I thank my parents, my friends, and my partner for their continuing love and support through my education.

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CHAPTER 1: Background and Introduction

Focal animal. The sailfin or leopard plecostomus *Pterygoplichthys pardalis* is a widely-known species in the catfish family Loricariidae (Order Siluriformes), being a popular hobbyist aquarium fish due to its algivorous diet. Loricariid catfish are endemic to the Neotropics, and *P. pardalis* is native to rivers in the Amazon river basin (Ferraris, 2007). The sailfin plecostomus *Pterygoplichthys pardalis* is often confused with the common plecostomus *Hypostomus plecostomus* (Linnaeus, 1758) due to its similar patterning and morphology. The most notable difference between the two species is the number of dorsal fin rays: *P. pardalis* possesses approximately 12-14 fin rays (Armbruster and Page, 2006). The resulting large dorsal fin gives *P. pardalis* its secondary common name: the sailfin plecostomus. In contrast, *H. plecostomus* possesses only 7-8 dorsal fin rays, resulting in a relatively smaller dorsal fin. The standard coloration of *P. pardalis* is a dark brown approaching black, with a spotted or vermiculated pattern. This coloration is cryptic, and may allow *P. pardalis* to hide from predators more effectively in their benthic habitat.

Pterygoplichys pardalis (Loricariidae: subfamily Hypostominae) is useful as a model species for the family Loricariidae as it possesses all the characteristics of the family, most notably including a sucker-shaped mouth that species belonging to the family Loricariidae use to scrape algae off hard surfaces; bony armor underneath its skin; sharp, backwards facing spines; and enlarged first fin rays in its pectoral, pelvic, and dorsal fins (Lujan et al., 2014). Behaviorally, *P. pardalis* exhibits behaviors typical of loricariids –

fish in this family are typically non-schooling and solitary, and are often territorial during mating, when males defend cavities in river beds in which females lay eggs. Loricariids are benthic fish that subsist on a diet of algae and detritus (Lujan and Armbruster, 2012). When exposed to stressful situations involving capture, as by a predator or in a fishing net, these fish often display a defensive fin spreading and fin spine locking behavior, and some, but not all, loricariids also produce a harsh stridulation sound (Kaatz, 1999). These sounds are audible in air, especially in larger individuals, and vibrations can be clearly felt in the hand.

Sound production. Sound production is a relatively common behavior among a wide variety of fish species. In fish – as in other groups of organisms – sound is generally used to communicate (reviewed by Amorim, 2006). Courtship sounds are common in many orders of fish, including in mormyrids, marine cod, toadfish, cichlids, damselfish, gouramis, and catfish. Many fish also use sound during aggressive intraspecific encounters: mormyrids and damselfish in particular employ sound during aggressive displays and fights, while cichlids use sounds in territorial and brood defense. The marine Triglidae (Scorpaeniformes) and many families of catfish produce distress sounds; these sounds are commonly observed when the fish is captured or chased by a predator, or when trapped in fishing nets. Fish also generate sound as a consequence of movement through the water. These hydrodynamic sounds are produced unintentionally on the part of the sender, but can be used by the receiver for a variety of different purposes.

SOUND PRODUCTION IN LORICARIID CATFISH

Fish as a taxonomic class do not possess any single organ dedicated solely to sound production in the way that amphibians and mammals have a larynx and birds have a syrinx. Rather, fish have developed several different sound production mechanisms, several of which may be present in a given species (Demski, Gerald, and Popper, 1973), and all of which involve a structure previously and primarily used for a purpose other than sound production. Most commonly, fish produce sound hydrodynamically as a byproduct of their movements through the water (Moulton, 1960). All fish produce hydrodynamic sounds to some degree, due to the turbulence caused by their movement through the water. These hydrodynamic swimming sounds are typically not made intentionally by the animal, but can be used for communication – for instance, schooling fish may use these hydrodynamic signals to help locate each other, or predators may use these signals to locate prey.

Some fish produce sound using their swim bladder, an air-filled organ used primarily to maintain and alter buoyancy in the water. These swim bladder sounds are produced by muscular activity that creates a “drumming” sound on the swim bladder. These muscles can either be dedicated to sound production, and only attached to the swim bladder, or they may be extrinsic body muscles that have some contact with the swim bladder (Demski et al., 1973) which may be primarily used for other body motions such as swimming. Certain widely-studied species of fish that produce sound use swim bladder mechanisms. These include the famous oyster toadfish *Opsanus tau* (Batrachoidiformes: Batrachoididae), which produces more than one distinct sound: a “grunt” sound that is evoked in territorial or disturbance contexts in male *O. tau*, and a “boatwhistle” sound that is used by male *O. tau* to attract mates (Gray and Winn, 1961); both sounds use the swim bladder. Sounds produced by the swim bladder may be variable, as multiple different sounds can be produced using the swim bladder even within a single species. This suggests that the signal can be modified, at least in some species, such that there is a different pitch or call pattern, and these differences are most likely as a result of differences in muscular activity (eg. the contraction of different muscles, or different patterns of contraction of the same muscles) of the muscles that attach to or come into contact with the swim bladder.

Stridulation sounds are a class of sound produced by the rubbing of hard surfaces against each other. In some fish, this can be the scraping of teeth against each other or on surfaces such as rock. In catfish (Order Siluriformes), stridulation sounds are most often produced by the rubbing of bone on bone; in almost all cases, the production of stridulation sound in this group is carried out by friction of an enlarged first ray of the pectoral fin (the pectoral fin spine) against the floor of the spinal fossa of the cleithrum, in the pectoral girdle (Fine and Ladich, 2003). Where stridulation sound is not produced voluntarily by the fin spine, it is produced involuntarily by the teeth, as by grinding them against food objects or hard substrates during feeding.

It has been remarked that the basal condition in loricariids is stridulation upon abduction of the fin spine (Fine and Ladich, 2003). However, some species of loricariids have been observed to produce sound on adduction or on both adduction and abduction

(Kaatz, 1999). Webb (2011) described two species of loricariid catfish that produce sound using pectoral fin spine movement that is not exclusively restricted to abduction. *Pterygoplichthys gibbiceps* (Loricariidae: subfamily Hypostominae) produces sound by both adduction and abduction of the pectoral fin spine, in “pulse trains” marked by different sound qualities on abduction versus adduction. *Macrotocinclus affinis* (Loricariidae) produces sound only by adduction of the pectoral fin spine, and then only in distinct “clicks,” in contrast with the full “grunts” or “squeaks” observed in *P. pardalis* and *P. gibbiceps.* Despite variation in the movements that produce sound among members of this family, *P. pardalis* is a good model species for the family Loricariidae insofar as sound production is concerned, as they produce sound only on abduction of the pectoral fin spine, the assumed basal condition of this family. With the variation observed in differential patterns of sound production based on action of the pectoral fin, the issue arises as to whether these differences in function may be due either to bone morphology in the pectoral fin spine or pectoral girdle, or alternately, from differential muscular activity in the muscles that insert on the pectoral fin spine.

There remains a question of the exact method of sound production at a more detailed level. How exactly does the ridged condyle of the pectoral fin produce the characteristic grunt-like stridulation sound? The current prevailing hypothesis is that each ridge of the dorsal condyle of the pectoral fin spine produces one “click” in the overall grunt sound (Fine and Ladich, 2003), although definitive evidence to support hypothesis has not been shown experimentally. Fine et al. (1997) suggested that the pectoral girdle itself is acting as a radiator for the sound produced by the friction of the pectoral spine ridges against the spinal fossa – that is, the audible and palpable sound produced by the fish is caused by the vibration of the pectoral girdle complex and not by the vibration of the pectoral spine itself. Hadjiaghai and Ladich (2015) found that in the callichthyid catfish *Megalechis thoracata,* pectoral fin spine length is inversely related to dominant frequency of stridulation sounds: the longer the fin spine, the lower the dominant frequency of the call. In *Pterygoplichthys pardalis,* the focal animal of the current study, there is a near 1:1 ratio of pectoral fin spine length to the width of the head (synonymous with the size of the pectoral girdle, as the bones of the pectoral girdle are fused with the bones of the skull in this family). There may be some effect of the size (as signified by the width) of the pectoral girdle on the quality of sound relative to frequency, but it is difficult to determine whether the size of the pectoral fin spine itself or the size of the pectoral girdle are affecting sound qualities more strongly, as these two measures of body size covary.

Loricariid catfish differ in morphology and functionality with respect to sound production. Though Kaatz et al. (2010) found *Macrotocinclus* sp. (Loricariidae: subfamily Hypoptomatinae) to be silent, this species possesses the ridges on their pectoral spine dorsal condyles necessary for sound production. *Macrotocinclus affinis* was found by Webb (2011) to produce sound on adduction of the pectoral fin spine – the opposite motion from that used by *P. pardalis* to produce stridulation sound. *Panaque maccus* (Loricariidae: subfamily Hypostominae) is known to produce sound using the pectoral fin spine (Kaatz, 1999). *Ancistrus* sp.(Loricariidae: subfamily Hypostominae) have been observed to be silent when disturbed, but possess pectoral fin spine morphology consistent with sound-producing catfish (Kaatz et al. 2010). *Rhineloricaria* sp. (Loricariidae: subfamily Loricariinae) also are silent, and it is not known whether they have similar pectoral fin spine morphology to that of sound-producing species. The purpose of the present study is to compare the mechanism and anatomy of sound production in the family Loricariidae, with a focus on *P. pardalis* as a model species for the family and comparison to other species with known differences in morphology and acoustic patterns. *Pterygoplichthys pardalis* is expected to possess well-defined ridges on the dorsal condyle of its pectoral fin spine for the purpose of sound production, as documented in other sound-producing catfish. This species will be compared to sound-producing species (*P. maccus*), silent species (*A. cirrhosus*), and species of undetermined sound-producing behavior (*Rhineloricaria*) in terms of the morphology of the dorsal condyle to determine if differences in the functionality of sound production mechanisms are due primarily to differences in bone morphology.

Purpose of stridulation sound production in catfish. Catfish display a number of antipredator defenses. Some catfish are known to puncture the skin of predators with their sharp fin spines during capture and release venom as a result of a fright reaction, intended as an alarm substance and a painful antipredator defense (Fine and Ladich, 2003); this defense mechanism is not present in *P. pardalis*. Many catfish display spine-locking behavior, in which the pectoral, dorsal, and/or pelvic fin spines are locked in a fully abducted position. In the pectoral fin spine in particular, this is accomplished by movement of the anterior process of the pectoral fin spine into a locking fossa of the pectoral girdle. These spines must be actively moved out of the locking fossa by the musculature of the animal, and the spines resist all attempts by outside forces to adduct the bones when locked in an abducted position. This spine-locking behavior results in a defensive posture in which the fins are spread wide, and would result in great difficulty in attempted swallowing of the individual by a gape-limited predator (Fine and Ladich, 2003). *Pterygoplichthys* sp. lock five of their fin spines in their defensive spine-locking posture – both pectoral fins, both pelvic fins, and the dorsal fin (Kaatz, 1999). In addition to this defensive posture, many catfish display “thrashing” behaviors during capture, which may be intended as an attempt to escape or as an attempt to injure their captor with their strong, sharp fin spines.

Among catfish, the sucker-shaped mouths and subdermal bony armor covering the dorsal surfaces of loricariids and callichthyids set these families apart from others (Lujan and Armbruster, 2012). This bony armor serves as a strong predator deterrent in these families as the skin becomes difficult to pierce, making the animal harder to consume. In addition, loricariid catfish typically possess several rows of backwards-curving spikes along the length of their body which are sharp and make it difficult to swallow an individual tail-first, as the spikes are likely to embed themselves in the mouth or throat of the would-be predator.

Sound production is also thought to be an antipredator adaptation in some catfish, though stridulation sound has also been observed to play a role in courtship in some species (Kaatz, 1999). Stridulation sounds as predator deterrents have been observed in some aquatic species, such as the Carribean spiny lobster (*Panulirus argus*, Malacostraca: Decapoda: Palinuridae), which produces a stridulation sound as part of an escape behavior that has been shown to deter predators (Bouwma and Herrnkind, 2009). However, Bosher et al. (2006) found that stridulation sounds by channel catfish (*Ictalurus punctatus*, Ictaluridae) did not have an effect on predation by largemouth bass. This suggests that stridulation sounds produced may be signals intended for specific predators, and not broadly effective on a wide range of predators. Kaatz (1999) observed that catfish stridulate more readily when removed from the water. This may be due to increased levels of stress when the animal is removed from the water, therefore causing sound production behavior to be released easier in air than underwater.

There remains a possibility that stridulation sounds could be signals to conspecifics, warning them of danger or calling for help. In order for sound to be a useful signal among conspecifics, the species in question must be able to hear in the range of frequencies of the sound produced by individuals of the species. The order Siluriformes belongs to superorder Ostariophysi, a taxonomic clade known to have particularly keen hearing among fish, displaying auditory sensitivity to frequencies between 100 and 5,000 Hz (Ladich, 1999). This is a relatively limited range of hearing compared to hearing specialists like mammals, in which some species may sense sounds as low as 14 Hz or as high as 100,000 Hz. Otophysan fishes (including loricariid catfish) accomplish their relatively sensitive hearing by the use of bony Weberian ossicles associated with the air-filled swim bladder to detect vibrations. However, many loricariids have swim bladders that are encased in bone (Weitzmann, 2005) and fewer Weberian ossicles than other catfish families, and both of these features skew hearing among loricariids towards lower frequencies (Lechner and Ladich, 2008). If sound produced by loricariid fish is composed of lower frequencies, then loricariids are likely to be able to perceive these sounds. If sound produced by loricariid fish is composed primarily of higher sounds, however, these sounds may not be perceived by loricariids at all, as high frequencies may fall outside their range of hearing.

Stridulation sound in most species of catfish is produced at frequencies between 100 and 4,000 Hz, within the hearing range of otophysan fish. Therefore, stridulation as a social sound among conspecifics is possible among loricariids. Smith et al. (2009) were able to train *Macrotocinclus affinis* to approach an underwater speaker in response to stridulation sounds produced by conspecifics and played back to fish. Though it is unknown whether naïve fish respond behaviorally to conspecific sound, the ability to train this species to respond to conspecific sound suggests that they do have the ability to hear sounds in the frequency ranges of the sounds produced by conspecifics. The possibility that stridulation sound among loricariids, and specifically in *P. pardalis*, may be a signal to conspecifics must be investigated. Evidence that naïve individuals of P. pardalis respond to conspecific stridulation sound would suggest that the common hypothesis that stridulation sound among catfish is an antipredator adaptation is not supported in all cases.

If the sound is indeed an antipredator adaptation, the question remains as to why *P. pardalis* (and other loricariid catfish) need so many antipredator adaptations. The development of armor, sharp spikes along the length of the body, a defensive posture, *and* predator-deterrent sound may appear excessive. If loricariids have so many other effective antipredator adaptations, why do they need yet another in the form of stridulation sound production?

CHAPTER 2: Methodology

Sound recording procedures. The main subjects of experiments to record distress calls in loricariids were 8 specimens of *Pterygoplichthys pardalis* (Siluriformes: Loricariidae), all of adult size (>11.5 cm standard length [SL] measured from the tip of the rostrum to the caudal peduncle), including 1 male and 7 females. Of these 3 (1 male, 2 female) were of an albino morph; the remaining 5 (all female) individuals were the standard dark brown coloration typical of the species.

To induce distress calls, individuals were captured in their home tank using a dip net and held manually by researchers to simulate predator capture. Individuals were held by the body between the pectoral and pelvic fins so as to allow free movement and posturing of all fins including the pectoral and dorsal fins (Fig. 1). Sound recordings were either made in the home tank or individuals were removed to a separate, smaller isolation tank for recording, the temperature and water condition of which was kept the same as the home tank.

Individuals were recorded underwater during distress calls using a hydrophone (Brüel & Kjær, model 8103), charge amplifier (Brüel & Kjær, model 2635), and band-pass filter (Krohn-Hite, model 3700). Signals were recorded using Spike2 recording software (Cambridge Electronic Design, Ltd., version 7.0.8). Recordings were made at 50,000 samples/second to ensure high-quality audio. The band-pass filter was set broadly (100-10,000 Hz) to filter out low-frequency noise at the low end of the frequency range, particularly 60 Hz electrical noise. The high end of the filter was set to capture possible frequencies that may exceed the hearing range of *P. pardalis*. Fundamental frequencies of *P. pardalis* sound lower than 100 Hz were able to be detected by examining the waveform trace (see details in *Sound analyses*) while low-frequency electrical noise was filtered out. At least five recordings were made of each individual so that sound quality could be compared within an individual over several time points.

For *A. cirrhosus, P. maccus,* and *Rhineloricaria sp.*, recordings were attempted to confirm the presence of sound production abilities in those species and the physical method of sound production by the pectoral fin spine (i.e. stridulation on adduction, abduction, or both) when applicable. Behaviors other than sound production displayed by these species during capture were noted.

Sound analyses. *P. pardalis* calls can be described as a series, or bout, of grunts. One grunt is the sound produced by one abduction of the pectoral fin spine. The intergrunt interval is the silence between the end of one grunt and the beginning of the next as the animal resets its fin position.

Analyses for primary frequencies (the most prominent frequency of the call, not including secondary frequencies of noise and reverberations) were made by examining a sample of five individual grunts within a single recording (Fig. 2). These groups of five grunts were chosen from the beginning of a bout of grunts, and consisted of the first five clear grunts (i.e. not overwhelmed by extraneous noise, such as splashes, hydrophone noise, or disturbance of tank contents) taken from a series of grunts in a recording. Though a bout of grunts can contain 60+ grunts, the first five grunts were used because hypothetically, these sounds would be the most likely to startle a predator during initial capture, and are therefore the most behaviorally relevant. Individual grunts were analyzed using a script for Matlab (MathWorks, version 2015a) to find major peaks in the audiogram of the grunt (Fig. 3). Each major peak represents one "click," and a series of these clicks produces the grunt-like stridulation sound. The minimum distance between peaks was set to 3 ms to avoid picking up noise and reverberations from the major peaks. Minimum prominence was adjusted for each recording depending on the volume of the recording. Prominence of peaks is higher in louder recordings than in quieter recordings, and therefore minimum prominence must be raised for louder recordings in order to avoid erroneously detecting peaks of noise as significant peaks. Primary frequency was determined for each grunt tested by determining the distance between each major peak (interpeak interval) and converting this distance to Hz ($\frac{1}{\text{average peak distance}}$).

The interpeak intervals of each sampled grunt in each recording were compared using a one-way ANOVA to determine if the mean interpeak interval (and therefore the mean frequency) of each grunt differed from the other grunts in the same recording. Analyses were then expanded to compare means among recordings taken from a single fish by comparing the total population of the interpeak intervals across all five sampled grunts in each recording to each other using a one-way ANOVA.

Data on temporal spacing of calls and call rate was gathered by examination of the first five clear grunts in a bout of grunts in each recording. The length of individual grunts and the length of silent time between each grunt (intergrunt interval) was measured, and the call rate (in grunts per minute) was calculated based on the average length of time from the beginning of one grunt to the beginning of the next grunt using the data for these first five grunts in each recording.

Data on possible muscle fatigue was collected by examining the length of the intergrunt interval between the first two grunts at the beginning of a series of grunts and the between the last two at the end of the same series. A *t*-test was carried out to determine if there was a significant difference in intergrunt interval between grunts at the beginning of a series versus at the end of the same series.

Behavioral response to sound. In order to examine the effect of stridulation sounds on conspecifics, 27 individuals of *P. pardalis* were placed individually in a shallow (~25 cm of water) round pool, 85 cm diameter, filled with water at the same temperature and pH as the home tank. An underwater speaker was placed on one side of the pool. Individuals were allowed 30 minutes to acclimate to the pool following transfer from the home tank to allow behavior to return to baseline levels.

Distress sounds previously recorded from *P. pardalis* for sound analysis were used in these playback experiments. A control sound was also created in order to ensure that any orientation to sound was due to the *P. pardalis* distress signal rather than to any sudden sound in the environment. The control sound was produced by rapping on the side of a fish tank at approximately the same rate as *P. pardalis* grunts. Such rapping on the glass of the tank shares some characteristics with *P. pardalis* distress sounds (similar frequencies and volume, though rate of sound pulses is approximately double in control sound versus *P. pardalis* sound; Fig. 4) but produces no behavioral changes in *P. pardalis* (personal observation).

Pre-recorded sounds from *P. pardalis* and control sounds were played to individuals in randomized sets of three (Table 1) and animals were observed for approach or avoidance behavior in response to each sound. Approach behavior was classified as any movement or orientation of the head towards the speaker following playback; avoidance behavior was classified as any movement or orientation away from the speaker. Non-reaction was classified as a lack of movement or orientation relative to the speaker. Five minutes were allowed between playing either distress or control sounds to allow the individual to return to baseline behavior state. Following the completion of playback experiments individuals were returned to their home tank.

Anatomy. Following sound recording experiments, individuals were euthanized by immersion in 2% MS-222 (tricaine methanesulfonate). Measurements of body weight, body length, head width and pectoral fin spine length were taken immediately post-mortem before any desiccation occurred. The sex of study animals was determined post mortem by dissection to determine presence of ovaries or testes. Following dissection, soft tissue was stripped from specimens by immersion of the specimen in 5% KOH. Bones were then rinsed with deionized water and air-dried prior to examination.

Bones from all species were subjected to preliminarily examination under a dissecting microscope. Measurements of the total length of the pectoral fin spine, the width of the pectoral fin spine at the “neck” (Fig. 11), and total length and width of the dorsal condyle were taken for all specimens (Table 6). The dorsal condyles of the pectoral fin spines for all species, as well as the spinal fossa of the cleithrum of *P. pardalis*, were imaged using a scanning electron microscope.

Ridge number was determined for each specimen of each species by examining the scanning electron micrographs. For specimens of *P. pardalis*, the interridge distance was calculated using the known measurement of dorsal condyle width and the pixel distance between the leading edge of each ridge to the leading edge of the next adjacent ridge. For *A. cirrhosus*, *P. maccus,* and *Rhineloricaria*, scanning electron micrographs were taken to determine overall similarity of the structure of the dorsal condyle. For these specimens, ridge number was counted, but interridge distance was not determined. The angular velocity of the dorsal condyle as it moves against the curved spinal fossa can be determined by determining the distance traveled (number of clicks times the average interridge distance of the individual in question) and dividing it by the total time of the grunt.

Two specimens of *P. pardalis* were preserved in 70% ETOH and subsequently dissected to examine the muscular attachments to the pectoral fin spine and associated surfaces of the pectoral girdle.

CHAPTER 3: Results

Sound producing behavior. *P. pardalis* produced stridulation sound in response to capture stress, triggered by the manipulation of the individual's torso by the researcher. Stridulation sound was not triggered by the dip net used to collect individuals in their home tank, suggesting that pressure on the body of the animal is one releasing factor for the sound-producing behavior. Most individuals began stridulation immediately upon manual capture, but some individuals required some manipulation before sound production was triggered. Other releasing factors likely to induce sound production in an individual that is already manually restrained include shaking (conducted by the researcher gently moving the individual either up and down or side to side in the water) or manipulation of the individual's tail.

Pterygoplichthys pardalis produces stridulation sound on abduction of the pectoral fin spine, and never on adduction. Individuals of *P. pardalis* produce stridulation sound only when taking their defensive posture (Fig. 5) – that is: if the dorsal and pelvic fins are not locked in their abducted position during manual restraint, the individual will be silent. It is likely that if this defensive posture has not been triggered, then the animal has not been exposed to sufficient stimulus to release the sound-producing behavior. Sound production is almost always accompanied by thrashing behavior, unless the individual is sufficiently restrained to prevent thrashing.

Pterygoplichthys pardalis produced stridulation sound when submerged and would continue to produce this sound when temporarily removed from the water. There appeared to be no effects on sound production (i.e. call rate, direction of fin movement) regardless of the individual's position in or out of water.

The other sampled loricariid species displayed behaviors that varied somewhat from the behaviors of *P. pardalis*. *Ancistrus cirrhosus* was not observed to produce sound when exposed to capture stress. Instead of displaying defensive fin-spreading behavior, *A. cirrhosus* instead displayed "pinching" behavior – the pectoral fins were adducted and slightly elevated, attempting to pinch the fingers of the researcher with the pectoral fins where the animal was being held posterior to the pectoral fin's joint with the pelvic girdle. *Rhineloricaria* sp. likewise were not observed to produce sound, but displayed fin-spreading behavior similar to *P. pardalis* instead of pinching behavior similar to *A. cirrhosus*.

Panaque maccus displayed similar behaviors to *P. pardalis*. Sound was produced by abduction of the fins, and thrashing behavior and attempts to escape always accompanied sound production. Sound produced by *P. maccus* was clearly audible and palpable despite the small size (3 – 5 cm) of the individuals tested relative to the body size of the primary specimens of *P. pardalis* (>11 cm).

Sound qualities. *Pterygoplichthys pardalis* stridulation sound consists primarily of frequencies between 50-500 Hz. The primary frequency of *P. pardalis* stridulation sound among the individuals sampled averages 121.87±34.46 Hz (Table 2).

There is a negative relationship between frequency and four measures of body size (body weight, standard length, head width, and pectoral fin spine length; Fig. 6). The frequency a fish produces appears to lower as the fish grows over time: a fish with a pectoral fin spine length of 3.7 cm may produce grunts around 320 Hz, and that same fish, six months later, produces frequencies closer to 160 Hz as its fin spine has grown to 4.1 cm.

The majority of recordings showed no significant differences between the five grunts, though differences were found in some recordings (summary: Table 3). Where significant differences existed, these differences were primarily driven by single grunts within the experimental sample with a different distribution of interpeak intervals (Fig. 7). However, despite the majority of the samples tested showing no significant differences among initial grunts, it cannot be said that every grunt produced by *P. pardalis* is identical to the others. Grunt frequency and length are slightly variable even from one grunt to the next, though they tend to remain within 30 Hz of the average for any particular recording (Fig. 8).

Significant differences also were found among calls of the same fish recorded on different days for half of the specimens tested (Table 4). This also suggests that *P. pardalis* are not completely consistent in frequency from one call to the next, and do not produce exactly the same call in all instances.

Call rate is determined by the length of the grunts ($\overline{X}$ = .22±.10 seconds) and intergrunt silences ($\overline{X}$ = .66±1.67 s); call rate averages 90 grunts/min^{-1} in *P. pardalis* (Fig. 9, Table 5). Call rate can vary with variation of either of these two measures and, as either grunt length or intergrunt interval increases, call rate will decrease (Fig. 10). However, differences in call rate are primarily driven by differences in intergrunt interval length (r^2= .92, $P < .00001$). Intergrunt interval length is highly variable, and ranges between .127 s and 6.59 s ($\overline{X} = .66$ s, $\sigma = 1.67$ s) . Grunt length is less variable than intergrunt length (ranging between .053 - .434 s, $\overline{X} = .22$ s, $\sigma = .1$ s), and does not drive call rate as heavily, but is nevertheless significant (r^2= .07, $p = .013$).

Call rate also appears to be affected by some level of muscular fatigue. A bout of grunts may contain as few as five grunts or over 60, as grunts typically continue until the animal escapes the hold of the researcher. Intergrunt intervals were significantly shorter at the beginning of a series of grunts than at the end of the same series ($t(164) = 8.01$, $p = .0001$). This suggests that the muscles that drive sound production are tiring, and the animal decreases call rate by increasing intergrunt interval to allow muscles more rest between grunts.

Behavioral response. The majority of playback sounds (76 of 81 sounds), either stridulation or control, elicited no behavioral response in the population of *P. pardalis* tested. The remaining five sounds were split nearly evenly between eliciting positive (2 responses) and negative (3 responses) behavioral orientation. There was no significant difference ($t_{82} = .49$, $P = .63$) in response by *P. pardalis* to conspecific stridulation sound compared to response to control sound. Given this wide disparity between non-response and response of any kind, we can assume that *P. pardalis* either cannot hear sounds at this frequency (though loricariids are ostariophysan fishes whose hearing is primarily low frequency, and stridulation sound is low frequency) or is not attending to sound in the frequency range of the stridulation and control sounds. If these signals are being received, they do not trigger a notable behavioral response in *P. pardalis*.

Bone morphology and pectoral fin spine function. The loricariid species surveyed in this study all possess similar (but not identical) morphology in the pectoral fin spine. In all cases, the fin spine is an enlarged, slightly curved fin ray, with a wider proximal end housing a roughly crescent-shaped dorsal condyle (Fig. 11). This crescent-shaped condyle has many small ridges on the surface of the condyle that meets the spinal fossa of the cleithrum (Fig. 12).

Measurements of both pectoral fin spines of each individual of *P. pardalis* were taken following cleaning of the bones. Measurements (Table 6) were taken of the length of the dorsal condyle (from end to end) and the width of the condyle (from the medial edge to the lateral edge of the condyle), as well as of the total length of the pectoral fin spine and the diameter of the neck of the pectoral fin spine (Fig. 11).

Pterygoplichthys pardalis have well-defined ridges on the dorsal condyle ($\overline{X}$ = 35.5, σ = 3.74, 30 – 41 ridges), oriented approximately vertically. These ridges are raised, with distinct valleys, and come to a point (Fig. 12). Ridges are generally complete, meaning they span the full width of the dorsal condyle. However, some ridges do not span the full width of the condyle, and such ridges were only counted as a full ridge if they spanned over half the width of the condyle. Some ridges appeared “broken,” with multiple interspersed projections forming a “chain” similar in shape and orientation to a ridge (Fig. 13). These broken ridges were counted as a ridge only if they form a distinct singular chain across the full width of the condyle. The ridges are an average of 179 microns apart, as measured from the leading edge of each ridge; there is some individual variation in the average ridge distance among individuals (Table 7), and variation within a single condyle, with interridge distances ranging from 81 to 423 microns. Condyle size slightly affects ridge number – larger condyles tend to have a few more ridges than smaller condyles, but no significant differences ($y = 1.4472x + 25.444$, $r^2 = 0.30$) are present (Fig. 14). Therefore, individuals are not typically gaining ridges as the dorsal condyle grows in size.

Of the four loricariid species surveyed, all four had ridges on their dorsal condyle. However, there are morphological differences in the shape of these ridges among species (Fig. 15). *Panaque maccus* appears most similar to *P. pardalis*. The ridges in this species are sharp and well-defined, as they are in *P. pardalis*, and this species has a similar number of ridges, ~30, as does *P. pardalis*. In *Rhineloricaria*, the ridges appear more rounded and the condyle has fewer ridges (~19) than *P. pardalis*. In *A. cirrhosus*, the ridges are flattened compared to *P. pardalis*; though it possesses a similar number of ridges to *P. pardalis* (~33 ridges), the ridges of *A. cirrhosus* are broader and flatter than the other species surveyed, and the valleys between each ridge are shallower. *Ancistus cirrhosus* also has a condyle distinctly different than that of *P. pardalis*: while the other species have a roughly symmetrical crescent-shaped condyle, that of *A. cirrhosus* is wider in the anterior half of the condyle (.88 mm at widest point). than in the posterior half (.57 mm at widest point).

During stridulation sound, the number of peaks in any given sound is normally less than the number of ridges on the dorsal condyle (Table 8). This suggests that *P. pardalis* does not use the full range of motion of its dorsal condyle during sound production, but instead only a part, and only the ridges in the part of the dorsal condyle being moved against the spinal fossa of the cleithrum are producing pulses in the overall grunt.

Interridge distance in *P. pardalis* increases with body size, pectoral fin spine length, and length of the arc of the dorsal condyle (Fig. 16). Therefore, as *P. pardalis* grows and the dorsal condyle of the pectoral fin spine grows, the peaks of each ridge grow farther apart. As interridge distance increases with the body size of the animal, the angular velocity of the fin spine moving in the spinal fossa of the cleithrum remains relatively constant (an average of 23.81±7.95 mm/s; Table 9) across all sampled specimens of *P. pardalis.* As interridge distance increases and velocity remains constant, grunt length increases (Fig. 17). This means that in a larger dorsal condyle it takes longer to move the dorsal condyle through its range of motion (e.g. past a similar number of condylar ridges) at the same rate, thus producing a sound that lasts longer (Fig. 18). Frequency also decreases as interridge distance increases (Fig. 19), as a function of increasing time between each ridge and therefore each intensity peak in the grunt.

Musculature. Upon dissection, and referencing muscles defined in Diogo et al. (2001) in other species of catfish with a similar body plan, it was found that a group of four muscles acts on the pectoral fin of *P. pardalis* (Table 10). The primary muscles acting on the pectoral fin spine itself, and therefore the muscles most likely to be involved in sound production, are the two divisions (ventral and dorsal) of the *M. arrector dorsalis*, and the

M. abductor profundis and *M. adductor superficialis* (Figs. 20, 21). The ventral division of the *M. arrector dorsalis* is responsible for pulling the fin spine ventrally by its attachment at the inferior edge of the neck of the pectoral fin spine. This also has the effect of raising the dorsal condyle of the pectoral fin spine slightly towards the rough spinal fossa of the cleithrum above the dorsal condyle of the pectoral fin spine. The dorsal division of the *M. arrector dorsalis* is involved in the abduction of the pectoral fin spine, as well as drawing the medial end of the pectoral fin spine closer to the midline of the animal. The *M. abductor profundis,* despite its name, is primarily responsible for adduction of the pectoral fin spine. This muscle also raises the fin spine dorsally. The *M. adductor superficialis* serves to rotate the pectoral fin spine in the anterior direction, though its action on the secondary fin rays lifts these bones dorsally. Lastly, though the *M. adductor superficialis* has no insertion on the pectoral fin spine, this muscle is used to depress the secondary fin spines, which may have a small effect in rotating the pectoral fin spine caudally if the membranes between the fin rays cause any tension where they attach at the posterior border of the pectoral fin spine.

During sound production, a pair of antagonistic muscles may potentially be used simultaneously: in this case, it is possible that the dorsal division of the *M. arrector dorsalis* is being used to abduct the fin spine as the *M. abductor profundis* lifts the dorsal condyle of the fin spine dorsally, despite the other action of the *M. abductor profundis* serving to adduct the fin spine. The *M. adductor superficialis* may also play a small role in sound production by rotating the dorsal condyle forward as it is abducted and held against the spinal fossa of the cleithrum.

CHAPTER 4: Discussion

The leopard pleco, *Pterygoplichthys pardalis,* produces upon capture a robust stridulation sound using its pectoral fin spine. This sound is low-frequency and reasonably regular, both in its frequency (~120 Hz) and in its temporal spacing (89.6 grunts/minute). As there are fewer clicks in a grunt than there are ridges on the dorsal condyle of the pectoral fin spine, and as *P. pardalis* does not use the full range of motion of the pectoral fin's joint for sound production, we can determine that each click in the overall grunt is made by one ridge of the dorsal condyle as it comes in contact with the spinal fossa.

The morphology of the dorsal condyle does not suggest the direction of movement required for sound production independently of behavioral observation: that is, it is not possible to tell by examining the bone features alone in what direction the animal moves its pectoral fin spine to create sound. The ridges on the dorsal condyle are not visibly oriented to suggest a direction – we might expect an animal that stridulates on abduction to have ridges which lean towards the anterior end of the condyle, as it might be advantageous to have a leading edge of an obliquely-oriented ridge come into contact with the spinal fossa of the cleithrum on abduction of the fin spine. Instead, these ridges are oriented roughly vertically in both sound-producing species examined, suggesting that stridulation could be accomplished equally well on either adduction or abduction of the fin spine if this behavior was determined solely by bone morphology. We can conclude that behavior (specifically muscular action) is responsible for the direction of spine movement which produces sound and not any directionality inherent in the bone itself, though the specialized ridges are required to be present and prominent for any sound production to occur.

Individuals of *P. pardalis* are not completely consistent in the stridulation sound they produce. Primary frequency tended to vary by a comparatively wide margin – though a small difference on the total scale of possible frequencies, a standard deviation of ~34 Hz from the mean of ~121 Hz still constitutes a difference of ¼ of the mean. Stridulation sound also decreases in primary frequency over time as the animal grows, and may vary from grunt to grunt within a single bout of grunts. This suggests that the stridulation sound produced by *P. pardalis* (and other loricariid catfish species) is not highly specialized, and therefore the interpulse interval of the grunts does not need to fit into a very narrow range. If it were important to maintain a constant primary frequency of ~121 Hz (the average primary frequency of the individuals sampled) as a communication signal, we would expect tighter control by the individual, and a primary frequency that does not vary so widely. *Pterygoplichthys pardalis* stridulation sound does not contain a highly specific and highly conserved primary frequency that may be targeting a specific predator with hearing attuned to such a sound. Rather, designed more to startle with a loud, disruptive noise than meant to communicate a complex signal to would-be predators. The primary frequency of stridulation sound is not held constant over the life history of an animal. Smaller fish make higher frequency sounds by virtue of their smaller anatomy. The dorsal condyle of smaller fish have ridges that are spaced more closely together and therefore will produce a higher frequency if the rate of movement of the condyle is kept constant. The angular velocity of the movement of the dorsal condyle remains relatively constant across the life history of the animal and this causes a reduction in frequency as the dorsal condyle ridges grow farther apart. In order for the velocity of the dorsal condyle to remain the same, muscle mass must be growing to maintain this velocity as the bone grows in size and weight. There does not seem to be a morphological or behavioral attempt by muscular modulation to keep the frequency of calls constant as the animal grows, only a proportional growth by the muscles to maintain a constant velocity. Therefore, the primary frequency of the stridulation call is likely not the most relevant factor in the effectiveness of the call produced by *P. pardalis*, as it is so highly variable from instance to instance and across the life history of an individual.

Although some catfish use stridulation sound as part of intraspecific mating displays and territorial interactions (Kaatz, 1999), *P. pardalis* exhibited no reliable behavioral response to conspecific stridulation sound. Sound production using the pectoral fin is always exhibited in capture conditions and accompanied by fin-spreading behavior in the dorsal and pelvic fins, an adaptation meant to reduce the ability of predators to potentially swallow an individual. Though ostariophysan fishes like loricariids are capable of hearing frequencies as low as 100 Hz, it is unlikely that *P. pardalis* is using stridulation sound to communicate with conspecifics, as stridulation sound elicited no behavioral changes in *P. pardalis*. This suggests that individuals of *P. pardalis* are not attending to this sound or that they have no reason to attend to this sound, and we would expect to see such a condition if attending to this sound by conspecifics confers no benefits (reproductive or otherwise) to the receiver. It is also possible that *P. pardalis* lacks hearing in the range of stridulation sound of its species: no studies have been conducted on the hearing range of this particular species. It can be inferred that stridulation sound is another part of this species' anti-predator defense system.

There remains a possibility that sound is not the primary signal being sent during stridulation. Many organisms including crustaceans, insects such as crickets, ants, and scorpions, mammals such as mole rats and elephants, and many species of lizards, snakes, frogs, and salamanders use vibrotactile signals to communicate (Hill, 2008), and vibration can be palpably felt in individuals of both *P. pardalis* and *P. maccus* as these animals stridulate. Sound is often produced as a consequence of vibrotactile communication, due to the vibrations of the substrate or structure producing vibrations in the air, water, or bone with which they come into contact. If indeed it is the vibrotactile signal which is most significant, this may explain in part why *P. pardalis* does not respond to sound generated by conspecifics: they cannot feel the signal as they could if they were the predator which has captured an individual of *P. pardalis*, and can only hear the resulting auditory signal produced by the vibrations of the water, and *P. pardalis* may never have developed a response to this auditory signal as they may have to the main vibrotactile signal.

If we accept the idea that stridulation is being used primarily (if not singularly) for predator defense in *P. pardalis* and not as a territorial or mating signal to conspecifics, the question remains as to why *P. pardalis* engages in stridulation at all, considering its multitude of other antipredator adaptations. Sound production behavior and the morphology of the fin spine necessary for sound production are present in 17 of the 31 families in the order Siluridae. Some species of loricariids have both the behavior and the morphology required for sound production – others only retain one or the other. Species such as *P. pardalis* and *P. maccus* show sound-producing behaviors even with their bony armor and sharp spines on their skin. Other representatives of the family Loricariidae did not retain sound-producing behavior (as in the case of *Rhineloricaria sp*., which display fin-locking but no sound production) or sound producing morphology (as in the case of *A. cirrhosus*, which performs a pinching motion instead of stridulatory motion or fin-locking behavior, and appears to have reduced ridges on the fin spine). In unarmored families (all those except Loricariidae and Callichthyidae), stridulation sound may serve as a more important antipredator defense than it does in loricariids, in which the bony subdermal armor is likely the most effective defense, though stridulation sound may have been retained as a behavior in some species of loricariids due to an effectiveness against predators that the bony armor does not confer an advantage against, due to a lack of selection against sound-producing behavior and morphology.

It is likely that the primary targets of sound-producing behavior are animals that may be attempting to swallow *P. pardalis* because stridulation sound and fin-spreading behavior is triggered by pressure on the body of the animal, as might be experienced when the fish is captured in the mouth of a larger predator and during an attempt by the predator to chew or swallow *P. pardalis*. Predators which target fish and share the same range as the native range of *P. pardalis* may include larger carnivorous fish, large wading birds, crocodilians, constricting snakes such as boas, and giant otters. If indeed the primary signal encoded by stridulation behavior is vibrotactile, this may be a very startling and aversive signal to a potential predator, as this vibrotactile stimulus may induce the perception of a very loud sound as this stimulus vibrates the bones of the jaw and skull as the predator manipulates a stridulating individual in its mouth. Target predators may not even need hearing in the specific range of *P. pardalis* stridulation sound, as the sound may even be a side effect of producing an aversive vibrotactile stimulus in the mouth of a would-be predator and not the primary communicative signal.

Examining potential predators of *P. pardalis* for their response to stridulation sound and vibrotactile stimulus from *P. pardalis* would provide a more complete picture of the usefulness of this behavior as a predator deterrent. Determining the reaction of potential predators to pre-recorded stridulation sound and to sounds and vibrations produced by live, captured *P. pardalis* would greatly further our knowledge of the purpose of stridulation sound as it is present in the family Loricariidae. If there are predators present in the environment of Neotropical loricariids like *P. pardalis* that in fact are deterred by the stridulation sounds of these catfish, then a compelling argument can be made for the preservation of this behavior in the lineage despite the presence of other antipredator adaptations, which may target different predators than does stridulation sound.

FIGURES

Figure 1. Manual restraint of *P. pardalis* as held during experiments. Individuals are held at the torso between the pelvic and pectoral fins, taking care not to pin the membranes of the pectoral fins to the body. This individual held out of water temporarily for illustrative purposes; recordings are taken from individuals while submerged.

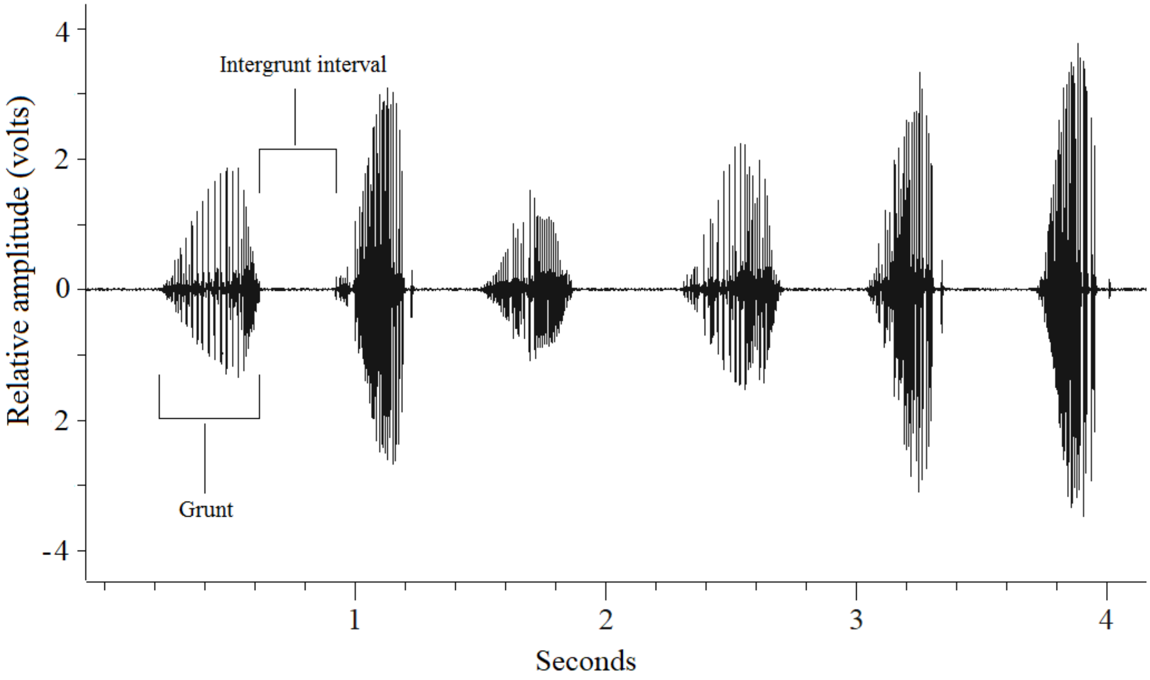

Figure 2. Audiogram of grunts recorded from *P. pardalis*. This represents part of a bout or series of grunts. Six grunts are present on this audiogram, though in the lab a bout can contain 50+ grunts before the animal tires or escapes from the hold of the researcher.

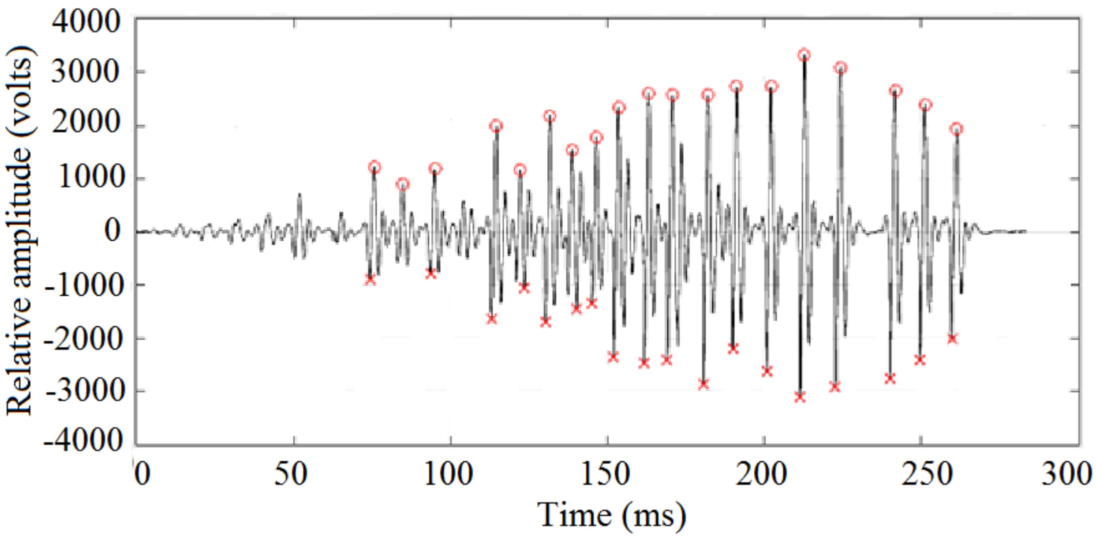

Figure 3. Audiogram of a single grunt. Each peak marked with an "○" is the major peak of one click. The distance between these peaks is used to determine the overall primary frequency of each grunt. The smaller peaks between the major peaks are typically reverberations of the previous click.

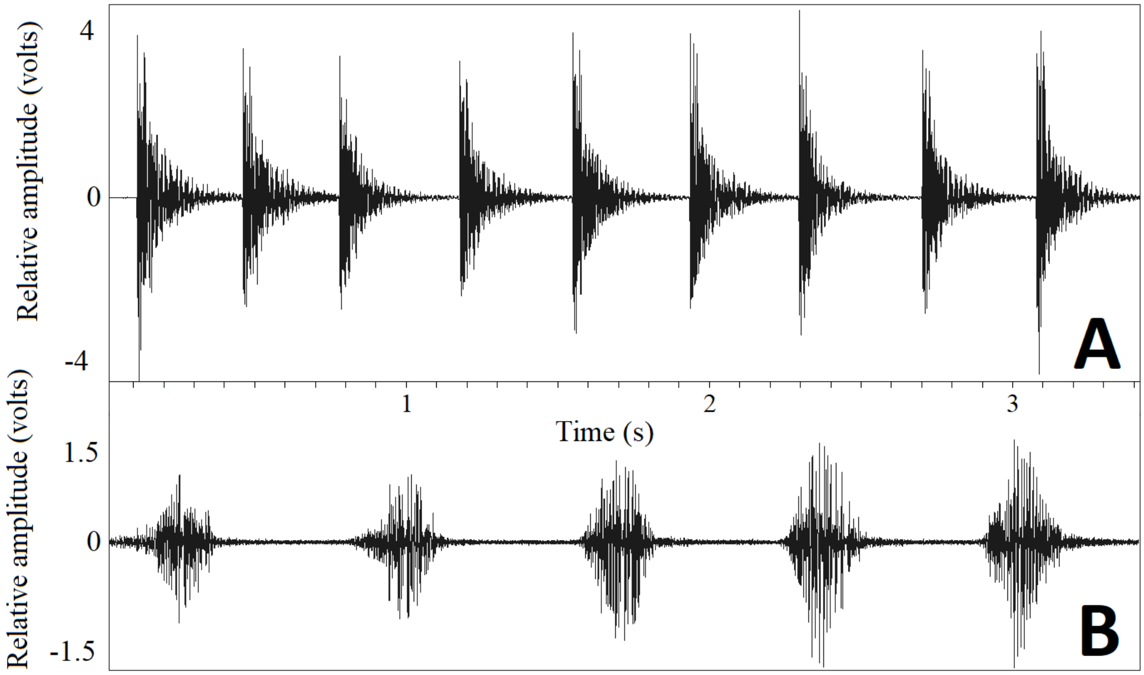

Figure 4. Sample audiogram of control sound (A) and conspecific stridulation distress sound (B) over time, as played to individuals of *P. pardalis* to test behavioral response to sound.

Figure 5. Defensive fin spreading posture. Dorsal, pelvic, and pectoral fins are locked in an abducted position. The fin spines resist manual adduction by outside forces.

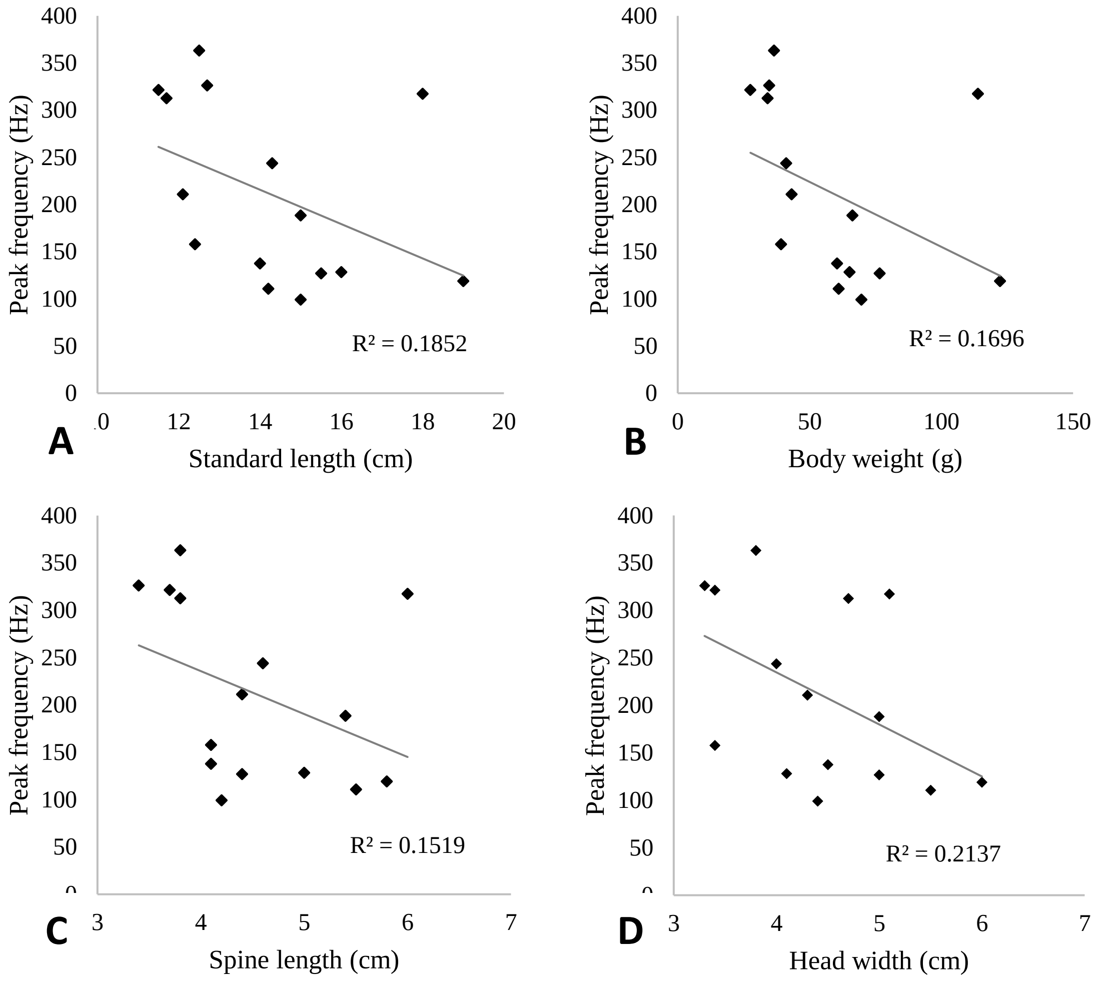

Figure 6. Peak frequency as it varies by standard length (A), total body weight (B), by length of the pectoral fin spine (C), and width of the head measured at the widest point (D). Peak frequencies were taken from the recordings of each fish taken closest in time to the time of body measurement.

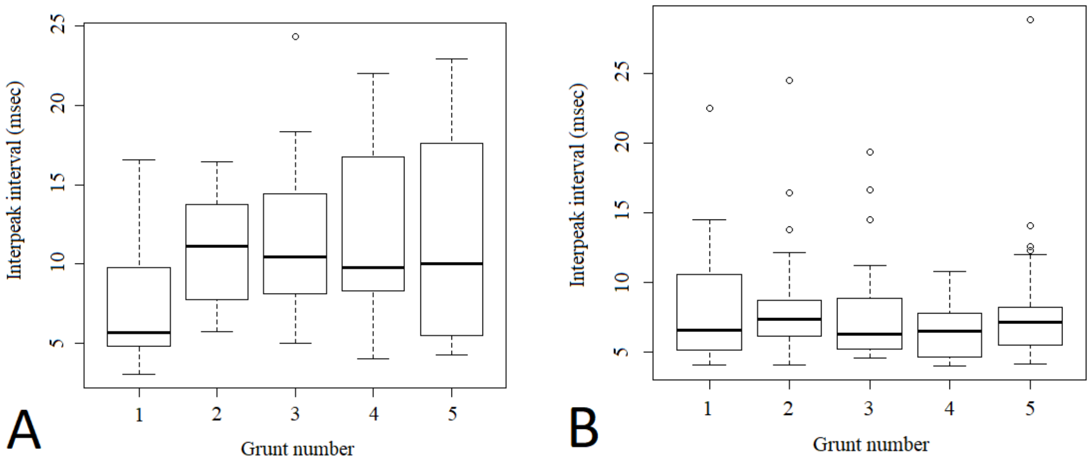

Figure 7. Distribution of means of (A) one set of significantly different interpeak interval means (p = .015) and (B) one set of non-different interpeak interval means (p = .798). Distributions are taken from the same individual of *P. pardalis* recorded on different days.

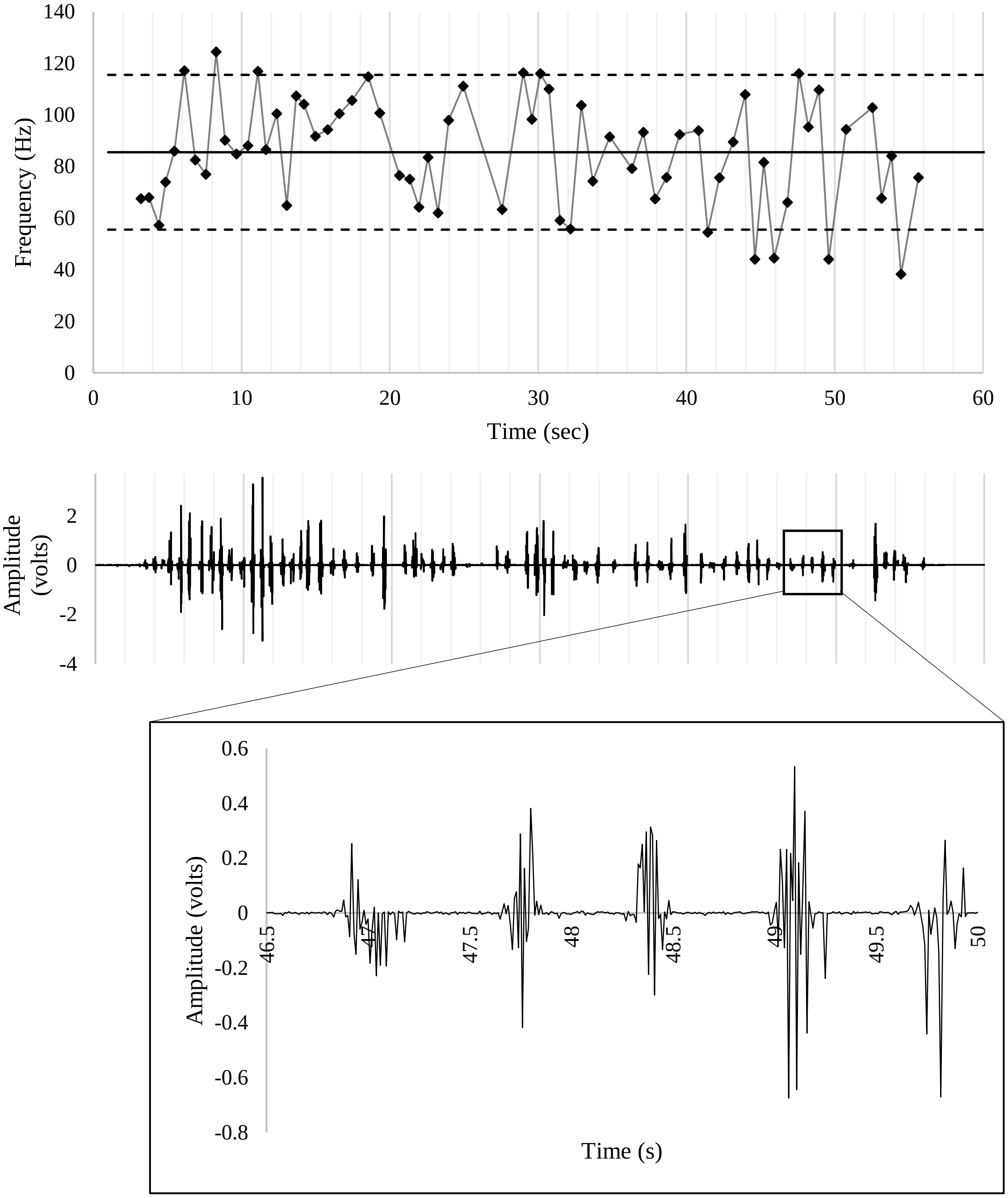

Figure 8. Average frequency per grunt (top) with waveform trace of recording of a bout of grunts (bottom). In the top graph, the solid black horizontal line represents the average frequency (85.51 Hz) across all represented grunts; the dashed lines represent the average ± 30 Hz. The selected bout of grunts contains 65 grunts in approximately 50 seconds.

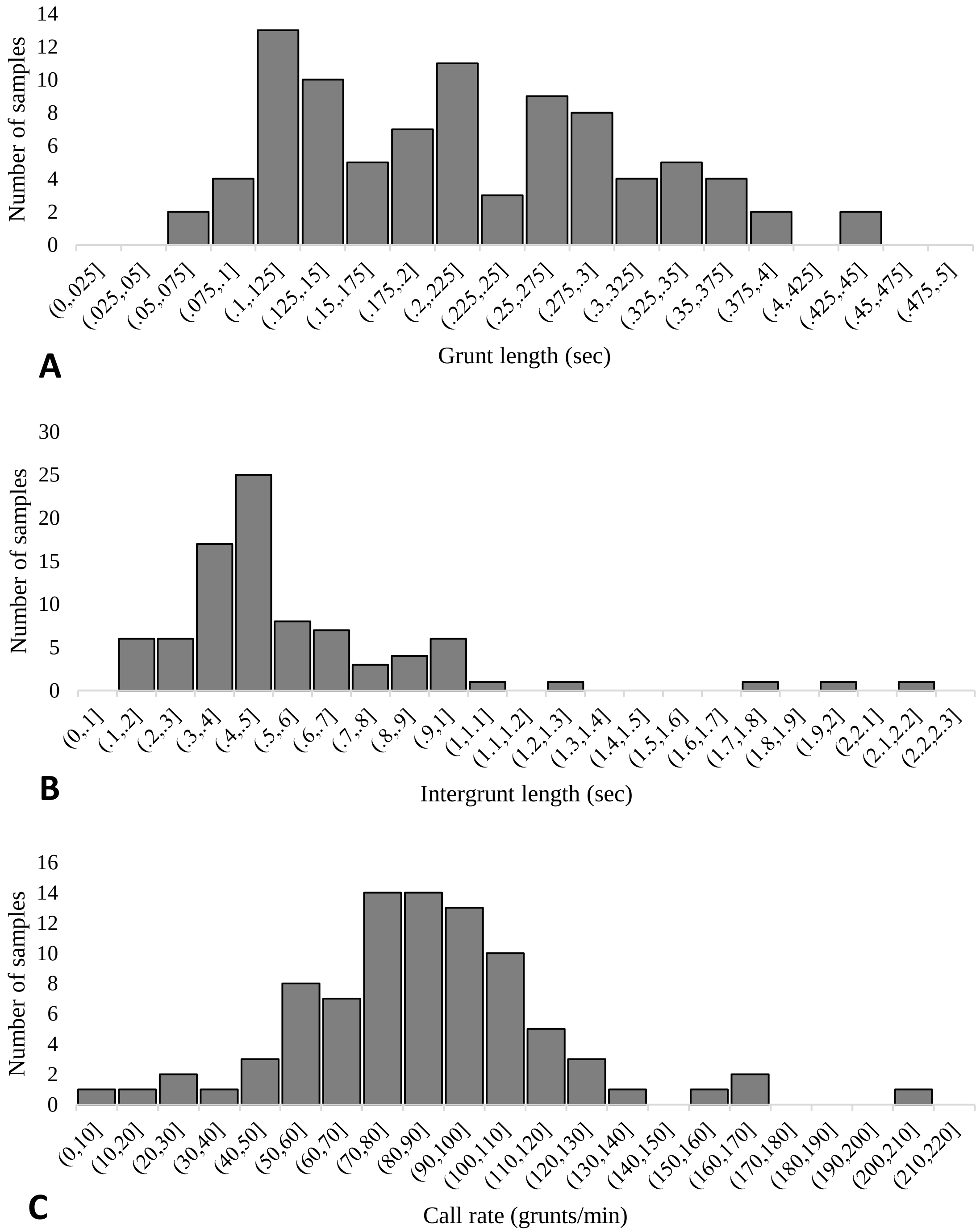

Figure 9. Distributions of average grunt length (A), average intergrunt length (B), and average call rate (C). n = 88 recordings.

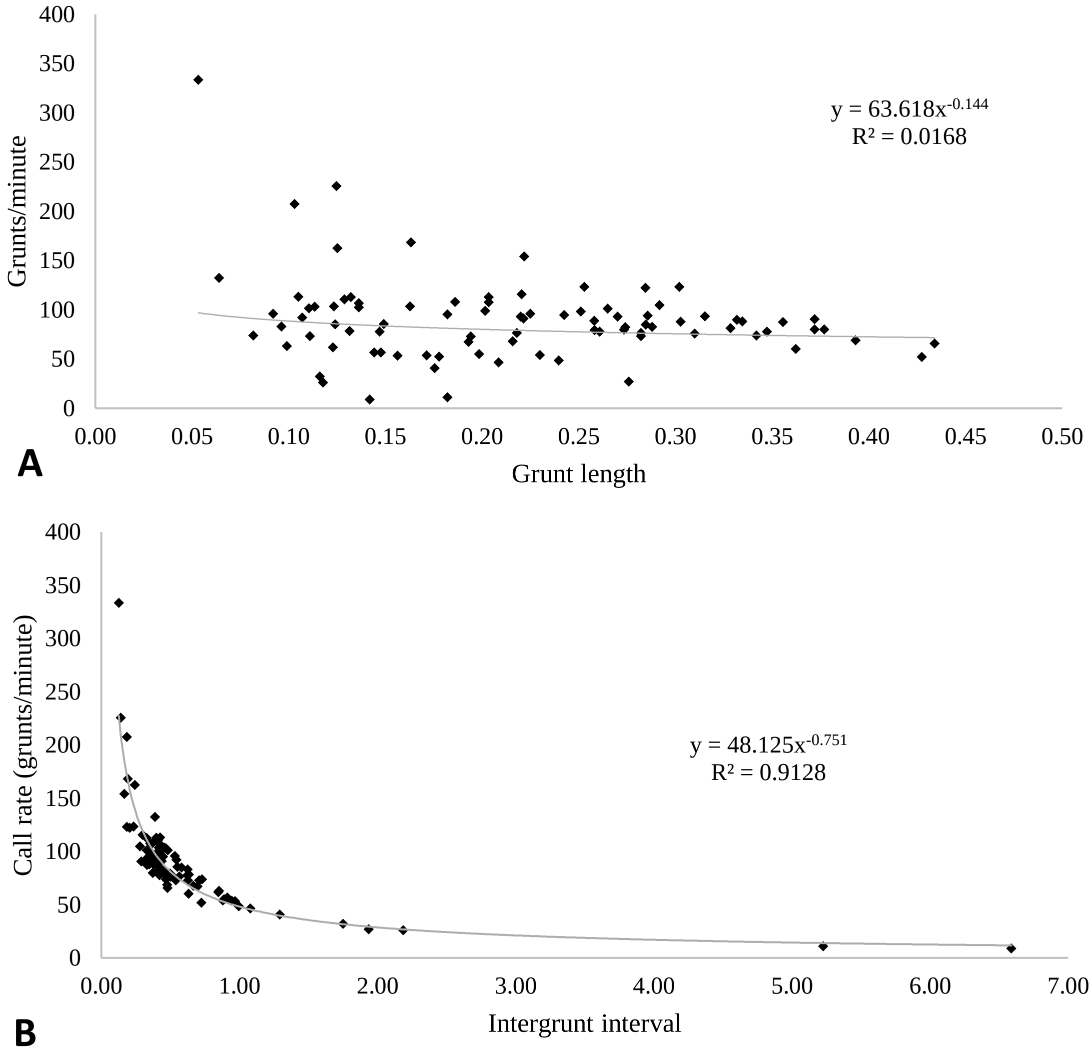

Figure 10. Relationship between call rate and its components – grunt length (A) and intergrunt interval (B). Call rate is more strongly related to intergrunt interval than grunt length. Intergrunt interval is more highly variable and has a wider range, and therefore affects call rate more heavily than grunt length.

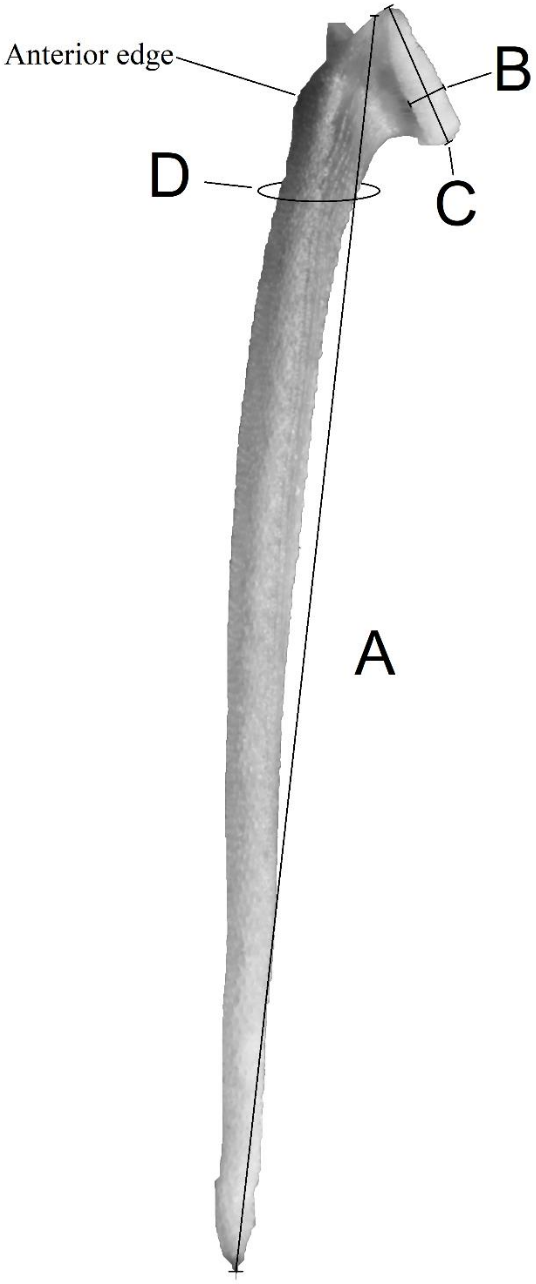

Figure 11. Pectoral fin spine of *P. pardalis* showing various features as measured. Measurements summarized in Table 3. A: Total length, B: condyle width, C: condyle length, D: neck of pectoral fin spine.

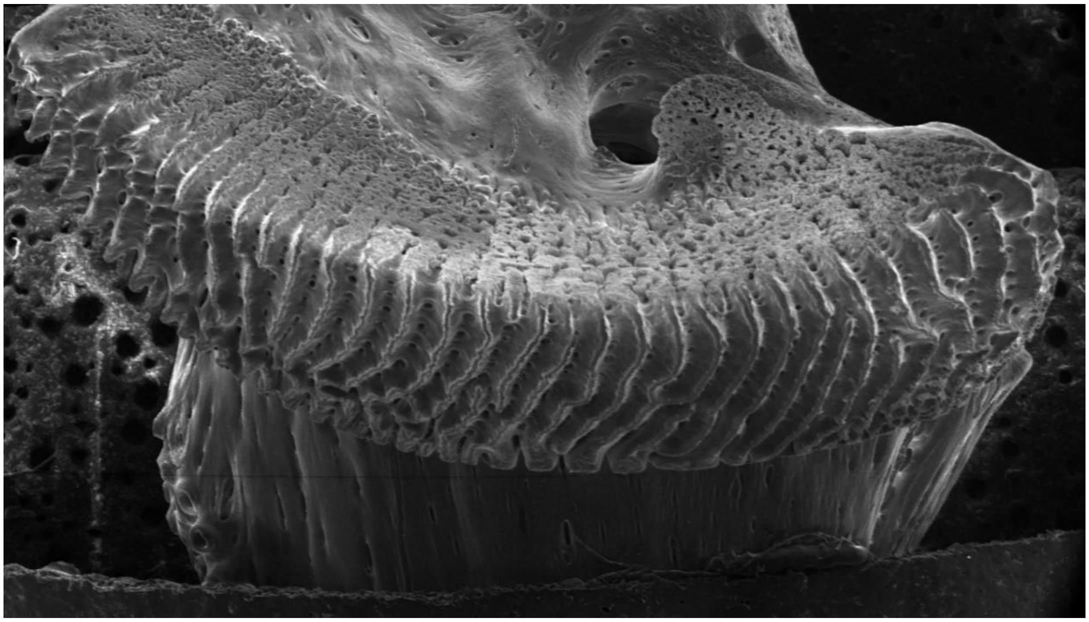

Figure 12. Scanning electron micrograph of dorsal condyle of pectoral fin spine of *P. pardalis*. This specimen shows typical shape and arrangement of ridges, with most ridges spanning the full width of the condyle.

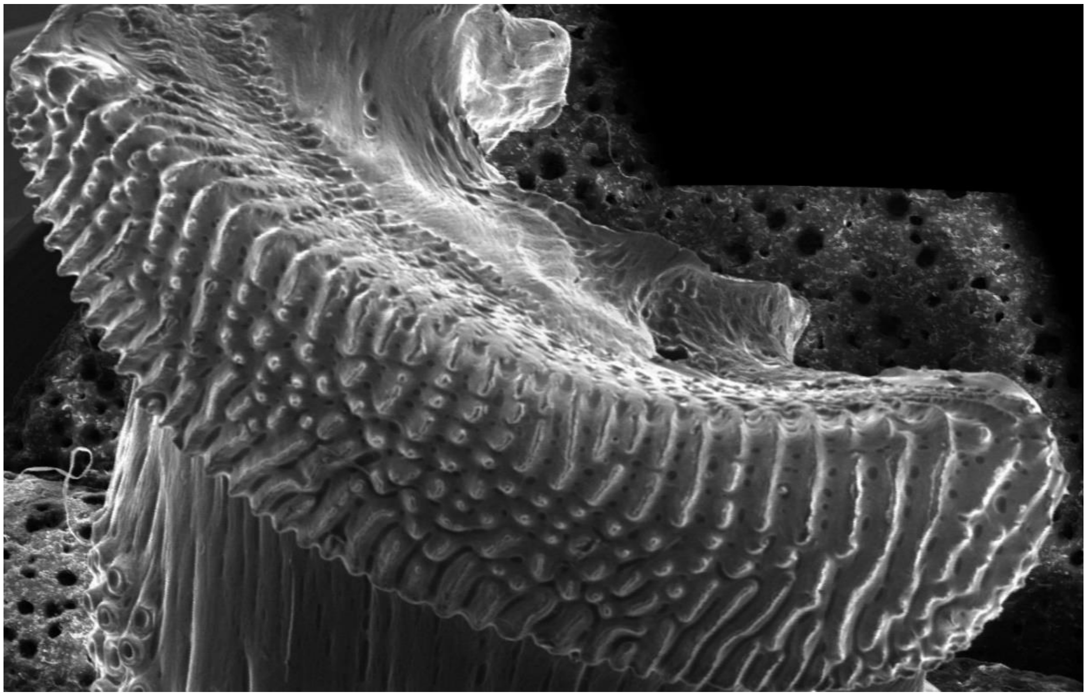

Figure 13. Scanning electron micrograph of the dorsal condyle of *P. pardalis* specimen 8. This specimen shows more “broken” ridges than is typical; however, these “broken” ridges often form a clear ridge-like pattern rather than showing a random distribution, and each of these clear rows of projections is counted as a ridge.

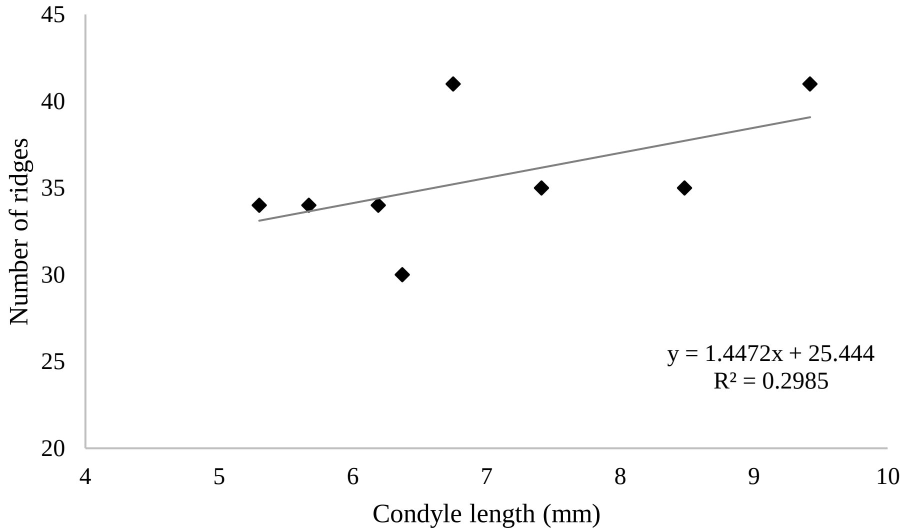

Figure 14. Number of ridges versus the size of the dorsal condyle in *P. pardalis*, as represented by total condyle length.

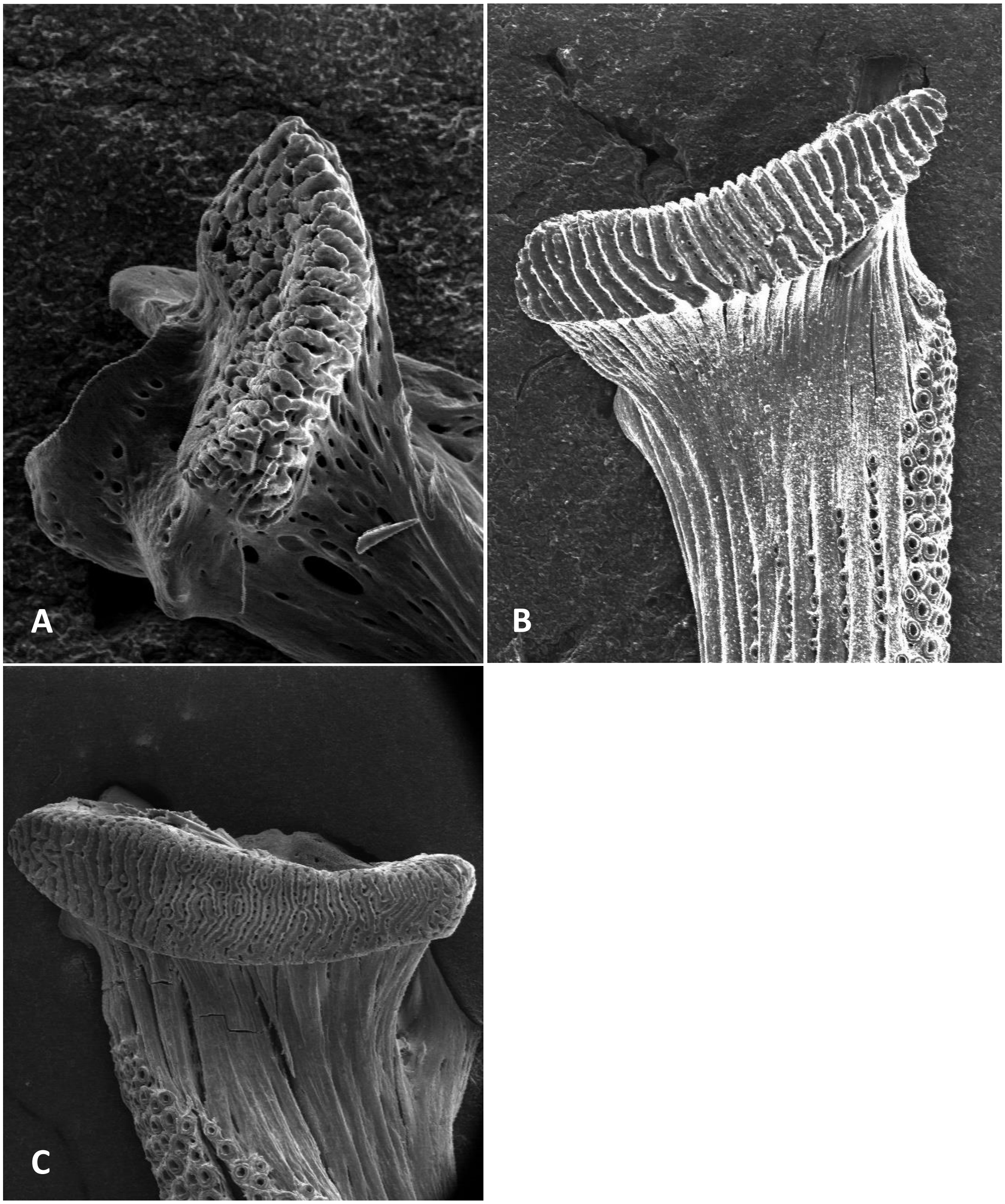

Figure 15. Scanning electron micrographs of dorsal condyles of pectoral fin spines of *Rhineloricaria* sp. (subfamily Loricariinae) (A), *Panaque maccus* (subfamily Hypostominae) (B), and *Ancistrus cirrhosus* (subfamily Hypostominae) (C).

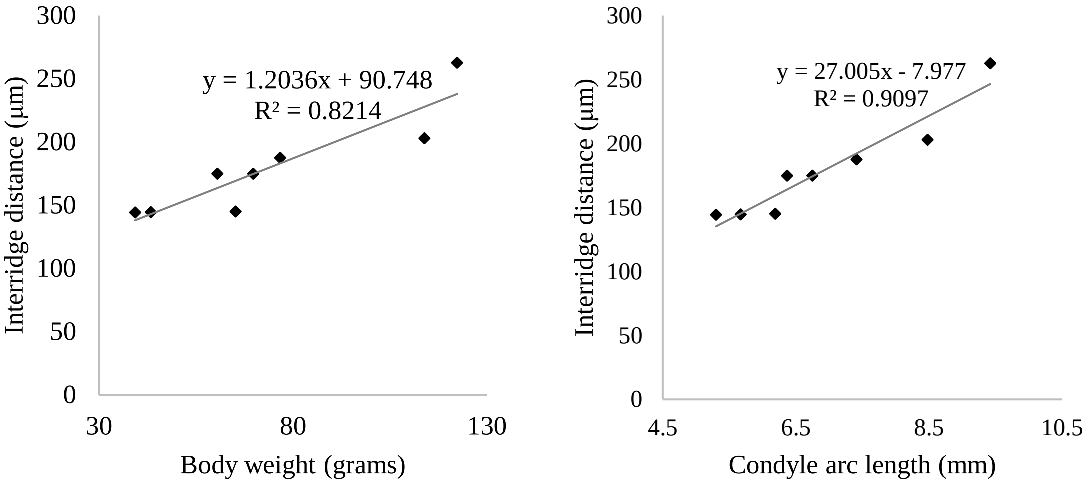

Figure 16. Relationship of interridge distance of ridges of the dorsal condyle of the pectoral fin spine to measures of body size and condyle size.

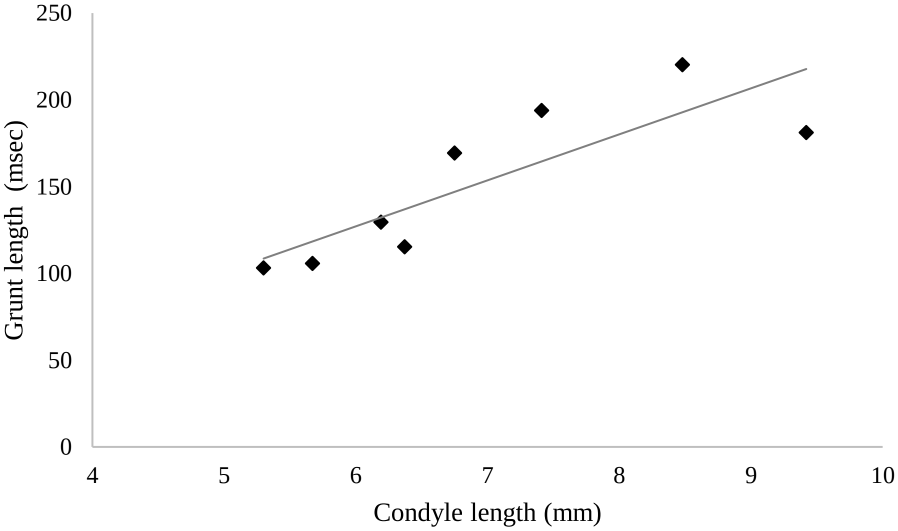

Figure 17. Size of the curvature of the dorsal condyle versus grunt length. As dorsal condyle length increases, the time of the grunt increases. Assuming a constant velocity, it takes longer to move a larger dorsal condyle through its range of motion.

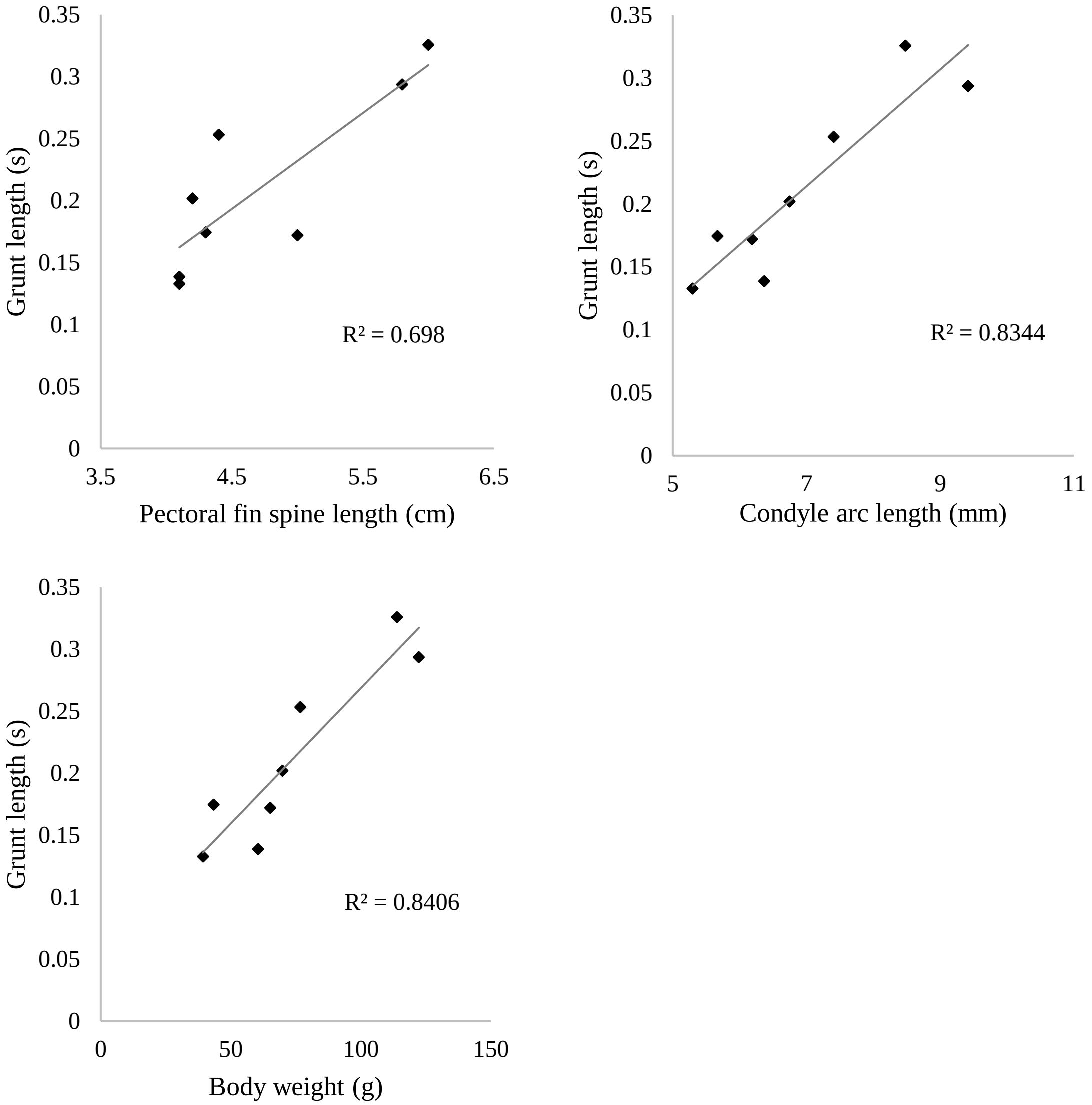

Figure 18. Relationship between grunt length and body size, and grunt length and pectoral fin spine dimensions.

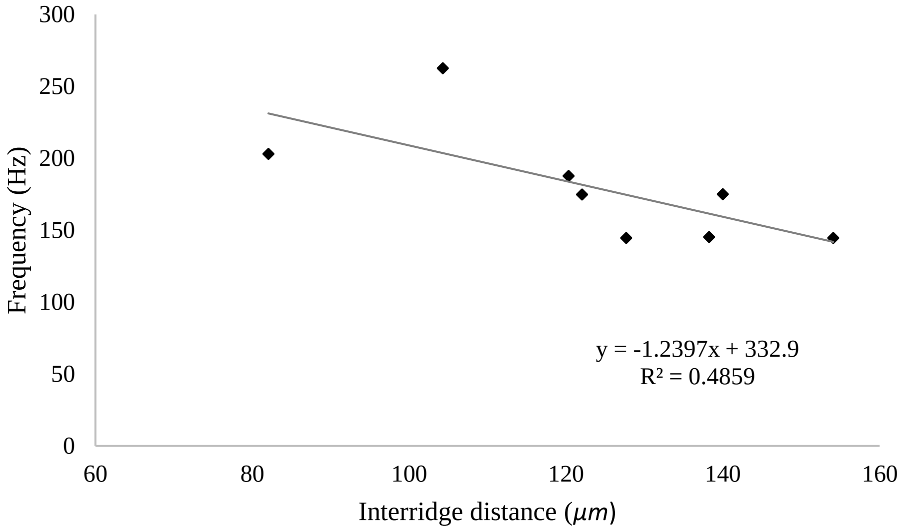

Figure 19. Regression between average frequency of grunts produced by individuals of *P. pardalis* and the interridge distance in the dorsal condyle of the pectoral fin spine.

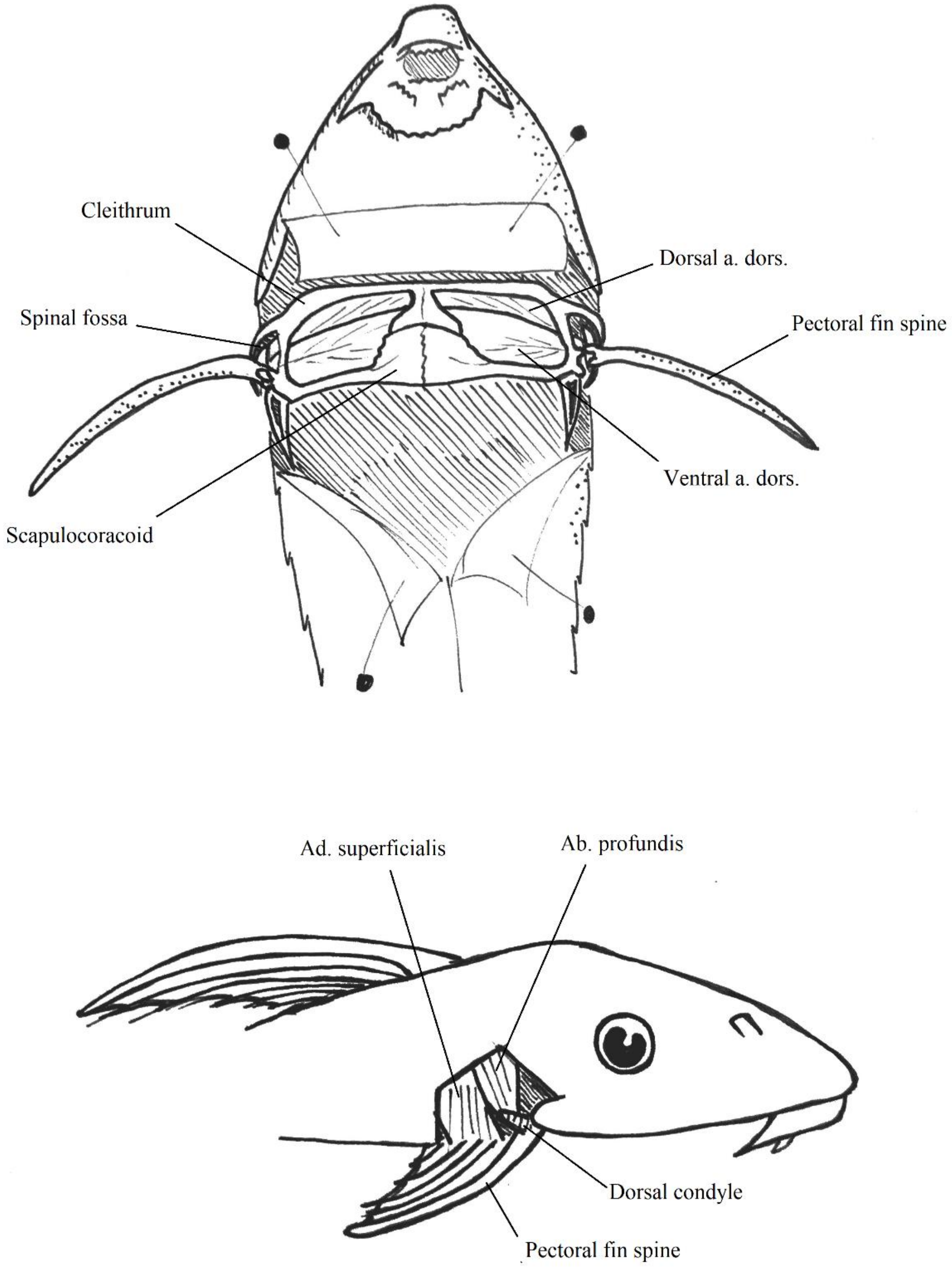

Figure 20. Muscles acting on the pectoral fin of *P. pardalis.*

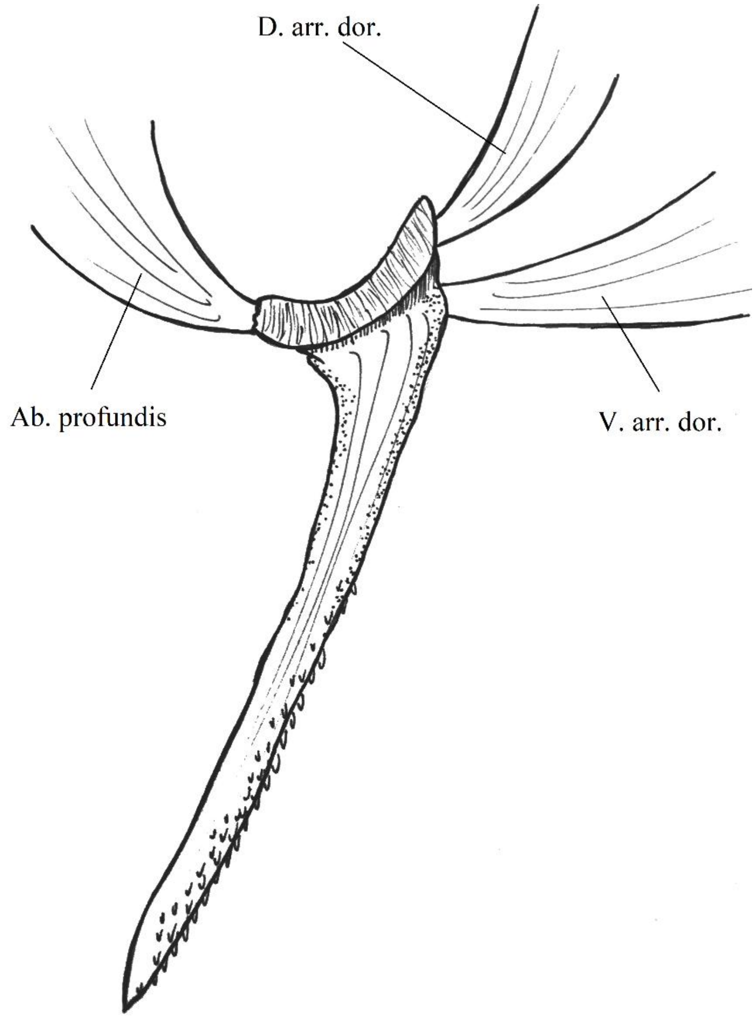

Figure 21. Diagram of primary muscular attachments to the pectoral fin spine of *P. pardalis.* See Table 5 for descriptions of muscle actions. *Ab. profundis = abductor profundis; v. arr. dor. = ventral arrector dorsalis; d. arr. dor. = dorsal arrector dorsalis*.

TABLES

Table 1

Distribution of sound sequences played to P. pardalis *during sound response behavioral experiments.*

Sequence	Number of individuals exposed
Control-Control-Control	1
Control-Control-Sound	4
Control-Sound-Control	4
Control-Sound-Sound	4
Sound-Control-Control	1
Sound-Control-Sound	5
Sound-Sound-Control	5
Sound-Sound-Sound	3

Note. “Sound” indicates *P. pardalis* stridulation sound, “Control” denotes knocking sound.

Table 2

Primary frequency of grunts by individuals of P. pardalis.

Fish Identity	N	Min.	Max.	Average	SD
2	38	41.10 Hz	162.88 Hz	120.34 Hz	33.08
3	30	54.76 Hz	163.91 Hz	122.04 Hz	27.94
4	40	33.62 Hz	141.76 Hz	104.30 Hz	23.87
5	30	93.73 Hz	182.70 Hz	138.26 Hz	16.41
6	35	32.46 Hz	150.38 Hz	82.06 Hz	29.17
7	24	115.16 Hz	173.15 Hz	154.07 Hz	13.03
8	20	89.07 Hz	183.51 Hz	127.68 Hz	30.84
9	39	49.56 Hz	207.15 Hz	139.99 Hz	33.49
Overall	256	32.46 Hz	207.15 Hz	121.87 Hz	34.46

Table 3

P values of one-way ANOVAs comparing means of five sample grunts in recordings of P. pardalis *stridulation sound.*

Recording number	Fish identification number							
	2	3	4	5	6	7	8	9
1	0.015 *	0.329	0.848	0.793	0.897	0.302	0.708	0.001 *
2	0.794	0.936	0.911	0.605	0.736	0.586	0.117	0.018 *
3	0.0006 *	0.657	0.006 *	0.776	0.199	0.871	0.841	0.119
4	0.013 *	0.566	0.392	0.017 *	0.169	0.173	0.119	0.794
5	0.206	0.225	0.976	0.107	0.146	0.189		0.691
6	0.431	0.337	0.867	0.322	0.836			0.807
7	0.798		0.874	0.303	0.004 *			0.132
8			0.483					0.424

Note. Values noted with an asterisk are significant ($p < .05$).

Table 4

Significance of one-way ANOVAs between recordings of specimens of P. pardalis.

Fish ID number	P
2	0.0002 *
3	0.032 *
4	0.034 *
5	0.889
6	0.0002 *
7	0.065
8	0.659
9	0.646

Note. Values marked with an asterisk are significant ($p < .05$).

Table 5

Temporal components of P. pardalis *distress sound.*

Fish identity	Grunt length			Intergrunt length			Grunts per minute		
	Min.	Max.	Avg.±SD	Min.	Max.	Avg.±SD	Min.	Max.	Avg.±SD
2	0.11	0.49	0.25±.06	0.10	2.60	0.49±.40	40.88	168.44	92.22±34.49
3	0.07	0.51	0.20±.09	0.12	1.73	0.49±.34	53.37	207.61	98.58±44.22
4	0.16	0.45	0.29±.07	0.14	3.25	0.47±.42	48.60	154.24	84.94±26.37
5	0.07	0.24	0.17±.04	0.26	31.12	1.56±4.53	8.91	107.72	67.16±35.51
6	0.16	0.49	0.33±.08	0.12	0.79	0.39±.16	52.07	122.35	88.57±20.03
7	0.05	0.34	0.13±.07	0.11	3.25	0.70±.59	26.05	132.39	83.51±43.29
8	0.06	0.29	0.17±.06	0.27	1.07	0.63±.29	53.72	113.04	82.08±28.09
9	0.04	0.31	0.14±.06	0.06	4.87	0.61±.70	27.13	333.70	115.69±83.88
Overall	0.04	0.51	0.22±.10	0.06	31.12	0.66±1.67	8.91	333.70	89.58±43.35

Note. All values are reported in seconds.

Table 6

Measurements taken of the pectoral fins of specimens of P. pardalis.

Fish identity	Left fin spine				Right fin spine			
	Total length	Width at neck	Condyle length	Condyle width	Total length	Width at neck	Condyle length	Condyle width
2	48.64	2.59	6.82	1.47	49.64	2.6	6.93	1.54
3	41.92	2.6	5.74	1.21	42.13	2.62	5.64	1.33
4	57.43	2.84	8.42	1.87	58.53	2.78	8.64	1.77
5	46.93	2.68	5.92	1.28	47.37	2.56	5.85	1.31
6	55.45	2.84	7.54	1.62	57.01	2.75	7.65	1.72
7	39.81	2.5	5.03	1.13	40.35	2.45	4.89	1.1
8	40.3	2.4	5.26	1.46	39.57	2.32	5.45	1.28
9	39.97	2.52	5.44	1.18	41.19	2.39	5.64	1.26

Note. All measurements are reported in mm.

Table 7

Measurements of dorsal condyle size and ridges in specimens of P. pardalis.

Fish number	Condyle arc length	Total ridges	Min ridge distance	Max ridge distance	Avg interridge distance	SD
2	7.41 mm	35	95.80 µm	373.26 µm	187.63 µm	63.95 µm
3	6.75 mm	41	104.38 µm	258.13 µm	174.87 µm	38.22 µm
4	9.42 mm	41	112.06 µm	422.96 µm	262.69 µm	77.20 µm
5	6.19 mm	34	97.61 µm	255.37 µm	145.20 µm	44.11 µm
6	8.48 mm	35	105.80 µm	334.35 µm	203.00 µm	51.24 µm
7	5.3 mm	34	103.45 µm	222.05 µm	144.46 µm	30.24 µm
8	5.67 mm	34	103.82 µm	229.11 µm	144.67 µm	30.56 µm
9	6.37 mm	30	81.45 µm	412.65 µm	174.91 µm	73.69 µm

Table 8

Number of clicks per grunt and ridges per condyle in P. pardalis.

Fish Identity	N	Min.	Max.	Average	SD	Ridge Number
2	38	7	45	24.2	10.4	35
3	30	6	39	22.6	8.5	41
4	40	5	40	21.7	7.8	41
5	30	9	33	19.0	5.9	34
6	35	4	38	14.2	8.7	35
7	24	6	27	17.0	6.8	34
8	20	7	21	14.6	4.5	34
9	39	7	30	16.8	6.4	30
Overall	256	5	45	19.7	8.2	

Table 9

Condyle length along the arc of the dorsal condyle, interridge distance, and angular velocity of the dorsal condyle in P. pardalis.

Fish ID Number	Condyle arc length	Interridge distance	Velocity±SD
2	7.41	187.6251	22.24±6.36
3	6.75	174.8719	23.38±4.52
4	9.42	262.6875	32.36±12.35
5	6.19	145.196	21.28±2.36
6	8.48	202.9976	17.71±5.92
7	5.3	144.4632	23.99±1.99
8	5.67	144.6658	19.94±4.39
9	6.37	174.9113	26.36±5.99

Table 10

Actions, origins and insertions of muscles acting on the pectoral fin of P. pardalis.

Muscle	Action(s)	Origin	Insertion
Arrector dorsalis:			
ventral division	Ventral extension of pectoral fin spine; slight dorsal raising of dorsal condyle of pectoral fin spine	Scapulocoracoid primarily, cleithrum secondarily	Neck of pectoral fin spine, anterior edge
dorsal division	Abduction of fin spine; draws dorsal condyle towards midline	Cleithrum primarily, scapulocoracoid secondarily	Inferior rostral border of dorsal condyle of pectoral fin spine
Abductor profundis	Dorsal flexion of fin spine; adduction of fin spine	Post-temporosupracleithrum	Superior caudal border of dorsal condyle of pectoral fin spine
Adductor superficialis	Dorsal flexion of secondary fin rays; rotation of fin spine anteriorly	Post-temporosupracleithrum	Shaft of pectoral fin spine, shafts of secondary fin rays
Abductor superficialis	Depression of secondary fin rays	Ventral scapulocoracoid	Last (medial-most) secondary fin ray

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