The Life of *Apteronotus rostratus*, a Panamanian Species of Weakly Electric Fish: A Field Study

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I. Executive Summary

English:

This study sought to provide insight into the life of *Apteronotus rostratus*, a species of weakly electric fish encountered in the rivers of Panama. Weakly electric fish have had their ability to actively generate electricity to sense their environment extensively studied in the laboratory, but little is known about their lives in the wild. A suitable study site was found at Piriati, in the Bayano region, where numerous *Apteronotus rostratus* were found in the river on an initial field outing. In order to fill the knowledge void about *Apteronotus rostratus* in the wild, four 200m transects were conducted in the Piriati river, and the location, habitat and frequency of every individual was taken (for a total of 240 *Apteronotus rostratus* sampled). Their distribution was tested and found to exhibit aggregation (i.e. clumping as opposed to individuals being randomly or uniformly distributed). 26 individuals were captured and were sexed as well as had their length and their electric organ discharge frequency measured, to determine if there was a relationship between the size of an individual and its' EOD frequency, but results suggest there is not a clear size to frequency relationship for males, and a negative relationship for females.

An overnight recording was conducted with two different artificial electric signals mimicking an *Apteronotus* EOD presented for 2 hours each. This was compared to a 2 hour recording that took place the same night without stimulation. It was found that there were significantly more fish surrounding the recording electrodes when there was stimulation than when there was not, suggesting that *Apteronotus rostratus* is attracted to EOD signals. All of this suggests that *Apteronotus rostratus* is a social species fish that likely uses its EOD, at least to some extent, to determine with whom to aggregate. Furthermore no clear size/length relationship was found, suggesting that previous explanations for the high EOD frequency of Apteronotids may need to be re-examined, especially in light of laboratory studies that could not confirm the higher frequency preference in *Apteronotus leptorynchus* females.

ESPAÑOL:

Este estudio intentó proporcionar penetración sobre la vida del de *Apteronotus rostratus*, una especie de pez débil eléctricos encontrados en los ríos de Panamá. Los pecez tienen la capacidad de generar activamente electricidad para detectar su ambiente. Los peces estan studiado extensivamente en el laboratorio, pero poco se sabe sobre sus vidas en el salvaje. Un sitio conveniente del estudio fue encontrado en Piriati, en la región de Bayano, donde numeroso *Apternotus rostratus* fue encontrado en el río en una inicial excursión a el campo. Para llenar el conocimiento vacío sobre el *Apteronotus rostratus* en el salvaje, cuatro transects de los 200m fueron conducidos en el río de Piriati, y tomaron datos sobre la localización, el habitat y la frecuencia de cada individuo (para un total de 240 *Apteronotus rostratus* muestreado). Su distribución fue probada y encontrada para exhibir la agregación (es decir agrupando en comparación con los individuos aleatoriamente o uniformemente distribuyendo). Capturaron y eran sexuados a 26 individuos así como tenían su longitud y su frecuencia de descarga del órgano eléctrico midió, para determinar si había una relación entre el tamaño de un individuo y su frecuencia del EOD.

Una grabación de noche fue conducida con dos diversas señales eléctricas artificiales mímicas un Apteronotus EOD presentado por 2 horas por cada señale. Esto fue comparada a una grabación de 2 horas que ocurrió la misma noche. Fue encontrado que había considerablemente más pescados que rodeaban los electrodos de la grabación cuando había estímulo que cuando no había, sugiriendo que *Apteronotus rostratus* está atraído al EOD señala. Todo el esto sugiere que el *Apteronotus rostratus* sea un pez social de la especie que las aplicaciones probables su EOD, por lo menos hasta cierto punto, de determinar con quién al agregado. Relación clara además no se encontró ninguna del tamaño/de la longitud, sugiriendo que las explicaciones anteriores para la alta frecuencia del EOD de Apteronotids pueden necesitar ser reexaminado, especialmente con los estudios de laboratorio que no podrían confirmar la preferencia más alta de la frecuencia en hembras del *Apteronotus leptorynchus*.

II. Host Information

The Smithsonian Institute has nine research facilities around the globe devoted to the study of the natural world. The Smithsonian Tropical Research Institute (STRI) in Panamá was set up in 1923 as a small field station on Barro Colorado Island, and has developed into "one of the world's leading centers for basic research on the ecology, behaviour and evolution of tropical organisms (http://www.stri.org/)." With 38 staff scientists and countless visiting scientists, there is a tremendous amount of research going on in STRI geared towards understanding many aspects of life in the tropics. The Bermingham Laboratory, part of the Naos subsection of STRI is focused on molecular population genetics, evolutionary biology, historical biogeography, molecular systematics of neotropical fish as well as Caribbean island birds.

While research in the Bermingham Laboratory is primarily molecular and genetic in nature, they were prime candidates for collaboration with the Krahe Laboratory of McGill because of a shared interest in diversity of freshwater fish, the maintenance of this diversity, and the history that has lead to the present species assemblages. The Krahe Laboratory at McGill is primarily focused on researching the mechanisms of sensory information integration used by weakly electric fish in order to generate spatial maps of their environment. Another recent development in the research conducted in the Krahe Neuroethology Laboratory is the behavioural aspect of their model organisms, with an interest in understanding how behaviour impacts evolutionary processes and a ethological approach to the study of *Apteronotus* species. Towards these ends they are utilizing electrophysiology, computational modeling, behavioural studies and respirometry in lab and recently field settings.

III. INTRODUCTION

Weakly electric fish are able to generate an electric field to monitor their environment and to communicate with conspecifics. This form of communication and generation of a spatial map of the environment has arisen independently several times in nature. Many different species exist in sympatry in Africa, South America and Central America, the Gymnotidae in South and Central America and Mormyriformes of Africa (Feulner et al. 2007). Electric fish have been extensively studied as a model system for understanding sensory information accumulation and integration on a neurological level. There are two main 'strategies' for electrolocation: to continuously produce a sinusoidal discharge (wave fish) or to produce brief pulses at an irregular rate (pulse fish) (Hagedorn and Heiligenberg 1985). Gymnotiforms are widely distributed throughout the humid Neotropics, from southern Mexico (15°N) to northern Argentina (36°S) (Crampton and Albert 2005a). The fish control their electric organ discharges (EODs) with a large number of pacemaker neurons that cause the synchronous firing of a group of electrocytes, which are responsible for generating the electric current of the EOD (Moortgat et al. 1998). Active electroreception (the active generation of an electric field as opposed to the passive electroreception of catfish or sharks), allows gymnotiforms to communicate, navigate, forage, and orient themselves in their environment, often at night (Crampton and Albert 2005b). Both pulse and wave type fish are thought to be present in Panamá (*Apteronotus rostratus*, *Sternopygus* and *Brachypomus*).

Electric fish are able to use their EOD to detect objects and animals that have conductivities different than water by using their sensory cells to detect changes in current flow. There has been extensive research seeking to understand the neuronal circuitry responsible for the creation of the spatial map of the fish's surroundings using electricity. At the time of this study a search of Web of Science of ‘weakly electric fish’ revealed more than 628 studies, with more than half of them containing some derivative of the word ‘neuro’ in their abstract or title. Behavioural studies are few and far between, and little is known about the life history and evolutionary past of weakly electric fish, of particularly interest is the Ghost Knifefish family, *Apteronotidae* (order <u>Gymnotiformes</u>), an extensively study set of model organisms for neural studies$. There haven’t been many studies of these fish in the wild, with very little being known about their natural population sizes, areas of habitation or overlap with heterospecific weakly electric fish.

> “Apteronotid species are readily recognized as the only gymnotiform fishes with a caudal fin and a dorsal organ (a longitudinal strip of fleshy tissue firmly attached to posterodorsal midline). Among gymnotiforms, apteronotid species also possess the following unique combination of characters: small eye (its diameter less than distance between nares); infraorbital and supraorbital laterosensory lines connected anterior to eye; infraorbital bones ossified as slender tubes; anterior nares located outside gape; no urogenital papilla; 1-2 rows of conical teeth in both jaws; anal-fin origin at, or anterior to, isthmus; no urogenital papilla. There are currently 72 names available in the literature for apteronotid genera and species. Of these, 13 generic and 52 species names are valid. There are in addition 12 manuscript names for species currently in preparation, bringing the known diversity of Apteronotidae to 64 species. Many more apteronotid species are anticipated, as the rate of description is accelerating. Like all gymnotiforms the shape is typically culteriform, with an elongate body and anal fin. Higher level apteronotid taxonomy emphasizes differences in snout length and shape, and shape and position of the mouth. The Apteronotidae is confined to the Humid Neotropics, ranging the Rio de la Plata of Argentina (35° S) to the Rio Tuira of Panama (8°N). Like many apteronotids, *Apteronotus* are aggressive predators of small aquatic insect larvae and fishes.” (Albert 2003)

The quasi-sinusoidal EOD produced by wave fish have been suggested to exhibit extreme sexual dimorphism between the sexes. It has been suggested that male brown ghost knifefish (*Apteronotus leptorhynchus*) produce discharges at a frequency of 800-1000 Hz while females discharge at a rate of 600-800 Hz, although some continuity between these ranges has been suggested and the extent of dimorphism remains to be demonstrated (Zakon et al. 2002, Bargelletti 2007). There is also a rather extreme physical dimorphism between the sexes.

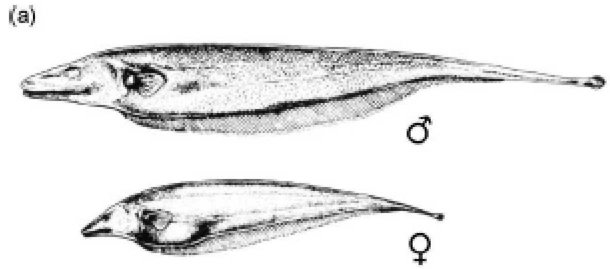

FIGURE 1: Male and female brown ghost fish (Zakon, Oestreich et al. 2002).

Meyer suggested that there is no clear sexual dimorphism in EOD frequency in any *Apteronotus* species and that the only species of Gymnotoid weakly electric fish that has had a clear sex difference in discharge frequencies is *Sternopygus*, which exhibits clear sexual dimorphism (1984). The underlying reason, genetic or environmental (or some combination of the two), for an individual *Apteronotus rostratus* fish to have a specific frequency EOD is still unknown. Meyer found that injection of male sex hormones consistently lowered the EOD frequency of a fish, suggesting stress and environment may have an impact on EOD frequency (1984).

Apteronotids can use modulations of their normally quite stable EOD frequency for communication. Several researchers have posited that the difference in EOD frequency, shape and modulation allowed for the intensive radiation event in weakly electric fishes and the ability of different species to evolve in sympatry (Moortgat et al. 1998; Zakon et al. 2002). There are six families in the order Gymnotiform, with more than 150 species described in detail, and many more are likely yet to be discovered. In *Apteronotus* alone there are 64 described species.

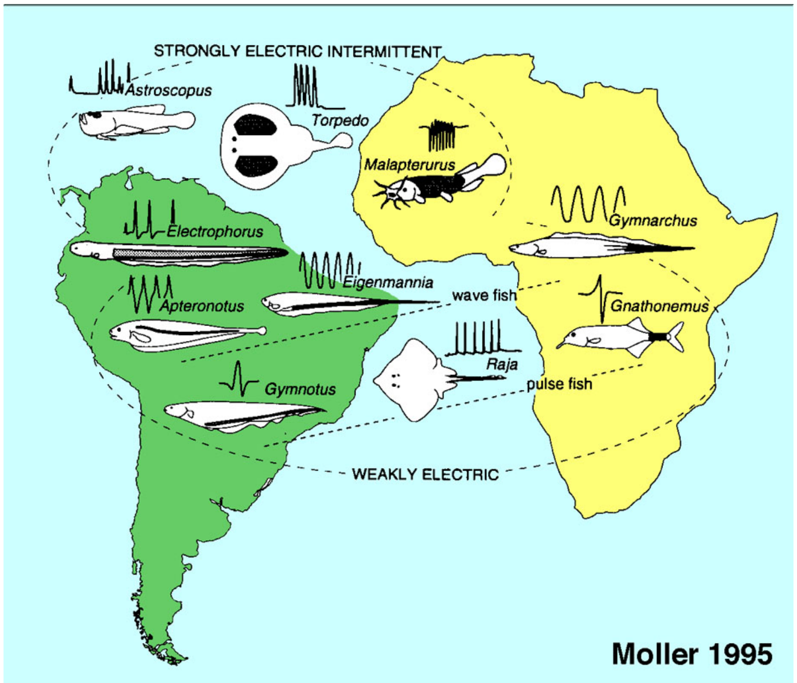

FIGURE 2: Example of species diversity in EOD wave form type across South America and Africa.

There are relatively few sexual preference and mating studies of weakly electric fish, they are limited to a study by Curtis and Stoddard (2003) on *Hypopomus pinnicaudatus*, a pulse fish, by Bargelletti (2007) on *A. leptorhynchus,* and a study on *A. leptorhynchus* by Krahe and Gogarten (unpublished data, 2007) using artificial male signals to measure female preference. Curtis and Stoddard (2003) found that females preferred larger males, which they found was correlated with the amplitude of the EOD signal. Bargelletti (2007) found no male preference for larger EOD amplitude in her experiments and rather that females preferred males with higher EOD frequencies. It is still unclear how specific communication signals may affect female mate preference.

It is unknown why weakly electric fish evolved wave type EODs of such high frequency, as there isn't likely to be a sample rate advantage between 100 and 1000 Hz and there is likely an energetic cost to higher frequencies. So what drove the frequencies to such levels and why is there a possible dimorphism between the sexes? Some researchers have suggested that a dimorphism between male and female EOD frequency might allow for females' preferences for higher EODs in males. The evolution of communication systems amongst conspecifics has attracted researchers studying a wide variety of organisms and it is a pertinent question for *A. rostratus* (Guilford and Dawkins 1991). Work by Dallaire and Krahe (unpublished data) on the energetic costs of chirping and maintaining a higher EOD may yield valuable insight into the selective advantage for a female to chose a male who is capable of chirping more or able to maintain a higher EOD. Yet it is not even known whether Apteronotids live in groups or are solitary. The vast majority of studies have been done in a laboratory environment, and in order to get a realistic picture of the lives of these fish and the potential evolutionary pressures they are subjected to, field studies are imperative. Moreover their habitat conditions are not known, and it is not know where they can be found in Panama. In order to allow for realistic laboratory studies of electrolocation and weakly electric fish behaviour much more information needs to be gathered about their lives in the wild. Given the intensive research effort that is ongoing in neuroethology attempting to understand the 'electric sense', it seems it can only benefit researchers to have an idea of the natural lives of their study subjects.

The spatial distribution of species can be driven by several factors; vertorial factors resulting from external environmental factors, reproductive factors related to mode of reproduction, social factors due to innate behaviour, coactive factors resulting from intra-specific interactions, and stochastic factors resulting from random variation in any of the previous factors (Jayaraman 2001). There are three basic types of spatial distribution patterns in communities; random, clumped and uniform. Generally random distributions are indicative of environmental homogeneity and/or non-selective behavioural patterns, while the other two distributions suggest there are some constraints on the population. Clumping is indicative of either patchy distribution of favourable habitat, gregarious behaviour, environmental heterogeneity, reproductive mode and many other factors which make it beneficial for individuals to group together. Uniform distributions tend to suggest negative interactions between individuals over food, space, resources or mates. There are many potential explanations for observing a given species distribution and careful experimentation is needed to elucidate the cause for a certain pattern (Jayaraman 2001). At the moment there is no information regarding the spatial distribution of *Apteronotus rostratus* available.

V. METHODS

GENERAL METHODS:

Electrodes were built using 3m long cable consisting of three wires. These were soldered to an audio jack on one end, and three wire mesh electrodes on the other that were fixed to a plastic pole(obtained from a plastic close hanger, provided the optimal flexibility and durability), with 12 cm between positive and neutral, and another 12 cm between neutral and negative electrode.

FIGURE 3: Picture of electrode set used to located and record from electric fish. There are 12 cm between electrodes, with the positive electrode on the right side, neutral in middle and negative on left side.

Electrode signals were sent to a RadioShack battery powered mini amplifier-speaker (cat. No 277-1008C), which could be listened to directly, recorded or connected to headphones. In this manner it was possible to detect electric fish signals at a distance. Signals were recorded as an audio signal using a Zen Nano Plus 1 GB MP3 player (using an encoding bitrate of 160 kbps). These MP3 recordings could be converted to .WAV files and then analysed using a MatLab routine written by Rudiger Krahe.

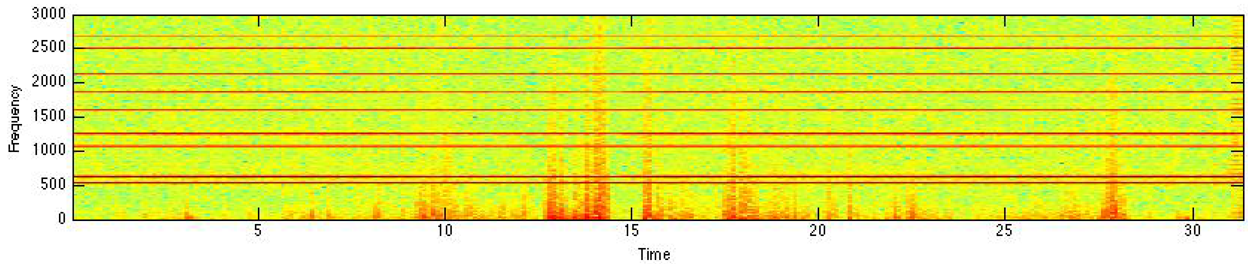

FIGURE 4: Frequency spectrogram of a fish captured in the Bayano's Piriati river generated using MatLab and the specgram function. Two fish appear to be present, one at 530 HZ and the other at 624 Hz.

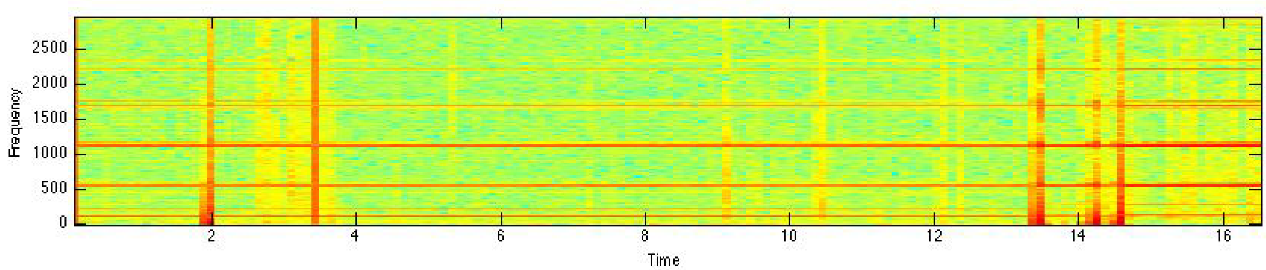

FIGURE 5: Frequency spectrogram of a fish captured in the Bayano's Piriati river generated using MatLab and the specgram function. The fish has baseline frequency of 570 Hz and there appear to be chirps (a type of communication signal) in the recording which might be indicative of its being a male.

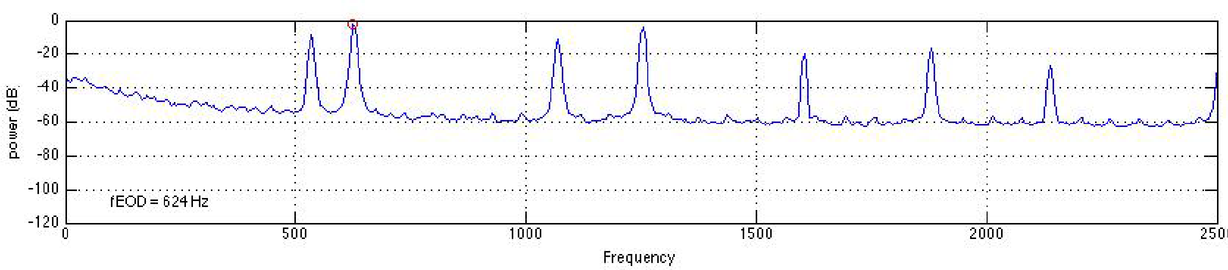

FIGURE 6: Powerspectrum of two fish recorded in the Bayano's Piriati river generated using MatLab and the discrete Fourier transformation. The two first large peaks represent the baseline frequencies of the two fish present. The next two peaks at ~1100 and 1300 represent the first harmonic of this frequency. The next two peaks represent the third harmonic. As expected the power component drops of with successive harmonics.

The above figures show typical outputs for tracks analyzed using the Matlab routine. As they suggest it was relatively easy to determine the number and frequencies of individuals present around recording electrodes.

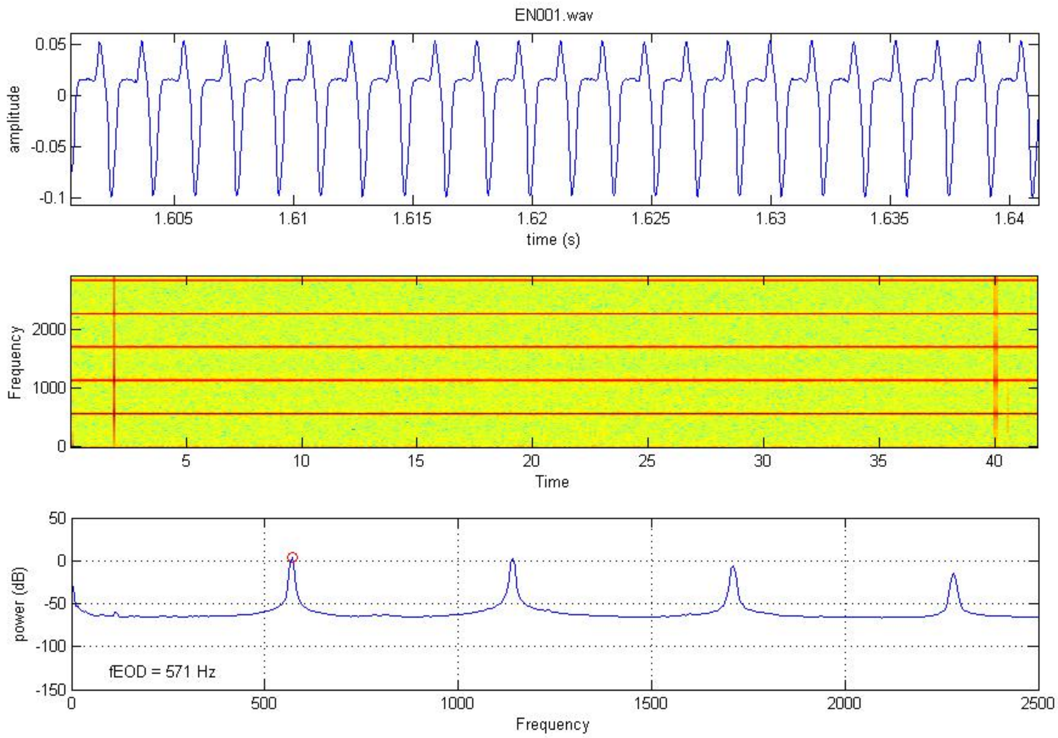

Figure 7: Figure showing output of Krahe Matlab program. Top graph shows direct EOD trace over time. The second figure is a frequency spectrogram of the recording. One can see two chirps. The baseline frequency of this fish is 571 Hz, and the red lines of high intensity above this line in the frequency spectrogram are the harmonics. The powerspectrum of fish recorded in the Bayano's Piriati river also shows the baseline frequency (first peak), and then the 2nd, 3rd, and 4th harmonics.

Geographical coordinates were obtained using a Garmin GPS 12 (software 4.53). Water conductivity, pH, and temperature were measured using an Oakton Waterproof pHTester 30, and an Oakton Waterproof ECTester 11+ Multirange. Photographs of fish were taken using a Nikon D80 body and Nikon AF Nikkor 28-80mm lens.

All frequency results were adjusted using a Q10 value of 1.62 (Dunlap et al. 2000). All results were adjusted to a temperature of 26°C, the average temperature of the river over many visits. This correction seeks to compensate for the fact that EOD frequency is temperature dependent, as most biological processes are, and that sampling took place in different temperature waters. $Q_{10} = (R_1/R_2)^{(10/(T2-T1))}$, R_1 is the rate of the reaction at initial temperature (in our case frequency before correction), R_2 is the rate of reaction at the new corrected temperature (in our case the frequency after correction), and T1 and T2 are the temperature at which the recording was done and to which it is being corrected to 26°C.

Panama is a neotropical country and the climate is seasonal, with a distinct 4-month dry season (January–April) during which precipitation rate falls off dramatically. Piriati, where most of the study described took place, was located on the frontier less than 30 years ago. In the past 30 years the rainforest has been completely cleared and converted to pasture and farmland. The town, of ~80 indigenous homes (Embera) and approximately the same amount of colonists dispersed along the Interamericana (Pan-American Highway). The surrounding area was developed in response to, or as a result of the Bayano Dam project in the 1970's which flooded more than 350 km^2. Indigenous groups were forced to leave the area being flooded and access-roads lead ultimately to the development of the area.

Due to the unknown location of *Apteronotus* species in Panamá at the start of this field study, initial research involved finding a suitable study site where *Apteronotus* species were present. An initial outing to Rio Frijolito, 4.1 km down Pipeline road, as well as two smaller streams in Parque National Soberania, revealed countless *Brachypomus* living in the rooted banks but no *Apteronotus* species. The second field expedition was to the Piriati River, crossed by the Inter-American highway at N 09° 02' 30.0" W 78°37'23.4", past Chepo and before Torti in the direction of the Darien if traveling from Panamá City. Here countless *Apteronutus rostratus* were encounter along with *Steronpygus* and *Brachypomus* (both pulse and wave-type fish were present in the river although no genetic analysis was completed, and EOD signals were used for species identification). All sampling was done during the Panamanian dry season between January and April.

Sensitivity of Electrodes

The sensitivity of the electrodes/amplifier was calculated by taking a captured *Apteronotus rostratus* in a net, and recording the strength of the signal at 20cm intervals away from the fish's side. Electrodes were held in a parallel position to the fish's side, as this seemed to be the way to maximize signal strength.

Transects

Four 200m transects were conducted at random locations along the Piriati river (Transect 1 coordinates; UTM X 761252.5953 and UTM Y1000269.993) (Transect 2 coordinates: UTM X 761472.724 and UTM Y 1000249.299) (Transect 3 coordinates: UTM X 761527.8646 and UTM Y 1000227.528) (Transect 4 coordinates: UTM X 761354.1144 and UTM Y 999883.3691. These consisted of walking a 1m spaced zigzag along the river with the electrodes, in order to detect every individual electric fish in the rivers. Distance along transect, information about living site of fish, distance from the left bank of the river, and the frequency of each individual encountered were recorded for each fish. 287 electric fish were recorded during these transects, 240 of them being *Apteronotus rostratus.* 14 were pulse type electric fish (believed to be *Brachypomus*) and 33 low frequency wave fish were also recorded (believed to be *Sternopygus).*

The transects were divided into sections in order to give a mean number per section of 1.0. To show that clumping was occuring the ratio of variance to mean was used. If the variance is < than the mean the distribution is said to be uniformly distributed. If the variance is larger than the mean than the distribution is said to be clumped, and if they are equal then the distribution is random. To test for aggregation in transects I compared the observed number of individuals that were in sections (i.e. 0,1,2,3,4…) to an expected poisson distribution of the same number of individuals (i.e. an expected random distribution of individuals in the transect). A chi-square test was done to test for difference between observed distribution and the poisson distribution.

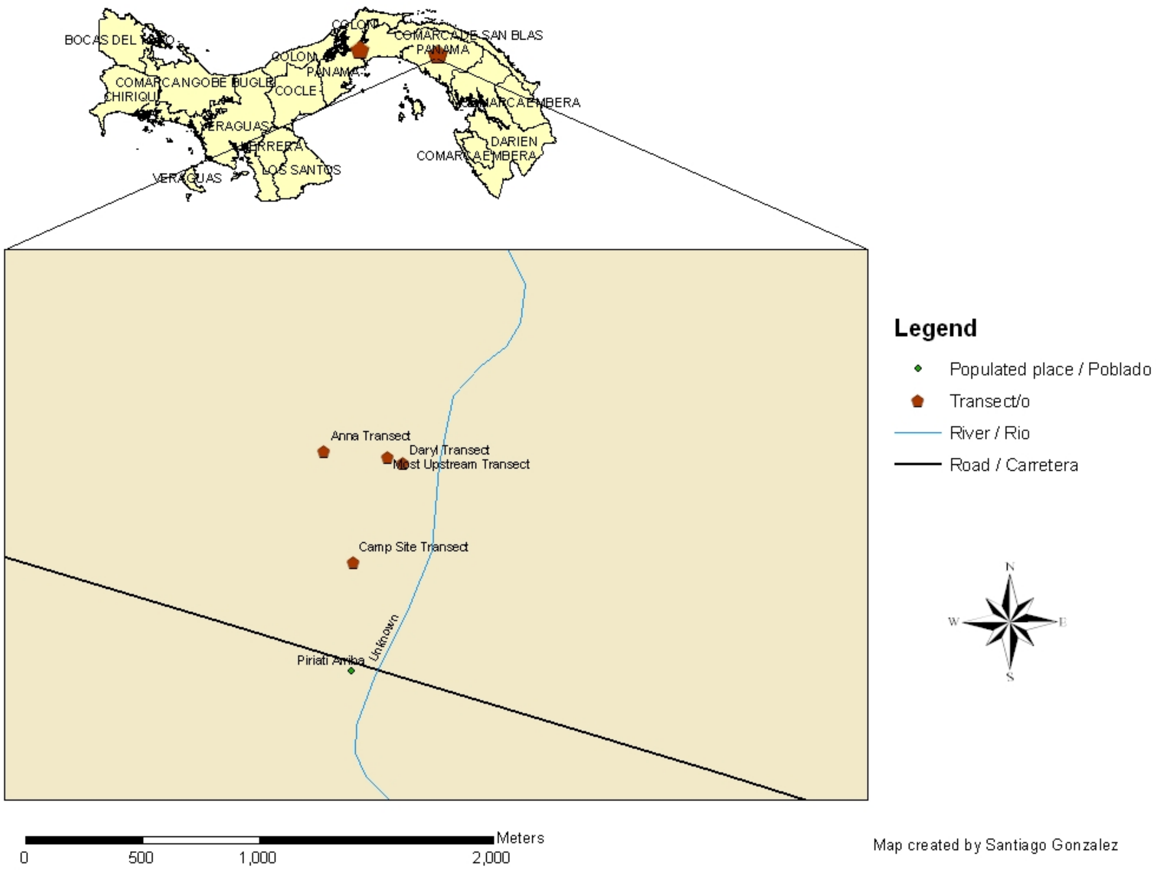

Figure 8: Map of transect locations in relation to Pan American highway. Either the river or the GPS coordinates do not line up exactly with the river, but the map gives a general idea of where in Panamá Piriati is located and relative position of study sites.

Overnight Recordings

One overnight recording was conducted at a site where many *Apterontus* were found during the days transect. The first two hours of recording were without any artificial stimulation, in order to observe the nocturnal behaviour of *Apteronotus rostratus*. Using two wire silver tipped electrodes, a Fortune Laboratory Industries Elecric Fish SIU Version 1.1 - Nov, 2006

Voltage to Current Board using the .2mV settings and a Zen Nano Plus 1 GB MP3 player and pure sine waves of 530 Hz and 700 Hz seeking to mimic *Apteronotus rostratus* EOD signals were applied (these two frequencies were chosen because they were the upper and lower maximum frequencies found during the first transect). These stimuli were each applied for two hours during the night in order to see if there was an attraction or repulsion fish towards the stimuli. Files were split into one minute segments (using computer program SPLICE), and frequencies of individuals present every five minutes were noted (i.e. every 5 minutes a 1 minute sample was analyzed to see what fish were present). An Anova, Single Factor, was conducted to test if there were significantly different number of individuals present during the 25 sample minutes of each 2 hour recording.

Length/Frequency/Sex Data

Twenty six fish were captured, and their length, frequency and pictures were taken. Fish were captured at random along the river, although it was decidedly easier to capture fish in stronger currents because they would be pushed into nets when disturbed as opposed to those living in the roots along the side of the river that tended to escape more often. When possible sex was determined using morphological cues (length of snout, presence of cloaca and shape of face. This was done independently by Jan Gogarten and Rudiger Krahe, and results were compared. Recordings were done in nets in the stream to ensure steady temperature, because EOD frequency is correlated to temperature. For the sake of trying to detect a relationship between length of fish and EOD frequency data between sexes was pooled. A linear regression was preformed, and an Anova was done on the regression to test for significance. The same analysis was conducted for males alone and females alone.

INFORMAL INTERVIEWS

Informal interviews were conducted with two colonist farmers and two Embera (indigenous group in Panamá) who had lived along the river their entire lives. All knew of *Apteronotus rostratus* upon seeing them. Information was obtained about the growth of the river during the rainy season as well as where fish had been spotted in the past and any information they had about the fish.

V. RESULTS

SENSITIVITY OF ELECTRODES

The electrodes were able to detect a fish in a net at 2m, both in terms of being audible in the field as well as being visible when frequency spectrograms were made of the recordings.

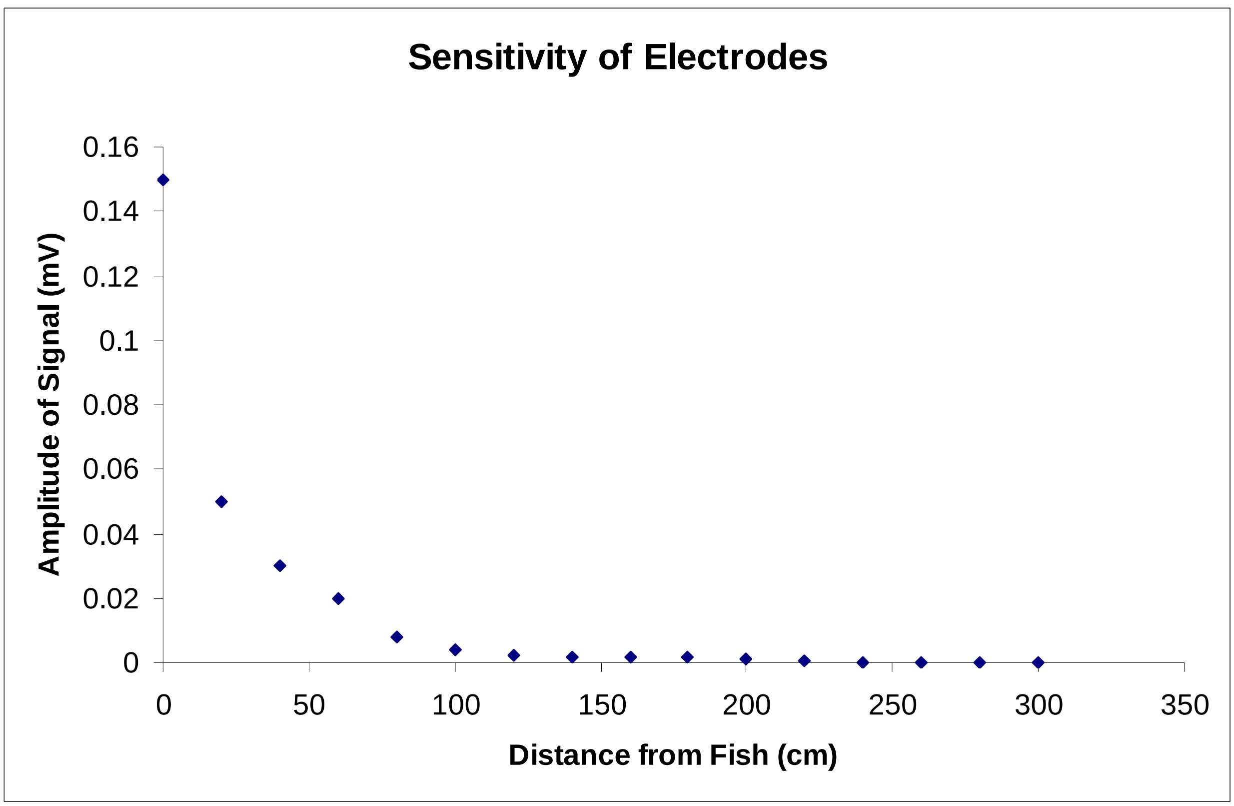

FIGURE 9: Shows sensitivity of electrodes to electric signal over distance. The amplitude of the signal drops off to close to 0 after 200cm. Signal was barely audible at 2m, and inaudible past 2m. Fish EOD was no

longer visible in frequency spectrogram after 220cm. This suggests that the electrodes are sensitive to electric fish up to 2m. When fish are under leaves and rocks detection distance was likely less than this ideal.

TRANSECTS

240 Apteronotus rostratus were encountered during the 4 transects. The range of EOD frequencies observed during these transects was 476-803 Hz (mean =628.9 Hz, Standard Deviation =73.8). 14 Pulse fish and 33 wave fish that were not Apteronotus rostratus were observed as well, for a total of 287 Electric fish encountered in 800m of river.

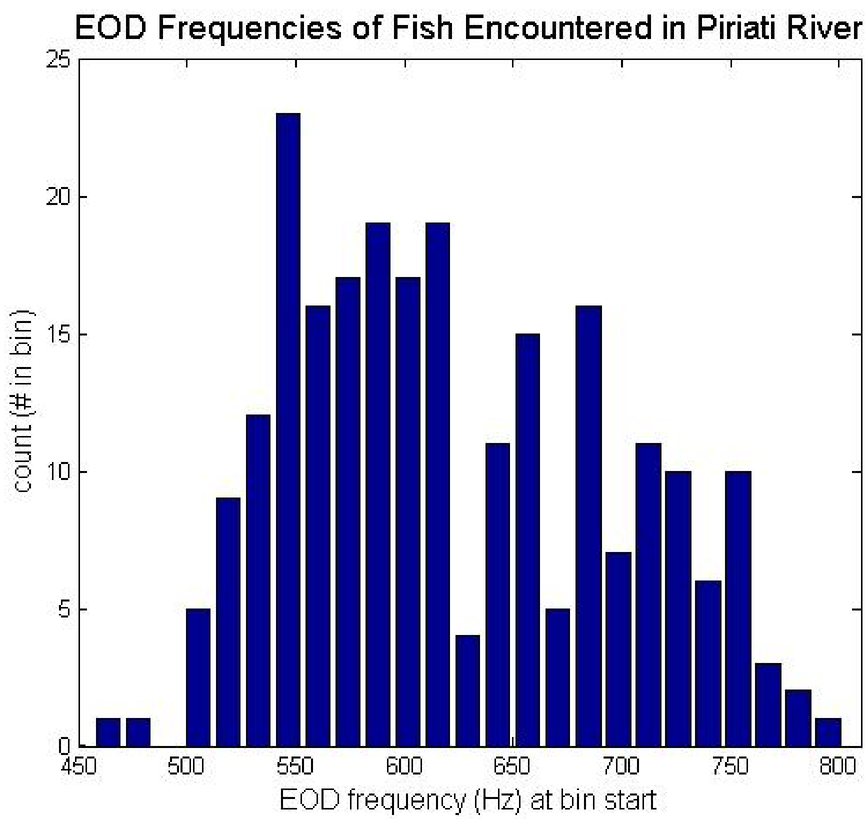

FIGURE 10: Historgram of EOD frequencies encountered.

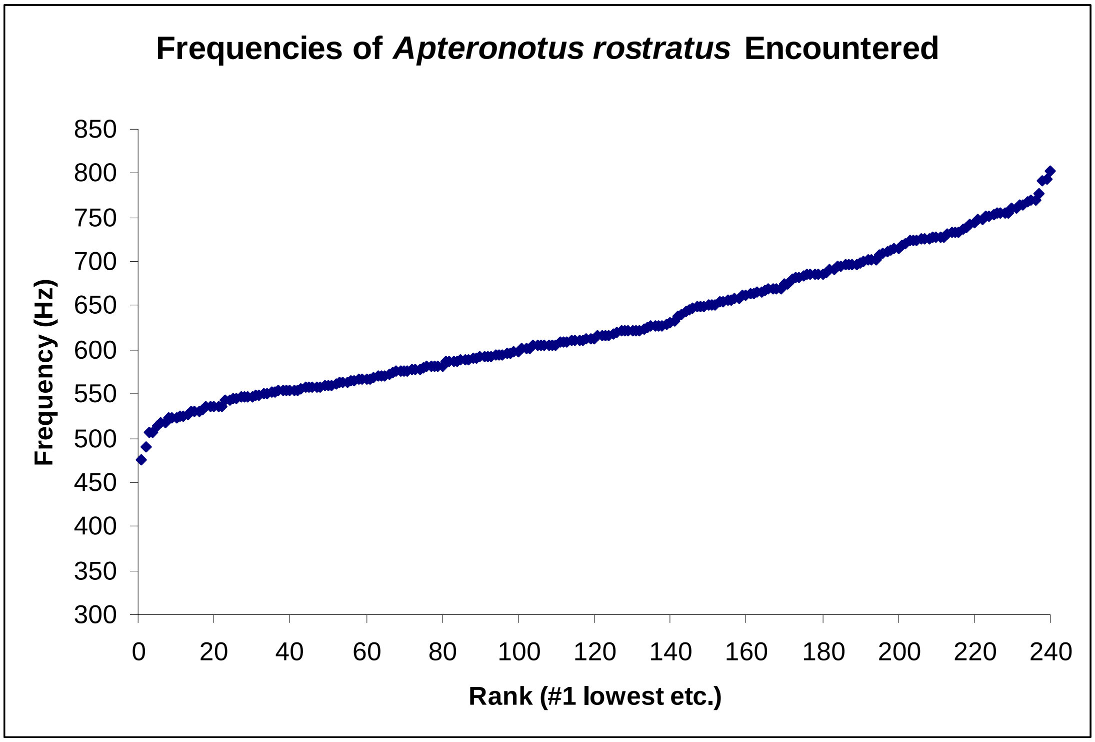

FIGURE 11: Frequencies of 240 fish recorded on field expeditions to Piriati. Mean frequency was 628.9 Hz with a standard deviation of 73.8. Maximum frequency found was 803 Hz and the minimum was 476 Hz.

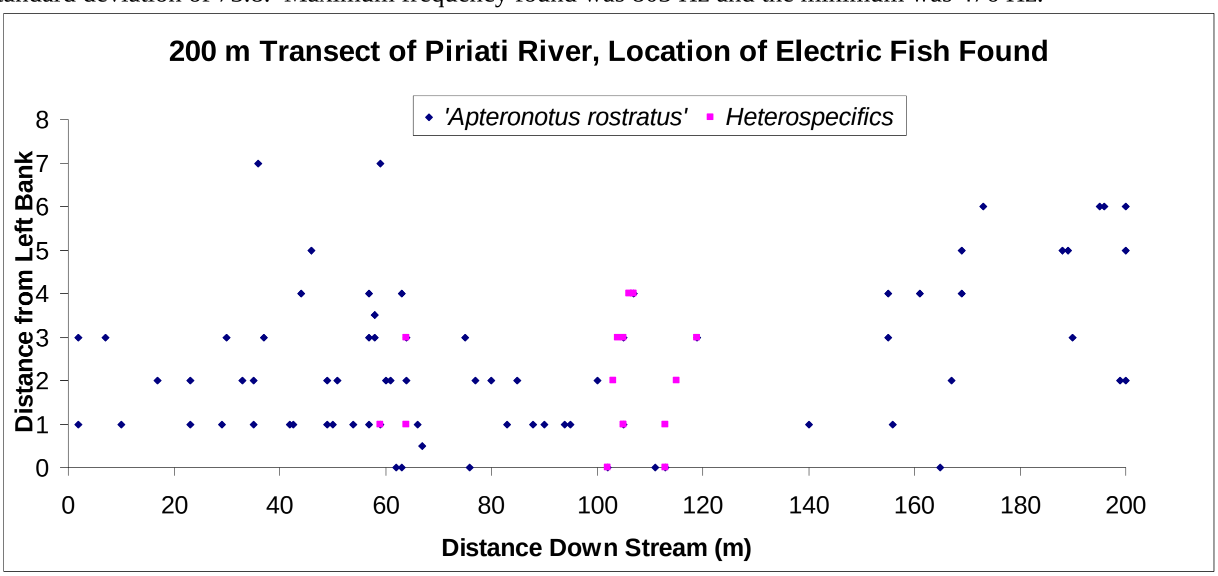

FIGURE 12: Position of *Apteronotus rostratus* in Piriati found during 200m transect. Visual inspection shows that the fish are not randomly distributed in the river and seem to cluster on the Left Bank and are generally found around other individuals. Some points represent more than one fish.

Transect broken into 103 segments for calculation of clumpedness (103 *Apteronotus* present). If they were randomly distributed one would expect every segment to contain 1 *Apteronotus* per segment (variation of number of fish per segment would be 1). For this transect the variation in number of *Apternotus* per segment was 2.33, strongly suggestive of a clumped distribution. A chai square test between the poisson distribution showed that they were significantly different (p<.0001, chi-square =335.92, df=11), suggesting a non-randomly distributed population.

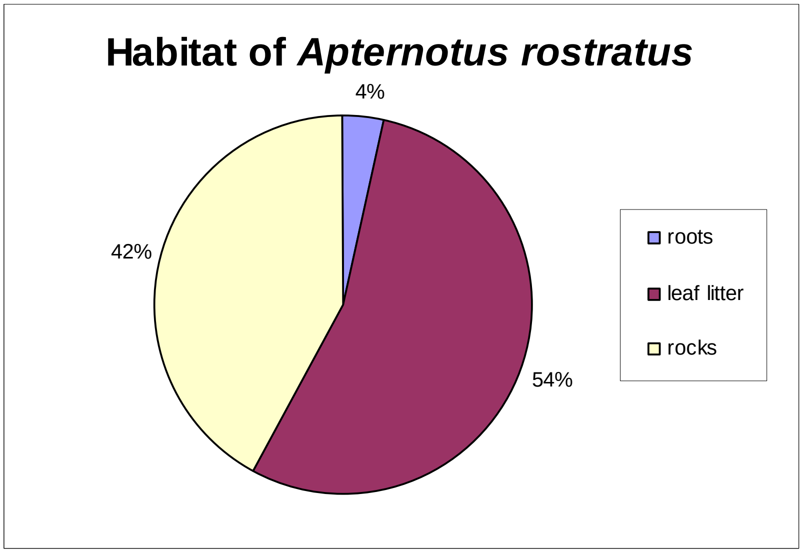

FIGURE 12: Habitat of *Apteronotus rostratus* encountered in one 200m transect.

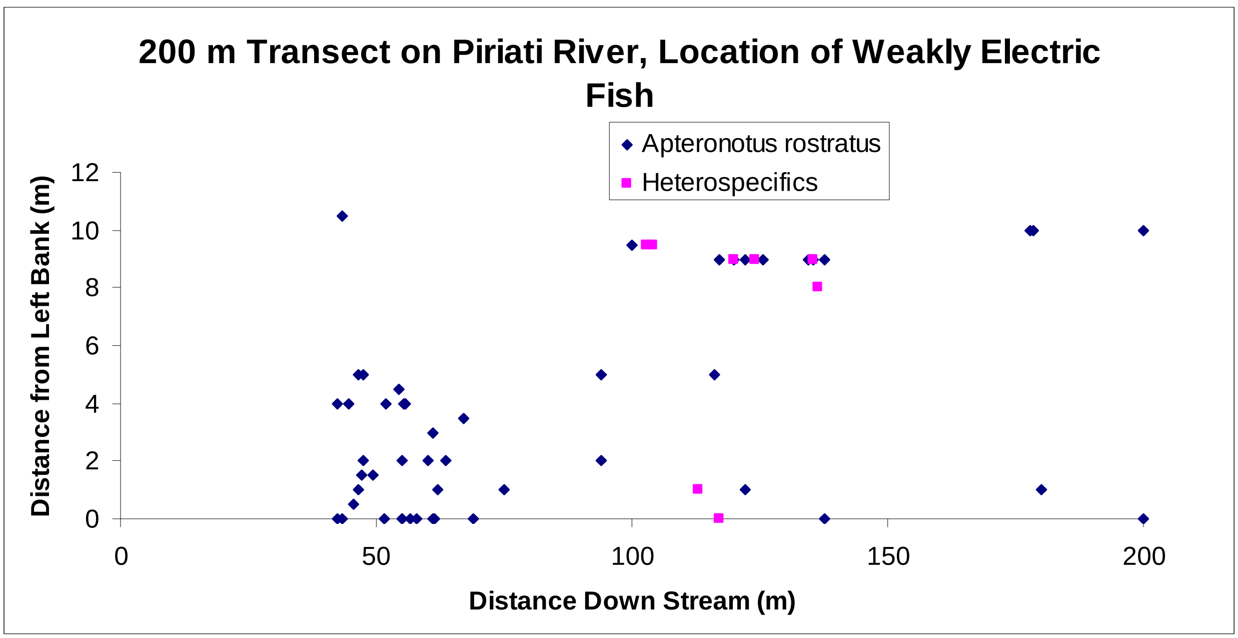

FIGURE 13: Position of *Apteronotus rostratus* in Piriati found during 200m transect. Visual inspection shows that the fish are not randomly distributed in the river and seem to cluster on the Left Bank and are generally found around other individuals. Some points represent more than one fish.

Transect broken into 72 segments for calculation of clumpedness (72 *Apteronotus* present). If they were randomly distributed one would expect every segment to contain 1 *Apteronotus* per segment (variation of number of fish per segment would be close to 1). For this transect the variation in number of *Apternotus* per segment was 3.94, strongly suggestive of a clumped distribution. Comparision with the poisson distribution using Chi-Square test suggested they were significantly different, suggesting a non-random distribution of fish in the river ($p<.0001$, chi-square = 1202.2, df=9).

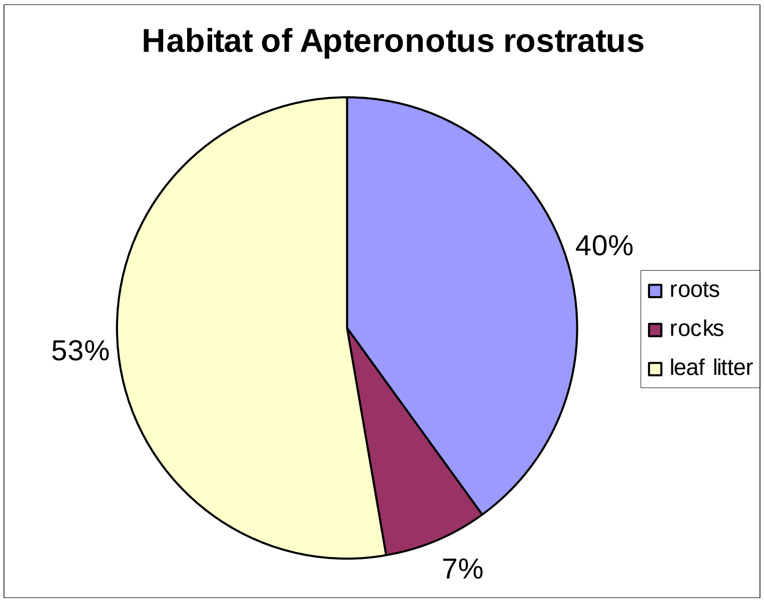

FIGURE 14: Habitat of *Apteronotus rostratus* in 200m transect of Piriati river.

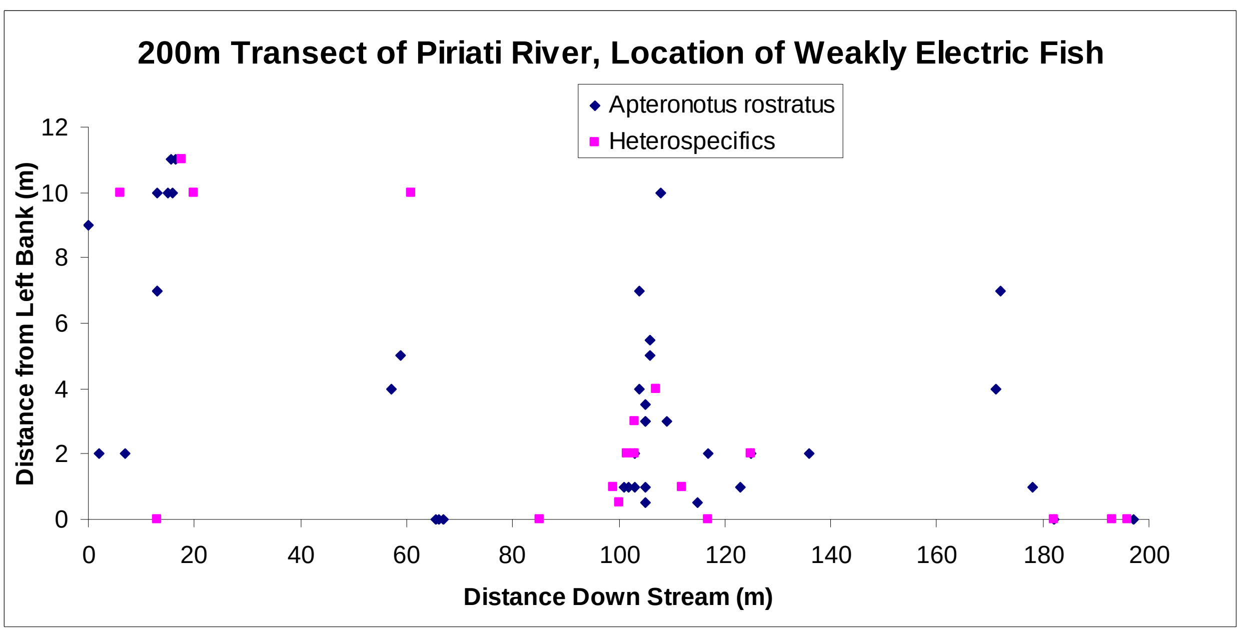

FIGURE 15: Position of *Apteronotus rostratus* in Piriati found during 200m transect. Visual inspection shows that the fish are not randomly distributed in the river and seem to cluster on the Left Bank and are generally found around other individuals. Some points represent more than one fish.

Transect broken into 50 segments for calculation of clumpedness (50 *Apteronotus* present). If they were randomly distributed one would expect every segment to contain 1 *Apteronotus* per segment (variation of number of fish per segment would be close to 1). For this transect the variation in number of *Apternotus* per segment was 4.78, very strongly suggestive of a clumped distribution. The chi-square test suggested significant difference between poisson distribution and observed distribution ($p<.0001$, chi-square = 10838, df=10)

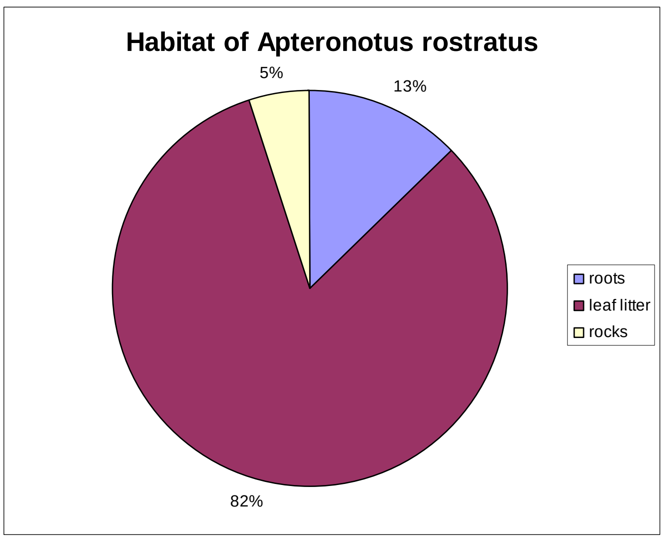

FIGURE 16: Habitat of *Apteronotus rostratus* in 200m transect of Piriati river.

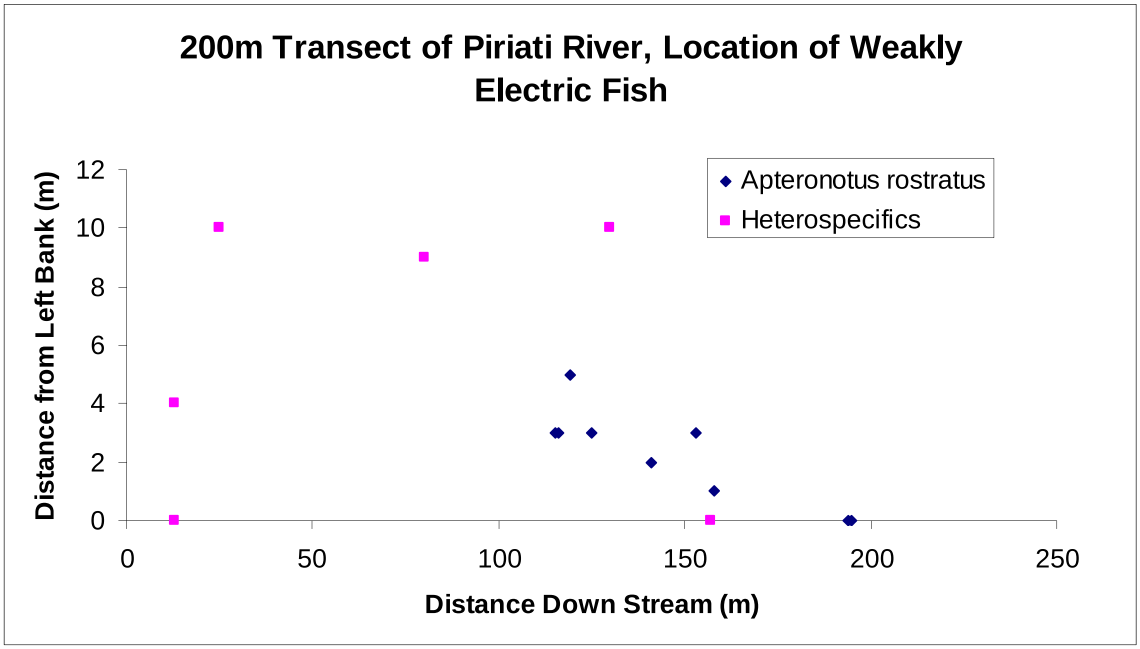

FIGURE 17: Position of *Apteronotus rostratus* in Piriati found during 200m transect. Visual inspection shows that the fish are not randomly distributed in the river and seem to cluster on the Left Bank and are generally found around other individuals. Some points represent more than one fish.

Transect broken into 15 segments for calculation of clumpedness (15 *Apteronotus* present). If they were randomly distributed one would expect every segment to contain 1 *Apteronotus* per segment (variation of number of fish per segment would be close to 1). For this transect the variation in number of *Apternotus* per segment was 4, very strongly suggestive of a clumped distribution. The chi-square test suggested significant difference between poisson distribution and observed distribution ($p<.001$, chi-square=23.221, df=6)

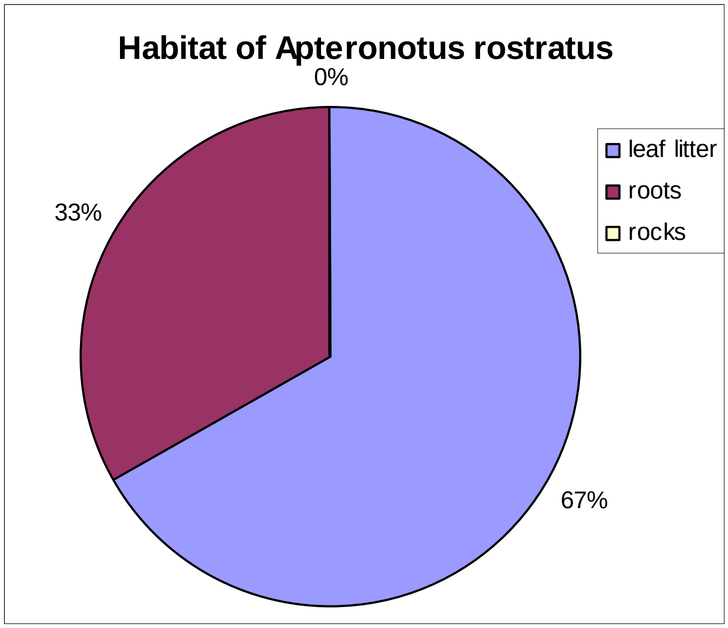

FIGURE 18: Habitat of *Apteronotus rostratus* in 200m transect of Piriati river.

OVERNIGHT RECORDINGS

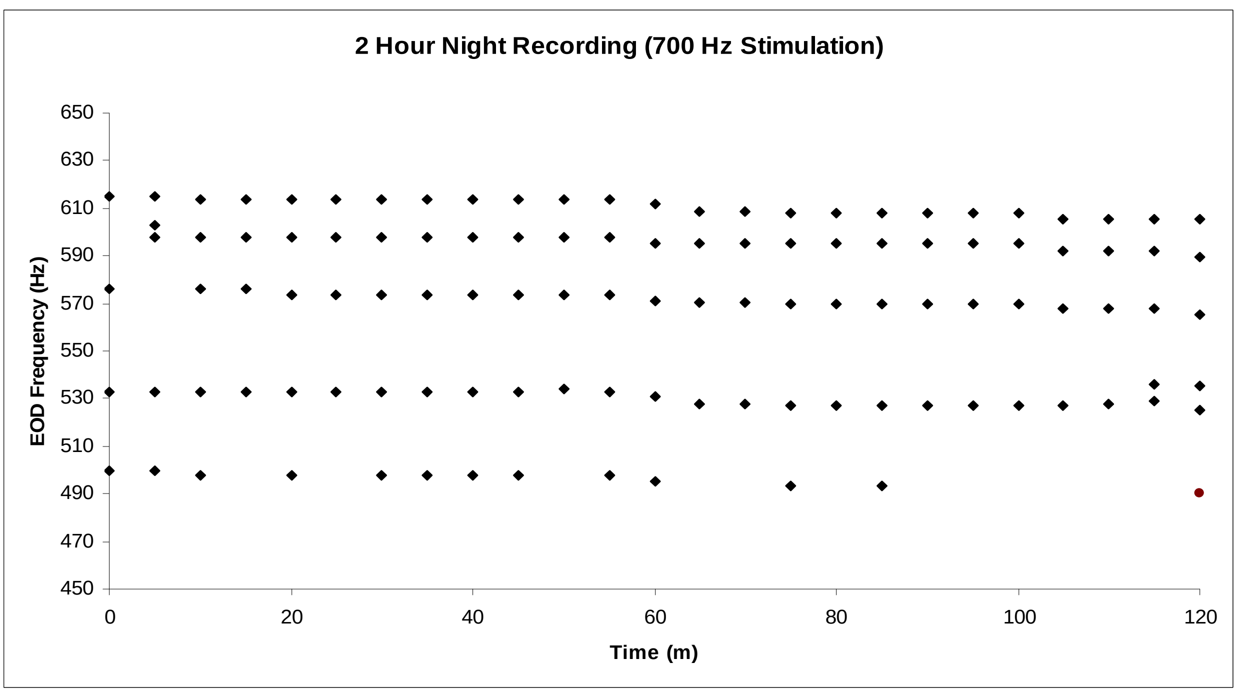

Figure19: Frequencies present in 1 minute intervals sampled every 5 minutes during one night for 2 hours while a stimulus of 700 Hz was being applied.

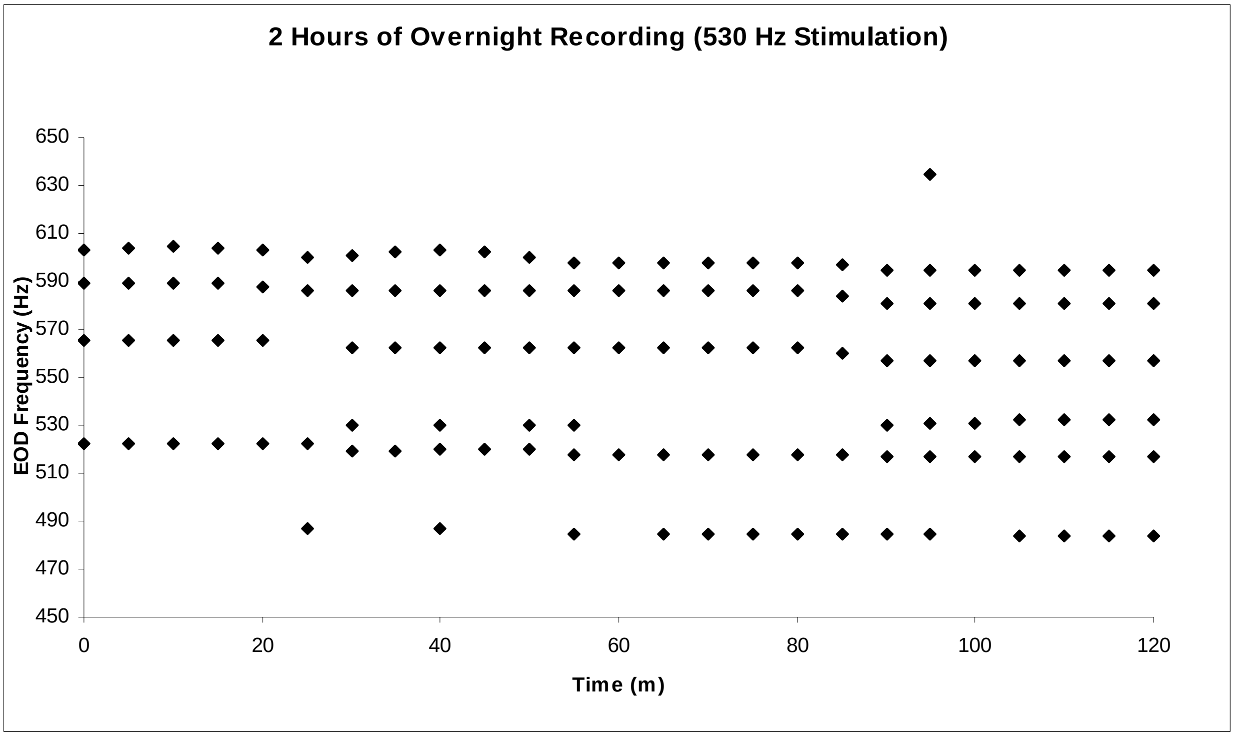

Figure 20: Frequencies present in 1 minute intervals sampled every 5 minutes during one night for 2 hours while a stimulus of 530 Hz was being applied.

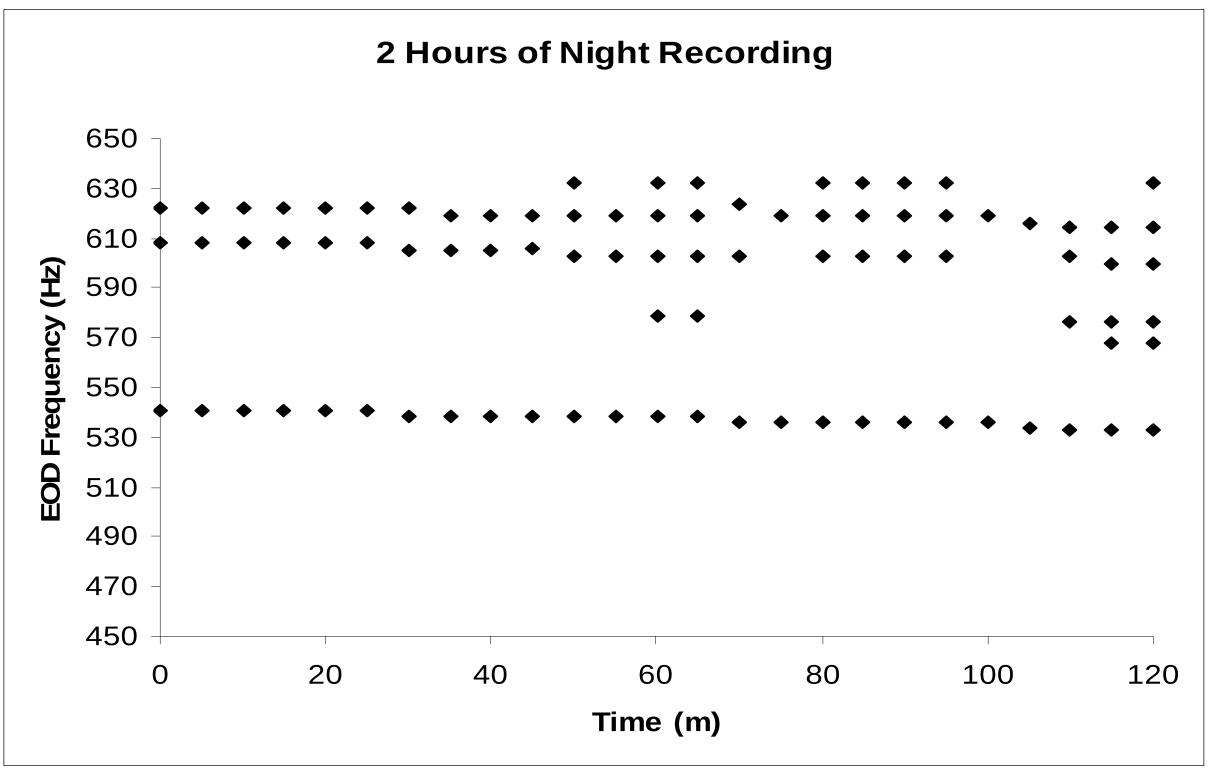

Figure 21: Frequencies present in 1 minute intervals sampled every 5 minutes during one night for 2 hours without applied stimulus.

Count	Sum	Average	Variance		
25	87	3.48	1.01		
25	125	5	0.833333333		
25	114	4.56	0.34		
SS	*df*	*MS*	*F*	*P-value*	*F crit*
30.58666667	2	15.29333333	21.01374046	6.48E-08	3.123907
52.4	72	0.727777778			
82.98666667	74				

Table 1: Results of Single Factor Anova, suggesting significant difference between the three conditions (530 Hz, 700 Hz and no stimulus).

LENGTH/FREQUENCY/SEX DATA

The Linear regression of the mixed sex data was negative, although an Anova of the regression suggests this result is not significant (N=26, R^2=.0763, Standard error = 65.67, p = .17). Visual inspection suggested that 15 females and 11 males were sampled, although several of these identifications come with large degree of uncertainty because individuals were immature and not gravid and thus difficult to sex. The longest individuals captured (>20cm), were all certainly male, and some have underestimates of actual length because pieces of the tail were missing. This suggests that mature males have lower frequencies than immature individuals (average male length = 16.36 cm with standard deviation of 3.35, average female length = 14.77 cm with a standard deviation of 3.28, average female EOD frequency = 636.22 with standard deviation of 73.5257, average male EOD frequency = 626.0837 with a standard deviation of 59.77). T-test between mean frequency of males and females showed they were not significantly different (t=-.38, p=.71), and a T-test between mean length of males and females showed they were not significantly different (t=1.239, p=.22).

This does not lay to rest the possibility that EOD frequency is involved in mate selection, but does indicate that EOD frequency is not a direct indicator of length (and therefore potentially fitness). Fitness of individuals is a very hard thing to measure, and age and length do not necessarily indicate overall fitness. Long term genetic studies that are able to identify parents of offspring are need to actually measure fitness.

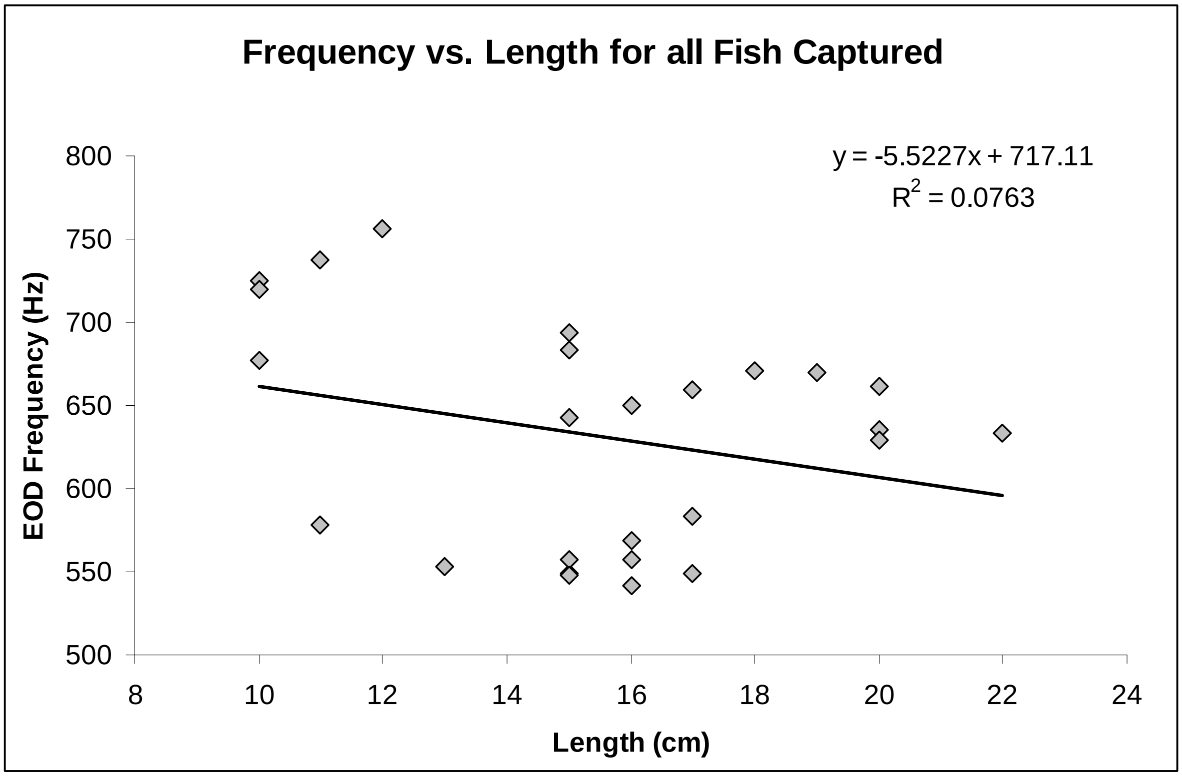

Figure 22: Length vs. frequency of 26 *Apteronotus rostratus* captured in Piriati River. Linear regression is negative with an R^2 = .0763.

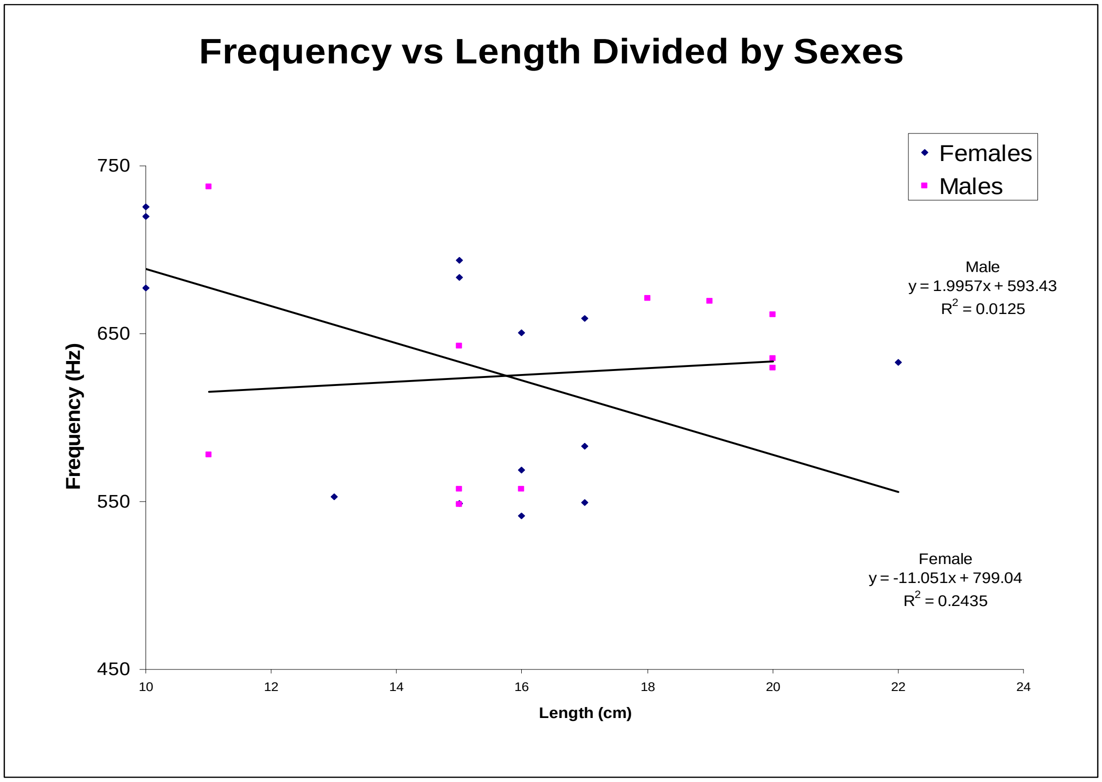

Figure 23: Length vs. frequency of 26 *Apteronotus rostratus* captured in Piriati River, this time with visual inspection to differentiate sexes.

Females had a strongly negative relationship between EOD frequency and length, whereas males now appear to have a positive relationship, especially when the one outlier at 11cm in length is excluded. The positive relationship for males between length and frequency does not appear to be very dramatic, and is not statistically significant (f= .75298), whereas the female is right on the border of significance (f= .06157).

SUMMARY OUTPUT: Male					
Regression Statistics					
Multiple R	0.112018				
R Square	0.012548				
Adjusted R Square	-0.09717				
Standard Error	62.60455				
Observations	11				
ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*

Regression	1	448.2474	448.2474	0.114368	0.74298
Residual	9	35273.97	3919.33		
Total	10	35722.21			

Table 2: Shows results of regression analysis for just males (frequency vs length).

SUMMARY OUTPUT: Female					
Regression Statistics					
Multiple R	0.493482				
R Square	0.243524				
Adjusted R Square	0.185334				
Standard Error	66.36494				
Observations	15				
ANOVA					
	df	SS	MS	F	Significance F
Regression	1	18431.8	18431.8	4.184952	0.06157
Residual	13	57255.96	4404.305		
Total	14	75687.76			

Table 3: Shows results of regression analysis for just females (frequency vs. length).

Informal Interviews

In Spanish *Apteronotus rostratus* is know as macana, while in the Embera language it is known as ‘veringo’. All interviewed people had experience with macana and stated that during the rainy season, when the river rises so dramatically that boats can use it and it can no longer be crossed on foot, the macana can be found living in the banks of the river. Several interviewees stated that they had eaten the fish, and claimed that they get up to 50cm during the rainy season. One interviewee stated that one could stand with a headlamp at night and watch them rushing out of the root matter. The fish are apparently delicious and are in high abundance and of much bigger size during the rainy season (May-December).

DISCUSSION

SENSITIVITY OF ELECTRODES

The ability to detect individuals at a distance up to 2m non-invasively offers an exciting approach for ecological and community studies. One is able to identify individuals without having to capture them because each fish in an area has a unique EOD frequency, which allows for exciting research possibilities. If projects are to take this kind of monitoring to the next level, a few major hurdles will need to be bounded over. Automation of identification of frequencies present in a recording is important, but can be difficult especially when multiple fish are present and are moving around. Triangulation between multiple sets of electrodes to estimate location should be possible but is also a potentially difficult programming venture. Furthermore the conditions between the dry and rainy-seasons in the rivers of Panama suggested that dramatically different electrode set ups will need to be in place between the two seasons.

Given these obstacles, the approach offers some very exciting potential for long term population studies. From manipulation of habitats to see if there is change in distributions, to see if removal of certain individuals will cause population phenotype changes, in principle one could monitor communication, movement and growth of a population continuously and non-invasively over the very long term. One could add or remove hetero or con-specifics in a section of river and monitor the effects of these additions. The possibilities of such a system, while costly, are nearly endless.

TRANSECTS

The results suggest that the distribution of fish in the river is not random. Something must be causing this non-random clumping. One factor that is a strong possible candidate is habitat availability. There were sections of river where there were no leaves and the water was much deeper and slower moving where encounters of *Apteronotus rostratus* fell off dramatically. Furthermore, the majority of fish were found living in leaf litter trapped by rocks and sticks, either because of food availability or because of a place to hide. While heterospecific weakly electric fish (pulse and wave) were found in the transects, the graphs suggest that they are sometimes found in different parts of the river (i.e. different constraints). The distribution of frequencies observed is not clearly bimodal, suggesting that there may not be a clear EOD frequency dimorphism between the sexes. There may be the possibility that there are two distinct different generations of individuals in the river which may be another potential reason for observing a bimodal distribution in EOD frequencies (although length which seems to be a good indicator of age, is not correlated with EOD frequency so this is not likely the case here). Further experiments could focus on habitat modification to see if fish can be made to occupy unoccupied areas (addition of food, changing of current-flow, addition of leaf-litter build-up etc.).

Overnight Recording

Attraction to artificial EOD stimuli suggests that perhaps part of the clumping behaviour observed in the transects might be due to attraction to conspecific signals. To confirm this further sampling is necessary with many more overnight recordings as well as randomization of the order of stimulation frequency (or simultaneous recordings in a stretch of river and see which stimulus (530, 700 or none) attracts the most fish.

Frequency/Length Relationship

No clear length/frequency relationship was suggested by our data. The only potential relationship (p=.17) was a negative one, which suggest if anything that the males and larger sized individuals have lower frequencies. This seems to be in agreement with the findings of Meyer, who injected male sex hormones into fish and observed a decrease in EOD frequency (1984). Sample size was not particularly large (n=26), nor was this study conducted during the rainy-season when *Apteronotus rostratus* is thought to be gravid and breeding. When the captured fish were separated based on sex (determined by visual inspection, independently by two people), the females appeared to have a negative relationship between length and EOD frequency whereas males had the opposite. Neither regression was significant, although the female regression was borderline. This result suggests there may be EOD dimorphism between the sexes, although the exact nature of this relationship is as yet still unknown.

Limitations and Problems

All sampling done during this study was conducted during the dry season when rivers were at their lowest. While the region where sampling was conducted was in a politically safe area, the area to the south, known as the Darien gap is not. There are likely many more pristine and ecologically rich rivers in the Darien gap, but they are largely unaccessible, due to lack of infrastructure and guerrillas from Columbia who control large sections of the region. The theft of a labtop provided another large hurdle, as much data and important programs were lost.

ACKNOWLEDGEMENTS

I would like to thank Ruth Reina, Anna Jamrozik, Darryl Singh and Adèle Michon for accompanying me to the field and providing much need hands and company by the riverside. Rüdiger Krahe has always been a tremendous help in terms of research ideas, advice, resources, encouragement and support. Without him none of this would have been possible and I would never have had the pleasure of learning about the sixth sense, electricity. Thanks are due to my family and friends who never stopped checking up on me making sure I had returned from field expeditions and not been bitten by one of Panamá's diverse poisonous creatures. Thanks are due as well to McGill University for putting the Panamá Field Study Semester abroad together and allowing me so much freedom in developing my own project, the Bermingham laboratory for hosting me at STRI and the wonderful people of Piriati who not only took me in as their local gringo but fed me fresh fruit and lent me their horses as well.

RECONOCIMIENTOS

Gracias a Ruth Reina, Anna Jamrozik, Darryl Singh y Adèle Michon para acompañarme al campo y para sus ayudas. Gracias a Rüdiger Krahe para ha sido siempre una enorme ayuda con las ideas, del consejo, de los recursos, del estímulo y de la ayuda de la investigación. Gracias al laboratorio de Bermingham por recibirme en STRI Gracias a la gente maravillosa de Piriati que me tomó no sólo adentro como su gringo cariñoso de los pescados locales sino me alimentó la fruta fresca y me prestó sus caballos también. Las gracias son debidas también a la universidad de McGill para organizar el semestre del estudio en el campo de Panamá

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APPENDIX

BUDGET:

- January 25, 2008 – 5 dollars spent on tolls to get to the Bayano.
- Transporation to and from Naos (as of April 16, 2008) – 20 dollars
- February 14, 2008 – 30 dollars on Mosquito netting, hammock, knife, camping supplies for the camping expedition in the Bayano.
- February 15, 2008 – Public transportation to and from Bayano – 10 dollars.
- February 29th – March 2nd – 30 dollars in food and transportation
- March 12th – 16th – 40 dollars in transportation and food and supplies
- April 8th – 11th – 50 dollars spent on transportation, gifts for children of friends, supplies etc.
- April 12th – 16th – 50 dollars spent on food, transportation, renting horses and a guide.
- The rest of the equipment has been provided by the Krahe and Bermingham labs (STRI and McGill), and whatever funding agencies have been generous enough to support them.

Net Spent = $235 (This includes food which I would have had to purchase anyway).

Without food this number is likely around $150.

CHRONOGRAM OF ACTIVITIES, TIME SPENT/WEEK

January 10, 2008 – Talked with the office at STRI in an effort to get onto the collecting permit of the Bermingham lab. They said it would be possible after I send some emails to the Bermingham lab and Dr. Oris Sanjur. Keycard access was also be arranged.

January 11, 2008 – Met with Ruth Reina and Dr. Oris Sanjur to discuss the project and its fesability. It was decided that I would try to find *Apteronotus* for the first month or so of fieldwork. They said they would help me on my quest, and if this did not work out that we would then start another project so that I would have something to work on over the course of the semester. Toured the facilities and the lab. Began planning field expeditions.

January 18, 2008 – Went to Pipeline road with Ruth Reina and spent the day recording signals from *Brachyhypopomus* along the side of the Friolito river. At least 20 individuals were recorded. Following this cursory examination of the population at Pipeline road, 6 individuals were captured using nets and electrodes to locate fish. These fish were brought back to STRI and are being kept as part of their fish collection and I will be doing detailed measurements on them there. Equipment and tested, and while all equipment worked, this allowed me to do a dry run of how to record with mp3 players and ultimately analyze recordings on the computer

January 24, 2008 – Went to lab and took recordings of individual fish captured at Pipeline road, and did some other things around the lab, including cleaning of field equipment, repairing of electrodes and charging of batteries. Had meeting with Ruth and Oris to discuss future study sites

January 25, 2008 – Collecting trip with Ruth Reina to the Bayano. Apteronotids found, 8 captured. 30 recordings made. Water properties measured along with GPS. Arranged for camping permission with farmer adjacent to the river. Will return to sample length, frequency, population structure and complete project.

January 28-29, 2008 – Analysis of recordings using Matlab program. Modified program to suit needs of current study. Figured out how to convert recordings to .WAV single channel (mono) using computer program. Designed methodology and worked on programs to complete project.
February 4th-5th, 2008 – Research at STRI library and discussion with Rudiger about potential for future study. Continued discussion with Fortune laboratory about potential of getting new stimulus circuit boards sent to Panama.

February 14, 2008 – Visit to STRI to examine captured electric fish. Apteronotids had perished over break. Collected supplies from Naos for field expedition this weekend. Bought supplies for camping in Bayano.

February 15-16, 2008 – Field visit to Bayano to do overnight recordings and field studies of *A. rostratus*. 72 individuals recorded. 200m transect of river completed and locations of all individuals and frequencies mapped.

February 29th – March 2nd, 2008 – Field expedition to Bayano/Piriati – Captured and recorded 10 individuals, and did measurements of sensitivity of electrodes at different distances. Overnight recordings done. Sadly the data from this field expedition were lost with the theft of the computer and backup.

March 12th – 16th – Field expedition to Bayano/Piriati – Informal interviews with indigenous fishermen and colonists about fish. Set up camp further along river. Overnight recording with stimulation. Capture of several fish. 2nd

200m transect completed. Stimulation experiment of one captured individual with 25 different artificial stimuli to get an idea of what reactions to stimuli are.

April 8th – 11th – Field Expedition to Bayano/Piriati – Collected sensitivity of electrode data again using captured individual. Captured many fish. 2 more 200m transects completed. More informal interviews with locals.

April 12th – 16th - Field Expedition to Bayano/Piriati – Horse expedition to Bayano lake to look and see if electric fish live in lake. Transect start sites GPSed and many fish captured.

Total Time Spent In The Field And In Transit to and in Piriati and Pipeline Road = **18 Days**
Total Time Spent Analysing Data, Planning and Meeting with people = **18 Days**
Net Time Spent on Project = 36 Days