Abstract

Can Passive Acoustics be Used to Estimate the Length of Atlantic Croaker (*Micropogonias undulatus*) within the Pamlico Estuary?

By

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The Atlantic States Marine Fisheries Council (AFMFC) attributes the decline in the Atlantic croaker (*Micropogonias undulatus*) stock to over-fishing practices. The majority of collected Atlantic croaker are small, young of year (YOY) individuals, which may lead to changes in age and size-class structure within the population. North Carolina and other southeastern states receive economic benefits from the fishing practices of Atlantic croaker and other important Sciaenid fishes. The goal of this research is to determine if Atlantic croaker populations can be monitored using passive acoustics by ascertaining if sound-production of a population will provide the listener with information such as length, maturity, or sex. An instrumented tripod (ITPod) was deployed to estimate the physical parameters (i.e. currents, water quality, waves, and turbidity) of the water and a passive acoustic recording system recorded environmental sounds (<10kHz) every 10 s at 15 min intervals at two sites in the Pamlico Sound from June to November 2008. Once a month at each site, Atlantic croaker were collected using a juvenile otter trawl and a gillnet, in addition an echosounder unit was simultaneously deployed to determine the size-selectivity of the nets. Laboratory results of

Atlantic croaker sound production revealed that the fundamental frequency is inversely correlated to the length of the fish. Based on these captive fish recordings, a linear regression analysis revealed that total length (*TL*, mm) was inversely related to fundamental frequency (F_0, Hz), where F_0= 1073.95 - 3.12 (*TL*), (R^2 = 0.84). Sex was not a significant factor influencing fundamental frequency for developing Atlantic croaker. Analysis of the length, mass, and area of the swimbladder, sonic muscles, and gonads revealed that all of these internal structures affected the fundamental frequency, but all were also highly correlated with the length of Atlantic croaker. Therefore, the data indicate that the fundamental frequency (Hz) in field recordings could be used to predict length (mm) with the same data using the following empirical relationship: *TL*=305.323 – 0.270 (F_0).

This linear regression equation collected from Atlantic croaker in captive conditions was used to predict the average length of Atlantic croaker from the passive recordings at the two field sites. These predictions were compared to the average lengths of Atlantic croaker collected in the trawl and gillnet, as well as the average length of the fish calculated from an active acoustic echosounder. When comparing the mean predicted length estimates from the passive acoustic device to the mean length of Atlantic croaker collected in the trawls, there was a significant difference for all months (ANOVA, $p<0.05$) except June (ANOVA, $p=0.37$).   An analysis comparing the lengths of all fishes collected in the trawl, gillnet, and active acoustics showed the selectivity of the nets. There was a significant difference in mean fish length among gear-types (ANOVA, $p<0.05$) for each month. Because each net was size selective when considered alone, I assumed they would not be size selective when the lengths of fishes collected in the gillnet and trawl net were combined. There was no significant difference between the fish community length estimates of the nets and that of the active acoustic echosounder (ANOVA, $p>0.05$) for all months except June (ANOVA, p=0.01). Next, a comparison was made between the Atlantic croaker the predicted mean length from the passive acoustic recordings, the mean lengths from the trawls, and the mean length predicted from the active acoustic echosounder surveys. There was no significant difference between the mean Atlantic croaker total lengths from the trawl and all of the fish in the active acoustics (ANOVA, p>0.05), except for June (ANOVA, p=0.001). Therefore, I conclude that the passive acoustic estimates of Atlantic croaker lengths (predicted from fundamental frequency) obtained in the laboratory did not correctly predict the observed length-structure of the Atlantic croaker population. This result may be due to the cutoff frequencies of the sites; in shallow water, the dominant frequency of an acoustic signal can change as it propagates. If the wavelength of the low-frequency component of a signal exceeds the water depth (the cutoff frequency), than these frequencies are essentially filtered from the recorded sound. Another factor influencing cutoff frequency is that of the resonance of trapped bubbles under the surface of the sediment. These bubbles can cause their own echoes when the sound reverberates off of the surface of the bubble. The bubble reverberations will add higher frequency components to the original sound. Together these phenomena would have increased the dominant frequency at the hydrophone, leading to an underestimate of length-structure for an Atlantic croaker population (the higher dominant frequencies would predict smaller fish observed). My research shows that Atlantic croaker length is inversely related to the fundamental frequency of the “croak” call, but the acoustic environment and the effects of the cut-off frequency need to be better understood prior to using this method in the field to estimate Atlantic croaker lengths.

Can Passive Acoustics be Used to Estimate the Length of Atlantic Croaker (*Micropogonias undulatus*) within the Pamlico Estuary?

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DEDICATION

I would like to dedicate this thesis to my family. To Jacqueline who taught me to listen to world around me and hear the music in the simple aspects of life. To Benedikt who showed me that hard work and dedication are always worth it. To Christina who was my first behavioral study. To Sebastian, who was not supposed to live; he has taught me that the simplicity in life does matter. Observation begins the process.

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LIST OF SYMBOLS AND ABBREVIATIONS

λ	Wavelength (m)
σ	Acoustic Cross Section
a	Radius of a Tank (cm)
ASMFC	Atlantic States Marine Fisheries Commission
ANOVA	Analysis of Variance
c	Speed of Sound (cm/s)
c_1	Speed of Sound in Water (m/s)
c_2	Speed of Sound in Substrate (m/s)
EL	Echo Level
f	Resonance Frequency of Sound
F_0	Fundamental Frequency (Hz)
f_0	Cutoff Frequency
FFT	Fast Fourier Transform
FMP	Fisheries Management Plan
GLM	General Linear Model
GSI	Gonadal Somatic Index
h	Depth of Water
Ha	Alternative Hypothesis
$I(r)$	Intensity of the Ensonified Target
I_s	Intensity of Target as Seen by the Transducer
L	Length (m)
LARS	Long-term Acoustic Recording System
N	Number in Sample
NCDMF	North Carolina Division of Marine Fisheries
OBS	Optical Backscatter Sensor
ITPod	Instrumented Tripod
PCS	Potash Corporation of Saskatchewan Site
PMS	Pamlico Mouth Site
SD	Standard Deviation
SL	Standard Length (mm)
SL'	Source Level of the Emitted Signal
SONAR	Sound Navigation and Ranging
STST	Selective Tidal Transport Model
TL	Total Length (mm)
TL'	Transmission Loss of the Emitted Signal
TS	Target Strength (dB re 1µPa)
W	Average Power of the Received Signal
Wt	Weight (g)
XBAT	Extensible Bioacoustics Tool
YOY	Young of Year

INTRODUCTION

The Atlantic croaker (*Micropogonias undulatus*, family Sciaenidae) stock is in decline on the United States' Atlantic coast. This commercially and recreationally important species is generally fished from Maryland to Florida. In 1987, a Fisheries Management Plan (FMP) was adopted to monitor the Atlantic croaker stock because the spawning-stock biomass crashed in the 1970s to an all-time low of ~12,000 metric tons. This crash was attributed to heavy fishing practices along the Atlantic coast of the United States (ASMFC 1987). The stock was able to make a recovery and peaked in 1997 at 91,000 metric tons but has continually decreased since 1997. The mid-Atlantic portion of the Atlantic croaker population is the core contingent of this species. Since commercial harvests on the Atlantic coast have increased since the 1970s, with both North Carolina and Virginia dominating the fishery (ASMFC 2005), there is a need to monitor the lengths, maturity and sexes that make-up Atlantic croaker on the Atlantic coast in order to prevent such a crash in the future.

In 2006 – 2008 Atlantic croaker made-up 20% of the total commercial finfish catch by weight in North Carolina (data from: NCDMF 2009b). However, it is considered to be a species of concern because there has been a continual drop in landings since 2003 (NCDMF 2009a). This decline has led researchers to explore the life history of this species, especially considering yearly abundance patterns are driven by recruitment (NCDMF 2009a).

Adults spend the spring and summer in an estuary and move offshore during the fall (Barbieri et al. 1994, AFMFC 2005), with spawning occuring both in the estuary and offshore (Diaz and Onuf 1985, Barbieri et al. 1994). Spawning locations have previously been extrapolated by collecting adults along a transect line that extended both inshore and offshore (Barbieri et al. 1994). Oocyte development and maturation of fish along the transect line were used to interpret the spawning location for this species; where fish with more mature oocytes or post-spawning follicles were assumed to be closer to the spawning habitat than those with less mature oocytes. Because Atlantic croaker are multiple spawners with asynchronous oocyte development and indeterminate fecundity (Barbieri et al. 1994 , Chittenden et al. 1994), and because spawning generally occurs over several months (Hettler and Chester 1990, Barbieri et al. 1994, Chittenden et al. 1994, Foward et al. 1999), the determination of spawning locations is problematic when using oocyte maturation and development. Barbieri et al. (1994) suggested that spawning by individuals begins in the Chesapeake Bay in July and the population continues to spawn as it slowly moves offshore and south until December or January. However, this is again inferred from gonadal maturation; not the direct observation of individual spawning behavior. The ASMFC (2005) states that there is a need to determine the exact location of Atlantic croaker spawning, to observe the behaviors associated with spawning, and to characterize the habitat and physical parameters that are associated with spawning in order to ensure proper management strategies of the stock. This will allow managers to close areas to fishing during Atlantic croaker spawning events and to monitor the spawning stock biomass directly, ensuring that adult Atlantic croaker have the opportunity to reproduce prior to being removed from the system.

Spawned eggs drift into the estuaries via active and passive transport through flood tides, bottom currents, and other oceanographic processes as discussed in the comprehensive review paper by Norcross and Shaw (1984). It is assumed that most marine fishes, including Atlantic croaker, spawn during fall and winter because larval collections of these species peak during these seasons (Hettler and Chester 1990, Warlen and Burke 1990, Forward et al. 1999). In general the larval collections for Atlantic croaker tend to be bi-modal, with a peak in November-

December and another from February to April (Cowan and Shaw 1988, Hettler and Chester 1990, Warlen and Burke 1990). These months seem to offer the best-possible survival time for these species because the waters are within the cold tolerance for Atlantic croaker (Joseph 1973), provide a zooplankton-rich food source (Thayer et al. 1974), which is the dominant food-source for postlarval Atlantic croaker (Currin et al. 1984), and there is little competition with resident ichthyoplankton (Walen and Burke 1990) and other zooplanktivores (e.g. Deason 1982). Additionally, the nursery grounds become a safe-haven from predation because their predators tend to need warmer waters for survival (Warlen and Burke 1990). Therefore, it is assumed that spawning would occur during these times, when conditions are appropriate for high larval survival.

Larval Atlantic croaker also tend to follow the Selective Tidal Steam Transport (STST) Model, which states that larvae move up in the water column during flood tides and down during ebb tides. This strategy would provide larvae with the maximum-transport power toward an estuary when spawned offshore because they would not have to swim against the ebb tide but instead wait until this tide passes before moving back to the surface and thus further towards shore (Forward et al. 1999). Larval Atlantic croaker have commonly been collected in the deep channels of North Carolina (Hettler 1989, Hettler and Chester 1990, Govoni and Pietrafesa 1994, Forward et al. 1999), the Gulf of Mexico (Cowan and Shaw 1988), and in the Chesapeake Bay (Norcross 1991); especially during night flood tides (Forward et al. 1999) and new moons (Warlen and Burke 1990, Hettler and Chester 1990). This suggests that spawning most-likely occurs offshore, but the collection of spawning individuals has yet to occur. Observations of how associated environmental parameters and behaviors are related to Atlantic croaker reproduction still needs exploration.

Sound Production in the Atlantic Croaker

Sound-production is a means of communication; a method of providing information to other animals that are able to interpret the signal (Delco 1960, Gerald 1971, Tavolga 1971, Myrberg et al. 1986, McKibben and Bass 1998). It can be produced as a result of an alarm response (Brawn 1961, Fish and Mowbray 1970, Amorim 1996), aggression (Fish and Mowbray 1970, McCauley and Cato 2000, Amorim et al. 2004), or as method to aid in reproduction (Grey and Winn 1961, Fish and Mowbray 1970, Myrberg et al. 1986, Luczkovich et al. 1999, Luczkovich et al. 2008b).

One method that may provide some insight into Atlantic croaker spawning behavior is the use of passive acoustics. Atlantic croaker contract their sonic muscles on their swimbladder to produce sounds (Tower 1908) with a fundamental frequency (F_0) between 300 and 1200 Hz (Fish and Mowbray 1970, Gannon 2007). Previous research on auditory sensitivity indicates that they can detect sounds between 100 and 1000 Hz (Ramcharitar and Popper 2004, Ramcharitar et. al. 2004). Atlantic croaker are unusual, because males and females, as well as juveniles and adults, are able to produce sounds (Hill et al. 1987, Gannon 2007) but juvenile's sounds are hypothesized to have a slightly higher F_0 than adults (Gannon 2007). Hill et. al. (1987) showed that both males and females of this species developed sonic muscles at 4 - 5 months of age but maturation does not actually occur until around 2 years old. Juvenile Atlantic croaker of around 4 – 5 months old (~ 45 mm standard length, *SL*) (Hill et al. 1987), should have the ability to produce sounds. Gannon (2007) re-examined the findings from Hill et al. (1987) by determining that Atlantic croaker produce sounds between 69 and 225 mm *SL*. However, he did not collect fish that were smaller than 69 mm *SL* and he stocked his fish in a shallow, concrete pond. Instead of recording the sounds of individual fish, he recorded the sounds of the stocked group of

Atlantic croaker, thereby being unable to identify neither specific individuals nor link sound-production characteristics to sex. Gannon (2007) showed that there was a negative correlation between the peak frequency of the group calls and the median length of the fish stocked in a pond but he did not measure individual Atlantic croaker sounds. It is clear that at the population level there is a relationship between the peak frequency of an aggregation and the mean length of an Atlantic croaker population but questions remain if length and F_0 are related in the individual fish, if the internal structures (i.e. swimbladder and sonic muscles) influence F_0, and whether sex and maturity play a role in the produced frequency of the acoustic call.

The shape and size of the swimbladder and sonic muscles are hypothesized to cause differences in the frequency of the sound produced by fishes of the family Sciaenidae (Chao 1978, Hill et al. 1987, Connaughton et al. 2000). In weakfish (*Cynoscion regalis*), it is hypothesized that larger sonic muscles have a faster velocity but take longer to create a single muscle twitch because the sonic muscle is longer for large males when compared to small males. The result is a longer call period and therefore a lower F_0 (Connaughton et al. 2000). Sexual differentiation of the swimbladder, sonic muscles, and gonadal weights becomes evident in Atlantic croaker that are 100 g or heavier (Hill et al. 1987). Mature females, on average, have lighter sonic muscles and swimbladders than mature males of the same size (Hill et al. 1987). Additionally, female Atlantic croaker have heavier gonads when compared to males of the same size (Hill et al. 1987). Therefore, it is speculated that females should produce higher F_0*s* than their male counterparts because of the smaller sonic muscles and swimbladder. In addition, sonic muscle development is believed to be limited in female Atlantic croaker because more energy is placed into gonadal development and maturation (Hill et al. 1987), which would result in a correspondingly higher F_0 when compared to males of the same size.

Sounds have been hypothesized to provide cues as to the reproductive success of the sound-producer, because large males have a higher gonadal somatic index (GSI) than small males of the same species (e.g. Duarte and Alcaraz 1989, Jennings and Philipp 1992). For example, large male seabream (*Diplodus sargus*) had a significantly higher GSI when compared to small males. If there is a direct relationship between length and F_0 (Fish and Mowbray 1970, Connaughton et al. 2000, Gannon 2007) and therefore reproductive success, a female may choose a mate on the basis of size or social status (e.g. Magnhagen and Kvarnemo 1989, Bisazza and Marin 1991, Nelson 1995). In Trinidadian guppies (*Poecilia reticulate*), female fish showed a preference for large males over small males because the large male fish sired offspring that grew faster than the offspring of small males. The result was daughters with high reproductive output and large sons, because the sons inherited their size from their fathers (Reynolds and Gross 1992). If females choose males on the basis of size and potential reproductive success then, in sound-producing fishes, a female fish may be able to obtain size information acoustically about males.

Swimbladder and Sonic Muscle Anatomy and Physiology

The swimbladder-sonic muscle mechanism occurs widely in sound-producing teleostean fish species but the anatomy can vary within and across species, which may result in a variation in the F_0. Some fishes have intrinsic sonic muscles, attached directly to the swimbladder wall, as is the case in the family Batrachoididae (Tower 1908, Tavolga 1964). They are typically composed to two muscle bands that extend laterally down the dorsal surface of the swimbladder (Tavolga 1964) with a sonic nerve that attaches to the swimbladder, while axons and motor neurons innervate the sonic muscles (Pappas and Bennett 1966, Bass and Baker 1990). The source of neuronal firing, initiating muscle contraction, is the “pacemaker’ neurons in the vocal-motor pathway (Bass and Baker 1990). Neurons extend from the forebrain to both the hindbrain and spinal cord. Stimulation of the forebrain yields an output from the hindbrain-spinal connection which produces a rhythmic response in the motor neuron, and then the pace-maker establishes the firing rate of the motor neurons. The firing rate and duration of the motor neurons determines the F_0 and the duration of the sound produced by the fish (Bass and Baker 1990). Other fishes, like Atlantic croaker, use extrinsic sonic muscles that attach both to the swimbladder and another structure such as the vertebrae, skull, ribs, and other muscles (Tavolga 1964). Extrinsic sonic muscles indirectly vibrate the swimbladder through an "elastic spring" mechanism (Sőrensen 1895). The sound-production pathway and how it affects F_0 for fishes with indirectly vibrating mechanisms remains to be explored.

Currently, there is a debate over the function of the swimbladder and sonic muscles in the production of the F_0 of an acoustic call. The majority of the conflict stems from the type of musculature (intrinsic vs. extrinsic) associated with the swimbladder. Fishes with extrinsic sonic muscles produce transient sounds, such as the "purr" of a weakfish or the "croak" of an Atlantic croaker. The frequencies they produce are believed to depend on the properties of the sonic-mechanism itself (Sprague 2000). Thus, the sonic muscle mass, the state of sonic muscle development, swimbladder size, and temperature will directly influence the F_0 since the sonic muscles vibrate the swimbladder (Connaughton et al. 1997, Connaughton et al. 2000, Sprague 2000). However, fishes that have intrinsic sonic muscles make steady-state sounds such as the "boop" produced by the oyster toadfish (*Opsanus tau*) (Sprague 2000). In these species, the muscle contraction rates determine the F_0 because muscle contractions are set and sustained by the neuronal pacemaker. These intrinsic-muscle species are able to modify the F_0 produced by the length of time the muscle contracts (Fine et al. 1997, Fine and Thorson 2008, Fine et al.

2009). However, the rate of muscle contraction can vary with temperature and spawning season (Fine 1978, Fine and Thorson 2008). Because temperature causes variations in the F_0 for weakfish (Connaughton et al. 2000), it is necessary to account for the internal sonic muscle sizes as well as temperature.

Using Passive Acoustics to Monitor Atlantic Croaker

One method that may be useful in monitoring the change in growth of a sound-producing fish population is passive acoustics (e.g. Luczkovich et al. 2008a). This is a method in which natural environmental sounds (e.g. fish sounds) are recorded using low frequency (<10 kHz) hydrophones and digital audio recorders. These systems are used to gather information about fishes and other marine organisms to infer information about behavioral patterns such as feeding, aggression, courtship, and spawning (Luczkovich et al. 1999, Luczkovich et al. 2008a, Luczkovich and Keusenkothen 2007, Luczkovich et al. 2008b). In addition, it is a non-invasive strategy, which provides a unique opportunity to monitor fish behaviors without the risk of human-influences (Rountree et al. 2006). Autonomous digital sound recorders that record fish sounds can be deployed for a long period of time. These systems record the sounds within the surrounding water column for a specified time-interval throughout the sampling period. Sound-production by fishes has been utilized by fishermen for hundreds of years to locate sound-producing fish stocks but passive acoustic technology has only recently been invented and applied to fisheries management strategies (Rountree et al. 2006).

For example, one may be able to determine the location and time of spawning for Atlantic croaker using passive acoustic methodology. Luczkovich et al. (2008b) showed habitat preferences during spawning behaviors for various fishes in the family Sciaenidae including silver perch (*Bairdiella chrysoura*), weakfish, spotted seatrout (*Cynoscion nebulosus*), and red drum (*Sciaenops ocellatus*) but they did not look at habitat preferences for Atlantic croaker. Acoustic responses have also been correlated with egg production in silver perch (Luczkovich et. al.1999), spotted seatrout (Mok and Gilmore 1983), white seabass (*Atractoscion nobilis*) (Aalbers and Drawbridge 2008), and red drum (Guest and Lasswell 1978, Lowerre-Barbieri et al. 2008). In addition, scientists may use the vocalizations of fishes to understand something about the size-structure and growth of the population. For example, Connaughton et al. (2000) showed that there is a relationship between F_0 and length in weakfish. It has also been noted that the call of the male bicolor damselfish (*Stegastes partitus*) exhibits a linear relationship between F_0 and *SL* as well as weight (*Wt*, g) (Myrberg et al. 1993) but these were laboratory studies. When we try to utilize the F_0 of a species to obtain information about the population, it is necessary to take into account additional physical and biological factors that may influence acoustic signals and their respective F_0.

Passive acoustics can also be used to understand the behavioral mechanisms associated with sound-production. For example, Luczkovich et al. (2000) noted that sound pressure levels of silver perch decreased significantly when exposed to bottlenose dolphin (*Tursiops truncatus*) calls. Because bottlenose dolphins are known to be predators of silver perch (Barros and Odell 1990), Luczkovich et al. (2000) hypothesized that the silver perch's response may be avoidance behavior. Acoustics has also helped researchers to observe movement patterns in the Goliath grouper (*Epinephelus itajara*) (Mann et al. 2009). The authors noted that during a hurricane the acoustic production of grouper did not significantly change.

Autonomous recorders allow researchers to observe behaviors under normal environmental conditions during times when human safety becomes an issue (e.g. hurricanes) and for time periods that are otherwise not feasible (i.e. days – months – years). If passive acoustics can be used to estimate growth and length distribution of sound-producing fishes, it will prove to be a valuable management tool, not only to understand Atlantic croaker size-structure and growth, but also fish behavior in association with natural and anthropogenic noise.

Using Active Acoustics to Monitor the Fish Community

Another method that is useful for monitoring fish communities is active acoustics. This method came about after World War II when researchers began to use it as a quick and efficient way to monitor fishes (Devold 1950). Active acoustics uses a transducer to produce a pulsed sound of a known sound pressure level, duration, and frequency which is received at the receiver (e.g. transducer). The signals can be obtained either as echoes off the bottom or as acoustic backscatter from organisms within the water column (MacLennan and Simmonds 1992, Gunderson 1993). This allows us to compute the range of an object using a series of equations, if the speed of sound and the time for the echo to return to the transducer are known. The process begins with the Sound Navigation and Ranging (SONAR) equation:

$$EL = SL' - 2TL' + TS, \qquad \textbf{(Eq. 1)}$$

where *EL* is the received echo level, *SL*' is the source level of the emitted signal, *TL*' is the transmission loss, and *TS* is the target strength (Kinsler et al. 2000). After an acoustic wave is emitted from the transducer, it ensonifies a "target" with a given intensity, the target then scatters the sound. Some of this reflected sound is sent back-up to the transducer (receiver) as an acoustic echo. The receiver modifies the acoustic echo to a distance of 1-m in front of the receiver. This corrects for the distance of the target from the transducer since some acoustic energy will be lost due to travel. Because the echo has less energy than the emitted signal, the received-strength is always less from the echo (i.e. the value received is negative). The transducer receives this echo as an acoustic cross section (σ) of the target:

$$\sigma = {}^{4\pi I_s(1)}\!/_{I(r)} = {}^{W}\!/_{I(r)}, \qquad \textbf{(Eq. 2)}$$

where W is the time averaged power of the received signal that is expressed in terms of 4π times the intensity of the target (I_s) as seen by the transducer (i.e. uncorrected) and where $I(r)$ is the received intensity of the ensonified target. The acoustic cross section does however vary with the orientation of the target and the angle of acoustic beam, both when emitted and after the echo is received from the target. Thus, the echo level of the target at the receiver is calculated using:

$$EL = 10\ log\left({}^{I(r)}\!/_{I_{ref}} * {}^{\sigma}\!/_{4\pi}\right) - TL', \qquad \textbf{(Eq. 3)}$$

where basically the echo level is the source level (10 log[$I(r)/I_{ref}$] minus the transmission loss (TL') of the acoustic signal. Hence the relationship between the acoustic cross section and target strength is as follows:

$$TS = log_{10}\ (\sigma/4\pi). \qquad \textbf{(Eq. 4)}$$

Because *TS* generally is a factor of size, shape, construction materials, orientation of the target with respect to the transducer, and the frequency of sound, Equation 4 needs to be modified accordingly to obtain *TS* for specific objects (Kinsler et al. 2000).

Two techniques have been developed to use the *TS* in an attempt extrapolate information about a fish population or community. The first method was developed by Dragesund and Olsen (1965) and is called Echo-Integration. Here the voltage from the backscatter of an acoustic signal is squared and summed over both depth and distance, thereby producing a *TS* for a school of fish (Misund 1997). This method estimates fish abundance and distribution within the water column when there are many targets (e.g. school of fish) that are causing a single echo (Foote 1983). The second method is called Echo Counting and was developed by Trout et al. (1952).

Echo Counting is used when fishes are less dense within the water-column because a single echo is used to individually count and estimate fish density. This method can also be used to determine the length-structure of the fish community (MacLennan and Simmonds 1992). The result is the ability to compare abundance and length distribution data obtained from both nets and active acoustics.

The benefit of active acoustics is that it is assumed to be both non-invasive and non-selective, unlike traditional fisheries gear (nets) (e.g. Dalen and Nakken 1983, MacLennan and Simmonds 1992, McCatchie et al. 2000, Axenrot and Hansson 2004). However, there are some problems with using active acoustics. It is necessary to know the *TS* of a fish and how the *TS* relates to fish length. The equipment is also expensive and it is necessary to train personnel to both run and interpret the results (MacLennan and Simmonds 1992).

However, if one can overcome the expense and use this method to estimate fish lengths, a better understanding to fish abundance, size-structure, and distribution will be more accurate than that of traditional fisheries methods (MacLennan and Simmonds 1992, Misund 1997). Presumably, simultaneous fish collections can be made in order for researchers to understand the species distributions. Active acoustics can also help researchers understand net avoidance behavior and the size-selectivity of their nets and how they influence community estimates (e.g. Fabrizio et al. 1997, McClatchie et al. 2000, Axenrot and Hansson 2004).

Love (1971) came up with an equation that represents the relationship between *TS* (dB) and length (*L*, ft) of fish ensonified by active acoustics. His equation includes an acoustic wavelength (λ) variable, where:

$$TS/dB = 19.1\ Log_{10}\ (L/ft) + 0.9\ Log_{10}\ \lambda/ft - 34.2, \qquad \textbf{(Eq. 5)}$$

however, there is much debate over whether this equation can accurately represent the lengths of all fishes within a community. Foote (1980) determined that the most influential factor in the production of a *TS* from a fish is the swimbladder but Frouzova et al. (2005) has recently shown that the axis of insonification explained up to 84% of the variability in the *TS* and that total length (*TL*) and species composition explained less of the variability (14% and 0.6%, respectively). Therefore, they determined that *TS* is more-likely to be influenced by body area rather than body volume (Frouzova et al. 2005). Several authors have stressed the importance of species-specific *TS* rather than using a community wide estimate like that of Equation 5 (e.g. McClatchie et al. 1996, Boswell and Wilson 2008) but there are limitations when using acoustics to measure the fish community rather than a specific species. Von Szalay et al. (2007) stated that species richness and dominance are important when considering the use of active acoustics. If a species is dominant in a system, then a species-specific echo-integration method should accurately measure the population structure for that species; however, if a species is not dominant in the system, the species-specific method will be erroneous. Therefore, Mehner (2006) showed that in general, Equation 5 performed similarly to that of the species-specific regression that they created using a computer modeling program (CMIX) (de la Mare et al. 2002) This implied that Equation 5 was an accurate representation of the length distribution of the fishes observed on the active acoustic device (Mehner 2006). Considering the limitations of the gear and the community structure, this research used Equation 5 to estimate *TL* from *TS* of all fishes detected on the active acoustic device. While this is not the most precise method, it is the method necessary for this research given the limitations of the gear, site, and species richness and dominance.

Objectives and Hypotheses

Atlantic croaker vocalizations were recorded in the laboratory to determine if the sex and length of an individual fish can be predicted from F_0. If a length and/or sex relationship with F_0 can be established, a non-invasive strategy to monitor Atlantic croaker populations in-situ in the Pamlico Estuary can be established, which may provide additional information about fish behavior, population size-structure, and growth to better manage the stock. To ensure the best possible length estimate for the fish community, active acoustics was used to measure the *TS* of fishes present in-situ. Additionally, nets (gillnets and trawls) were set to collect fishes in order to monitor the length-structure of the community to compare with both acoustic techniques. The length estimates across the four gear-types (active acoustics, passive acoustics, gillnets, and trawl nets) were compared to determine the validity of using passive acoustics to measure the length-structure of the Atlantic croaker population within the Pamlico Estuary. The goal of this study is to determine if Atlantic croaker length-structure can be monitored using passive acoustics. The specific objectives are:

Objective 1: To measure the length and F_0 of individual Atlantic croaker over a range of sizes in the laboratory.

Objective 2: To measure the F_0 of male and female Atlantic croaker over a range of sizes in the laboratory.

Objective 3: To determine the effect of maturity on the F_0 of Atlantic croaker in the laboratory.

Objective 4: To determine the effect of the size of the internal structures (gonads, sonic muscles, and swimbladder) on the F_0 of Atlantic croaker over a range of sizes in the laboratory.

Objective 5: To estimate the mean length of Atlantic croaker using trawls and passive acoustics at two sites in the Pamlico Estuary.

Objective 6: To estimate the mean length of Atlantic croaker using nets (gillnet and trawl) and active acoustics.

The hypotheses of this study are:

Ha_1: The F_0 of an Atlantic croaker call is inversely related to fish length.

Ha_2: The length-adjusted F_0 of an Atlantic croaker call is higher in female fish when compared to male fish.

Ha_3: The F_0 of an immature Atlantic croaker call will be higher than a mature Atlantic Croaker call.

Ha_4: Atlantic croaker with longer sonic muscles will have a lower F_0 when compared to croaker with shorter sonic muscles.

Ha_5: Atlantic croaker with a large swimbladder will have a lower F_0 when compared to croaker with a small swimbladder.

Ha_6: Atlantic croaker with large gonads will have a higher F_0 when compared to croaker with small gonads.

Ha_7: The mean length of Atlantic croaker using trawls and passive acoustic recorders will not be the same.

Ha_8: The mean length of Atlantic croaker using nets and active acoustics will not be the same.

MATERIALS AND METHODS

Long-Term Monitoring Field Stations

The Albemarle-Pamlico Estuarine System is the second largest estuary in the U.S. and is located in eastern North Carolina. This system is comprised of seven sub-estuaries, one of which is the Pamlico Sound that connects to the Atlantic Ocean through several inlets in the Outer Banks and has its freshwater inputs from both the Pamlico and Neuse Rivers as well as their various tributaries. Generally, the Pamlico Sound is considered to be a shallow-water estuary with an average depth of 4.8 m (Wells and Kim 1989). This brackish water system is the spawning and nursery ground to a variety of soniferous fishes, including the spotted seatrout, red drum, weakfish, silver perch (Luczkovich et al. 2008b), and Atlantic croaker (Currin et al. 1984, Hettler 1989, Hettler and Chester 1990, Warlen and Burke 1990, Forward et al. 1999).

Two sites (Figure 1) were chosen in the Pamlico Estuary to monitor both fish acoustics and water quality with Instrumented TriPods (ITPods) (Figure 2) from June to November 2008. One site, the Pamlico Mouth Site (PMS), was in the Pamlico Sound at 35.250702° N latitude and 76.435620° W longitude. The second site was Potash Corporation of Saskatchewan (PCS) and was located at 35.385650° N latitude and 76.742567° W longitude in the Pamlico River. Both sites had mud bottoms with similar depths, average temperatures, and average salinities (Table 1). At both sites Long-term Acoustic Recording System (LARS, <10kHz, Loggerhead Instruments Inc. Sarasota, FL) digital recorders were used to record ambient sounds, Hydrolab DS5X (Hach Environmental, Campbell Scientific Inc. Edmonton, AB Canada) Sondes recorded temperature, salinity and dissolved oxygen, Optical Backscatter Sensors (D&A OBS-3, 8Hz sampling rate, Campbell Scientific Inc. Edmonton, AB Canada) recorded turbidity, and Nortek Aquadopp Acoustic Doppler Current Profilers (2Hz sampling rate, Nortek Inc. Rud Norway) recorded currents hourly, and Nortek Vectors (8Hz sampling rate, Nortek Inc. Rud Norway) recorded waves hourly. Each instrument contained an internal hard drive to record information about the sites at specified time intervals (Table 2).

Atlantic Croaker Collection

Mature and young of year (YOY) Atlantic croaker were collected at each site once a month from June until November 2008 (ECU-AUP #D222). An experimental gillnet and a juvenile otter trawl were deployed on the bottom at each location to collect fishes. The 45.7-m long and 2-m tall experimental gillnet was deployed at approximately 3-m depth between 1830 and 1230 hours local standard time and contained five panels (38.1, 50.8, 63.5, 76.2, 88.9-mm stretch mesh). Three 120-s trawls were deployed between 2000 and 0400 hrs local standard time. The trawl extended approximately 20 m behind the stern of the vessel and had a 7-m head rope (3.2 m opening) and tickler chain with a bag stretch mesh of 10-mm and a cod-end stretch mesh of 3.2-mm.

All collected fishes (gillnet and trawl), except Atlantic croaker, were euthanized in MS-222 (250-mg/L), placed in a marked plastic bag, and put on ice in a cooler. Atlantic croaker were kept alive, transferred to a live-well, and transported to an aquarium holding facility at East Carolina University (ECU-AUP #D222). All other fishes were identified to species, and the total number (N), *Wt*, *SLs*, and *TLs* were recorded for each specimen. If more than 20 individuals of a single species were collected in a sample, a subsample of twenty fishes was selected at random to obtain length and weight information.

Individual Fish Analyses

Laboratory Atlantic Croaker Passive Acoustic Recordings

Atlantic croaker were allowed to acclimate to the ECU facility for a minimum of 24 hrs prior to acoustic data collection. The temperature of the laboratory water averaged 23 ± 3°C (standard deviation, SD). Fishes were fed frozen brine shrimp, freeze-dried shrimp, or freeze-dried blood worms twice daily. Any uneaten food was removed from the tank. Each fish was individually transferred into a 20-L bucket with water from the aquarium system and an InterOcean omnidirectional low-frequency hydrophone (model T-902, frequency range 20 Hz - 10 kHz, hydrophone sensitivity -195 dB Nominal re 1 V/μPa). The hydrophone was connected to a SONY DAT recorder (model TCD-D8) with a digital audio tape and placed in the center of the bucket, halfway between the surface of the water and the bottom of the bucket. The fish was held by the caudal peduncle between the thumb and forefinger approximately 1 to 2 cm to the right of the hydrophone. Any elicited sounds were recorded for a 60-s period. Each fish was then euthanized using a mixture of MS-222 (250 g/L) and aquarium water in a second 20-L bucket. Fish were tagged, placed on ice, and dissected.

Dissections

After euthanization each Atlantic croaker was measured for *TL* (mm), *SL* (mm), *Wt* (g) and dissected to obtain the length (mm) and weight (g) of the gonads, sonic muscles, and the swimbladder. Sex was determined by visual examination for all mature fishes. For non-mature individuals (if they could not be visually assigned to a sex group), the gonads were fixed for 24-hrs using 10% formalin. Following the 24-hr fixation, the gonads were preserved in 95% anhydrous ethanol for 24 hrs with liquid changes at 24, 48, and 72 hrs. Gonads were held in 95% anhydrous ethanol prior to histological preparation. To prepare the gonads for paraffin penetration, each gonad was held in a new 95% anhydrous ethanol fluid for 1800 s, followed by two 1800 s 100% anhydrous ethanol washes, two 1800 s 50:50 mix of 100% anhydrous ethanol and xylene mixture, two 1800 s 100% xylene washes, a two 50:50 mix of xylene and paraffin that were placed in a paraffin oven held at 129°C for 1-hr to allow xylene evaporation, and last the tissue was transferred into 100% paraffin and held in the oven for 1 hr (this step was repeated three times). After the final paraffin wash, the tissue and remaining paraffin were poured into a metal mount, covered with a plastic cover and allowed to sit for 24 hrs. Next, the gonads set in paraffin were removed from the plastic mount after being placed in the freezer for a period of 10 min. A sharp razor was used to trim away the excess paraffin from the area where the gonad was preserved. Each set-gonad was trimmed in the shape of a trapezoid and trimmed at an angle (where, the top-most portion was slightly in from the bottom-most portion) to make sectioning easier. Each sample was placed in a microtome and cut into 10 μm sections.

Slides were prepared to receive these gonadal sections by placing a single drop of albumin on the slide and wiping it off using lens paper. Next, enough distilled water was placed on the slide so that the entire slide was covered. The gonadal sections (in paraffin) were put on top of the water on the slide (this step prevents folds within the sections) and set on a hotplate at 47°C until dry (usually for 24 hrs). Next, all dried slides were placed in the paraffin oven for 1 hr to help adhere the paraffin to the albumin.

The last step in this process was to stain the slides. First, the paraffin had to be removed from the sections. To do this, the slides were placed in xylene for 120-180 s, then in 100%, 95%, and 70% anhydrous ethanol (each for 120-180 s). To rehydrate the gonads so that they could receive the stain, the slides were placed in water for 180-240 s, stained with hematoxylen for 60 s, then in eosin stain for 30 s. Next, the gonadal sections were dehydrated and covered with a cover slip. The stained gonadal sections were dehydrated by 2 dips in 70%, 70%, 95%, 95%, 100%, and 100% anhydrous ethanol each, then two dips in xylene and another xylene bath. It was necessary to dehydrate the sections in multiple ethanol and xylene baths with the same concentrations because over time the concentrations became slightly watered down and multiple baths ensure that the slide preparations were properly dehydrated. The last step in the process was to use crystal bond to adhere the cover slip to the slide over the sectioned tissue.

Multiple sections of the same fish's gonad were microscopically examined for evidence of oocytes or sperm at 40x, 100x, 400x, and 1000x (if needed) by two observers. The presence of oocytes indicated a female (Figure 3a) and the presence of sperm cells indicated a male (Figure 3b). Each observer made his/her own sex determination by looking at the slide sections. If a disagreement arose between the two observers, another slide was prepared with additional sections of the gonad.

Sound Recording Analysis

Recordings from the digital audio tapes were converted to computer 16-bit Waveform Audio File Format (.WAV) files at 44-kHz using a "Creative Sound Blaster" card (Audigy 2 ZS, Creative Technology Ltd. Singapore) on a Windows XP computer. Atlantic croaker sounds were selected based on the pulse length and characteristic waveform as observed in an oscillogram. Recorded sound files were sectioned into individual pulses (Figure 4) using Creative Wave Studio (version 5.01.2600, Creative Technology Ltd. Singapore). Laboratory fish sound recordings were selected for frequency analysis using the following procedure: 1) the fish had to produce a minimum of five pulses within a 60-s recording period; 2) the pulses could only contain a single sound (i.e. any pulses that also contained additional fish movement sounds or background noises were eliminated from analysis); 3) the entire pulse had to be made in the water (if the fish was making the pulse while being placed in the water, the pulse was eliminated); and 4) the pulse could not be muffled (i.e. position of holder's hand could not be over the abdomen). Any pulses that met the above criteria were numbered 1 – n (where n is the total number of pulses that a given fish produced), generally there were less than twenty pulses that met these criteria for any individual fish. Five of these pulses were randomly selected using a random numbers table generated in Microsoft Excel. Each selected pulse was clipped from the 60-s recording using Creative Wave Studio and imported into Matlab (version 7.0.4.365, The MathWorks Inc. Natick, MA) for F_0 analyses using a Fast Fourier Transform (FFT) in the Matlab signal processing/spectral analysis toolbox. Each spectrogram was analyzed visually by a power spectral density curve to determine the F_0 (Hz) of the captive fish (Figure 4). The average of the five randomly selected pulses' $F_{0\text{s}}$ was determined and plotted against *TLs* and *SLs* (mm) using Systat (version 11, SigmaPlot, Systat software Inc. Chicago, IL). A general linear model (GLM) was then estimated to determine the relationship between F_0 and *TL* and *SL*.

The bucket's resonance frequency was determined using the estimate derived from Akamatsu et al. (2002) for a cylindrical fish aquarium:

$$f circular\ minimum/Hz = \frac{c/cm/s}{2}\sqrt{\left(\frac{2.405}{\pi a/cm}\right)^2 + \left(\frac{1}{h/cm}\right)^2}\ , \qquad \textbf{(Eq. 6)}$$

where *f* is the resonance frequency, *a* is the radius of the tank, *h* is the depth of the water in the tank, and *c* is the velocity of sound in water. Therefore, the resonance frequency of the bucket used in this experiment to record Atlantic croaker was 4528-Hz, using the above equation with the speed of sound at 1500-m/s, a tank radius of 0.15-m, and a water depth of 0.31-m. This information allows the removal of the resonance frequency of the tank from the frequency analyses for the captured Atlantic croaker sound-production samples.

Atlantic croaker *TLs*, *SLs*, and *Wts* were used to predict the F_0 produced using the sample of laboratory fish. First, the *Wts* of the fish were $\log_{10}$-transformed. Then, GLMs were created to predict F_0 from the three independent variables: *TL*, *SL*, $\log_{10}$-transformed *Wt*.

Next, an analysis was completed to assess the relationship between F_0 and both maturity and sex in Atlantic croaker. For maturity, a GLM was made with F_0 as the dependent variable and *TL* as the independent variable and with maturity as a grouping variable. Another GLM was created with F_0 as the dependent variable and *TL* as the independent variable with sex as a grouping variable.

Analyses were made to determine how the weight, area, and length of the internal structures (swimbladder, sonic muscles, gonads) of Atlantic croaker influenced the F_0. Weights and areas were $\log_{10}$-transformed. Then GLMs were used with the length, $\log_{10}$-transformed internal structure areas, or $\log_{10}$-transformed internal structure weights as the independent variables and F_0 as the dependent variable. For the internal parameters, the GLM that had the highest R^2 for each internal structure was chosen to compare with *TL*, rather than comparing all lengths and areas for each structure.

Trawl and LARS Length Comparisons

Field passive sound recordings from the passive acoustic device (LARS) were analyzed for Atlantic croaker sounds using the Extensible Bioacoustic Tool (XBAT, see http://www.xbat.org, Cornell Bioacoustics Lab, Ithica, NY) for Matlab. Recordings for Atlantic croaker did not always correspond directly with gillnet and trawl net collection times because of recording instrument failures or environmental background noises. As a result, 24-hrs worth of passive acoustic recordings that contained Atlantic croaker sounds close to the field collection times were selected for analysis (Table 3). In some instances recordings and collections occurred days apart but were within the same week. Therefore, comparisons were made on a monthly, rather than daily basis. For each recording, individual Atlantic croaker pulses were identified within a single 10-s recording and each pulse in the recording was sectioned using Create Wave Studio. The majority of these recordings had less than 7 pulses within a given 10-s recording period. Therefore, a maximum of 7 pulses were analyzed for each 10-s recording period. For recordings with Atlantic croaker pulses where N>7, the 7 best recorded pulses were selected out of the recording (based on the above criteria for selecting laboratory Atlantic croaker pulses in the individual fish analyses section) to evaluate for F_0. Individual pulses within the 10-s recordings were chosen and analyzed for F_0 using the same method as in the laboratory recordings in the individual fish section. The GLM was created to predict *SL* and *TL* as well as *Wt* from the F_0 of an Atlantic croaker "croak" call using the collected laboratory recordings. From the recordings made by the passive acoustic recording device, F_0*s* were used to estimate the *TLs* of the Atlantic croaker in the field. Month and site-specific analyses were made for both the trawled Atlantic croaker and the estimated *TL* of Atlantic croaker on the passive acoustic recordings using an Analysis of Variance (ANOVA). Each gear-type was first analyzed with *TL* as the dependent variable and month as the factor, then for site. Lastly, an ANOVA was used to compare the total lengths for each gear type across both month and site.

Estimating the Lengths of Fishes in the Pamlico Estuary

Length Comparisons of Atlantic Croaker Using Trawls and Passive Acoustic Recordings

In order to compare the fish size-distribution to a method that is not size-selective, a BioSonics (Leary Way Seattle, WA) DT-X echosounder with a 200-kHz transducer (hereafter called active acoustic device) was deployed (Figure 5). It was set to a ping duration of 0.4-ms and a ping rate of 5-Hz. Received pings were recorded on a Panasonic Toughbook's (CF-27) hard drive using BioSonics Visual Acquisition Software (version 5.0.3, BioSonics Inc.). Then BioSonics Visual Analyzer Software (Version 4.1.3.6, BioSonics Inc.) was used to remove the bottom profile from the analysis and to analyze the data for fish distribution and target strengths. Equation 5 was utilized to convert *TS* values into *TL* for individual targets. The length distribution of fishes in the active acoustics, gillnets, and trawl nets were separated into 77 total-length bins (Table 4) because a *TS* represents a range of lengths rather than a single value. These bins allowed for direct comparisons between the gear-types. ANOVA analyses for each gear-type (active acoustics, trawl, and gillnet) were made with month as the factor and the *TL* as the dependent variable. Then the *TL* for the trawl and active acoustics were compared for each month using an ANOVA with gear as the factor and the *TL* as the dependent variable. The same analysis was conducted to compare the gillnet and the active acoustics *TLs*. Next, because trawls and gillnets are known to be size-selective, the fishes TL for each *TL* bin were combined for both the gillnet and the trawl into a "nets" category. An ANOVA was used to determine if there was a significant monthly effect on the *TL* estimate for the nets. Lastly, an ANOVA was used to compare the *TLs* of the active acoustics to nets by month, using *TL* as the dependent variable and the gear as the independent variable.

Comparison of Atlantic Croaker Lengths Based on Nets and Passive Acoustic Recordings

The last step in the process was to compare the trawled Atlantic croaker *TL*s, the estimated *TLs* based on the passive acoustic recordings, and the active acoustics *TLs*. Here again, all *TL* data were placed into the 77 *TL/TS* bins (Table 4) created from Equation 5. First, an ANOVA was used to explore the monthly effect on the *TL* for each gear type (active acoustics, trawls, trawled Atlantic croaker, and passive acoustics), with month and gear as factors and *TL* as the dependent variable. Next, an ANOVA was used to compare the trawled

Atlantic croaker and the passive acoustic device for each month to ensure that the new groupings of *TL* bins did not affect the analysis. Then, an ANOVA, with gear-type as the factor and *TL* as the dependent variable, was utilized to compare the estimates for the trawled Atlantic croaker and the active acoustics by month. Lastly, an ANOVA compared the active acoustics and the passive acoustic recordings by month with *TL* as the dependent variable and gear-type as the factor.

RESULTS

Laboratory Recordings of Individual Atlantic Croaker

Atlantic Croaker Sound Types

Atlantic croaker demonstrated three types of sound-production. The first sound (called a "scrape") (Figure 6a) was elicited by Atlantic croaker with lengths between 50-mm *TL* (38-mm *SL*) and 162-mm *TL* (126-mm *SL*). Of those Atlantic croaker recorded in the laboratory (N=115), 30% of them produced this "scraping" sound. A single pulse from the "scrape" sound was not clearly defined (Figure 6a) and lasted for generally 0.03-0.07-s in length. In addition, the F_0 for this sound was relatively high (5000 – 9000 Hz). Because the "scrape" sound did not predict length in the Atlantic croaker (Figure 7), it was eliminated from further analyses.

A second sound (called a "croak") (Figure 6b), is the same call described as a "disturbance call" by Fine et al. (2004) and as "croaking" by Fish and Mowbray (1970). Both authors reported that the "croak" sound was made by the contraction of the sonic muscles against the swimbladder. Of the 115 analyzed laboratory Atlantic croaker recordings, two fish did not make the "croak." One of these was the smallest recorded individual (50-mm *TL*/38-mm *SL*) and the other was a 84-mm *TL* (65-mm *SL*) fish that was having problems swimming in an upright position (i.e. it was stressed due to handling). Histological examination of the fish revealed that the sonic muscles of the 50-mm *TL* fish were in the early stages of development (Figure 8a), whereas the 84-mm *TL* fish had well-developed sonic muscles (Figure 8b) and therefore should have been able to produce the “croak” sound. The smallest fish collected that was able to produce this sound in the laboratory was 60-mm *TL* (47-mm *SL*); the largest fish caught in the study was 250-mm *TL* (212-mm *SL*) and also produced a “croak” sound. Generally, the duration of a single pulse for the “croak” was less than 0.02-s and had a much lower F_0 (300 – 1000 Hz) than that of the “scrape” sound. This “croak” sound was used in all further analyses.

The last observed call-type produced by Atlantic croaker was not a sound that was elicited from the fish during the holding period. Instead, this call was observed and recorded when the Atlantic croaker were freely swimming around in their tanks. There were two instances where this call (called a “pop”) (Figure 6c) where recorded. The first was after the fish were fed. A 100-L tank was set up with between three and ten Atlantic croaker between 60 and 150 mm *TL*. Video and audio recordings were made when food was introduced. The Atlantic croaker in the tank would carefully circle the piece of food, swimming towards it and picking at it several times before one fish would finally obtain and hold the piece of food in its mouth. Some of the other fish in the tank would follow after the one holding the food and attempt to gain control over the food item. The fish holding the piece of food would sometimes emit a "pop," which was observable from the movement of the abdomen from the contraction of sonic muscles. The other fish then would respond either with a “pop” or would lunge in towards the food item. If an aggressive encounter occurred between two of the fish, several “pops” may have been produced. This behavior would persist until one fish swallowed the prey item or when the other fish no longer attempted to steal the prey item. While the majority of “pop” detections were observed during feeding encounters, these “pop” sounds were also elicited at other times from one fish to the other. Following the acoustic encounter one fish would move away from the other or engage in an aggressive response. These “pop” sounds had the same frequencies at the “croak” call (300 – 1000 Hz) and were similar in duration but they generally contained only a single pulse.

Relationship Between Fish Size and Fundamental Frequency

The F_0 of the Atlantic croaker “croak” sound varied inversely with length; larger fish had a lower frequency call (Figure 9). Standard and total lengths predicted F_0 quite-well (R^2 values of 0.831 and 0.840, respectively; Table 5):

$$F_0/Hz=1066.63 – 3.81\ (SL/mm), \qquad \textbf{(Eq. 7)}$$

-or-

$$F_0/Hz= 1073.95 - 3.12\ (TL/mm). \qquad \textbf{(Eq. 8)}$$

To be consistent with other measurements of fish length in the fish acoustics literature, I will use *TL* as the measure of fish lengths for all further analyses.

Additionally, there was a strong linear-relationship between the F_0 and the $\log_{10}$-transformed *Wt* of the fish (Figure 10). The following equation (R^2 = 0.868, Table 5) can be used to calculate the F_0 of the call from the *Wt* of the fish:

$$F_0/Hz = 981.37 – 263.58\ [\mathrm{Log}_{10}(Wt/g)]. \qquad \textbf{(Eq. 9)}$$

Relationship Between Fish Maturity Stage and Fundamental Frequency

Fish were separated into mature and developing categories based on visual inspection of the gonads to determine if maturity had a role in the F_0 produced by a fish. Fish without developed gonads (i.e. unable to determine sex upon macroscopic observation) were considered developing, while those with developed gonads were called mature. Mature Atlantic croaker ranged from 147 – 250-mm *TL*, while developing individuals were between 63 – 165-mm *TL* (Figure 11a). Mature individuals (N=15) with an average *TL* of 175.3-mm (± 23.5-mm SD) had an average F_0 of 525-Hz (± 71-Hz SD), while developing fish (N=41) with an average *TL* of 87.5-mm (± 29.3-mm SD) had an average F_0 of 792-Hz (± 117-Hz SD) (Figure 11a). While maturity did help predict F_0 (GLM, R^2= 0.561, F<0.001, Table 5), it is highly correlated with *TL* (Pearson Correlation Matrix, N=56, R=-0.818, p<0.001).

Relationship of Sex and Fundamental Frequency

Atlantic croaker of both sexes produced sounds in the laboratory. Male fish were the greatest proportion of the mature individuals tested (0.87) and female fish were the greatest proportion of developing individuals (0.67). Overall, sex did predict the F_0 of Atlantic croaker (GLM, R^2=0.203, F=13.54, p=0.001, Table 5) but this was based on only two mature females. Therefore, developing Atlantic croaker were separately analyzed and it was found that sex did not strongly predict F_0 (GLM, R^2=0.02, F=0.75, p=0.39, Table 5). It is evident that the F_0*s* of developing Atlantic croaker do not differ between the sexes (Figure 11b), while more research is needed on adult Atlantic croaker.

Relationship of Fundamental Frequency to Internal Structures

The next step was to look at how the length, area, and weight of the sonic muscles, swimbladder, and gonads influenced the F_0 of Atlantic croaker's "croak" sound. The general trend for both sexes was an increase in the length and weight of all measured internal structures with an increase in fish *TL* (Table 6) and *Wt* (Table 7) and a decrease in F_0 with an increase in internal structure length (Figure 12) and *Wt* (Figure 13). Log_{10}-transformed swimbladder area, sonic muscle length, and $\log_{10}$-transformed gonad area were the most important in the prediction of F_0 [GLM, R^2=0.856 (F<0.001), 0.838 (F<0.001), and 0.817 (F<0.001), respectively, Table 5]. In addition, the logarithmic_{10}-transformations of the sonic muscle, swimbladder, and gonad weights were the most influential on the F_0 [GLM, R^2=0.891 (F<0.001), 0.838 (F<0.001), and

0.826 (F<0.001), respectively, Table 5]. However, all of these internal structures are highly correlated with the length (Table 6) and *Wt* (Table 7) of each fish. Individually, the $logarithmic_{10}$-transformation of the sonic muscle weight (mg) and the $logarithmic_{10}$-transformation of the swimbladder area (mm^2) are the two most influential internal structures in the production of the F_0 (Table 5).

Estimating the Lengths of Fishes in the Pamlico Estuary

Length Comparisons of Atlantic Croaker Using Trawls and Passive Acoustic Recordings

Based on the laboratory observations of Atlantic croaker both the length and *Wt* of the fish are good predictors of the F_0 produced by the "croak" sound (Table 5). In this section, I used the F_0 recorded on the passive acoustic recorders (LARS) placed at the two locations in the Pamlico Estuary to estimate the lengths of the Atlantic croaker producing the sounds. This prediction was made by using the F_0 as a predictor of *TL* or *SL* in a new linear regression equation derived from the laboratory measurements:

$$SL/mm = 248.691 - 0.221\ (F_0/Hz), \qquad \textbf{(Eq. 10)}$$

-or-

$$TL/mm = 305.323 - 0.270\ (F_0/Hz), \qquad \textbf{(Eq. 11)}$$

Additionally, one can also estimate the weight (*Wt*) of an Atlantic croaker from its F_0 using:

$$Log_{10} Wt/g = 3.330 - 0.003(F_0/Hz). \qquad \textbf{(Eq. 12)}$$

First, the focus was on the comparison between the mean *TL* of Atlantic croaker estimated using Equation 11 from the passive acoustic recordings and the mean *TL* of the Atlantic croaker collected in the trawl. Then, Equation 5 was used to estimate the *TL* of fish from *TS* from the active acoustic device (BioSonics DT-X echosounder). All estimated lengths were put into 77 *TS/TL* bins (Table 4) with a range of 1.6 mm–12.6 mm (mean 5.0 ± 2.9 mm SD) for each bin. The lengths of all of the fishes collected using the trawl and gillnet were also placed into the 77 *TS/TL* bins, based on the *TLs* measured in the laboratory, and compared to the active acoustics estimate. The last set of analyses compared mean lengths of all Atlantic croaker captured in the passive acoustic recordings, and the mean lengths of fishes collected on the active acoustic device. The predicted Atlantic croaker *TLs* from the passive acoustic recordings were placed in the 77 *TS/TL* bins and compared to the known Atlantic croaker lengths collected in the trawl and all possible Atlantic croaker lengths from the active acoustics device.

Comparison of Atlantic Croaker Lengths Based on Nets and Passive Acoustic Recordings

Atlantic croaker were collected in trawls and recorded on the passive acoustic recording device (LARS) at two sites from May through December 2008. A total of 239 Atlantic croaker were collected using the trawl with an average *TL* of 91-mm (± 32.4-mm) and 571 Atlantic croaker pulse recordings (most were "pop" sounds) were analyzed with an average *TL* predicted of 114.2-mm (± 37.9-mm). There was a strong monthly effect on the mean *TL* of the fish caught at the sites for both the passive acoustic recordings (ANOVA, F=3.82, N=571, df=2, p=0.022) and the trawl (ANOVA, F=652.14, N=239, df=3, p<0.001) (Figure 14). The mean *TL* collected in the trawl net gradually increased (Table 8) from June (74.0 ± 10.3-mm, N=183) to August (122.3 ± 23.2-mm, N=4), August to September (135.9 ± 8.4-mm, N=12), and then again from September to October (150.4 ± 11.5-mm, N=40). The LARS did not show this same relationship. There was an increase in the estimated mean *TL* of Atlantic croaker from June (109.8 ± 33.7-mm) to September (123.6 ± 46.9-mm), but the mean length from September to October (112.8 ± 35.2) decreased (Table 8). The mean *TL*s of Atlantic croaker in the trawl and predicted by the passive acoustic recordings for June and October were significantly different (p<0.001); but for September, the mean lengths were not different (p=0.368, Table 9). The *TL* estimates derived from the passive acoustic device were larger than the lengths of Atlantic croaker caught within the trawl for all observed months.

Both trawls and passive acoustic recordings suggested that there were more Atlantic croaker at the PCS site when compared to the PMS site (Table 8). The trawl collected 197 Atlantic croaker with a mean length of 83.7 (± 26.2-mm) at PCS and 42 Atlantic croaker with a mean length of 123.5 (± 38.1-mm) at PMS from June to December 2008. In comparison, 462 Atlantic croaker sounds were recorded on the LARS in a 24-hr period each month (June, September, October) at PCS, while the LARS at PMS only recorded 109 Atlantic croaker sounds within a 24-hr period each month (June and October). Estimated lengths from these recordings showed that the mean *TL* of Atlantic croaker at PCS was larger (117.3 ± 38.4-mm) than that of PMS (101.0 ± 33.0-mm). Hence, there was a strong site effect for the mean *TL* of fish in the trawl (ANOVA, F=67.05, N=239, df=1, p<0.001) and the passive acoustic recordings (ANOVA, F=16.74, N=571, df=1, p<0.001). Sites were analyzed separately to compare mean *TL* estimates from both gear types. There was a significant difference between the mean *TLs* collected in the trawl and the mean *TL* predicted by the passive acoustic recordings for both PCS (p<0.001) and PMS (p<0.001) (Table 9). Therefore, I conclude that the mean *TL* estimated from the F_0, using Equation 11, of an Atlantic croaker "croak" sound is not accurately predicting the mean *TL* of the trawled population. Because trawls are also known to be size-selective (e.g. Huse et al. 2000, Lauth et al. 2004, Battaglia et al. 2006), I next compared the overall fish community length estimates using the trawl, gillnet, and active acoustics in order to determine if the trawl was selecting a specific size-range.

Length Comparisons of the Fish Community Using Active Acoustics, Trawls, and Gillnets

The fish community was compared by creating *TL* distributions for the gillnet, trawl net, and active acoustics using the 77 *TS/TL* bins (Table 4) obtained from using Equation 5. These data were separated by both gear and month but sites were grouped. There was a significant change in the size distribution across months for the active acoustic device (ANOVA, N=190, F=6.18, df=3, p<0.001), the trawl (ANOVA, N=102, F=3.75, df=3, p=0.014), and the gillnet (ANOVA, N=54, F=3.12, df=3, p=0.034). Therefore, all the data were analyzed on a monthly basis. A comparison between the trawl and the active acoustic device revealed a significant difference in estimated *TL* for June (p=0.001) and November (p=0.040) but not for September (p=0.258) or October (p=0.066) (Table 10). The trawl collected fish (all species combined) had a smaller mean *TL* than that of the mean *TL* estimated using active acoustics (Table 11). Next, a comparison between the gillnet and active acoustics device indicated that the there was a significant difference in the size distribution for September (p<0.001) but not for June (p=0.344), October (p=0.074), or November (p=0.648) (Table 10). Generally, the gillnet collected fish (all species combined) with a mean *TL* that was slightly larger than that of active acoustics (Table 11).

Since the gillnet tended to overestimate and the trawl tended to underestimate the mean *TL* of the fish community, these gears were combined into a “nets” category for comparison with the active acoustic device. There was no significant monthly difference in *TL* (ANOVA, N=137, F=0.45, df=3, p=0.715) when both the gillnet and trawl net were combined, but there was a monthly difference in the mean *TL* measured using active acoustics (ANOVA, N=190, F=6.18, df=3, p<0.001) so the data were analyzed on a monthly basis. When comparing the mean *TLs* from the nets and the *TLs* estimated from the active acoustics, only June exhibited significantly different mean *TLs* (p=0.015) for the fish community. Therefore, it is concluded that active acoustic (echosounder) measurements of the fish community mean *TL* agreed with the mean *TLs* of the fishes captured in the gillnets and trawl nets combined.

Length Comparisons of Atlantic Croaker Using the Trawl, Active and Passive Acoustics

Since the active acoustics accurately represented the fish community length structure in most of the months, the predicted *TLs* of Atlantic croaker from the passive acoustic recordings and the *TLs* of the Atlantic croaker collected in the trawl were placed into the same *TL* bins as the active acoustics (Table 4). Because the trawl tended to be selective towards smaller fish and the active acoustics seemed to appropriately estimate the *TL* of the fish community, a comparison between active acoustics and passive acoustic recordings should help determine if the passive acoustic method is accurately representing the mean length-structure of the Atlantic croaker population. The gillnet did not collect any Atlantic croaker and therefore it was not included in these further analyses.

Because Atlantic croaker can reach over 500-mm *TL* (Ross 1988) and the active acoustics device estimated the largest fish in the community to be 492 mm, all of the *TL* bins (Table 4) were included in the following analyses. Atlantic croaker were not collected in the trawl nor heard on the analyzed passive acoustic files for the month of November so, all analyses were limited to comparisons between June, September, and October. Finally, data from both sites were combined and analyzed. There was a significant monthly effect for the trawled Atlantic croaker (ANOVA, N=41, df=2, F=58.84, $p<0.001$) and active acoustics (ANOVA, N=145, df=2, F=6.22, p=0.003) but not for the passive acoustic recordings (ANOVA, N=100, df=3, F=1.28, p=0.284). Because there was a significant monthly effect for two of the gears, it was necessary to compare data by month.

Both the mean *TL*s of Atlantic croaker in the trawl and the mean *TLs* estimated from passive acoustic recordings were generally smaller than that of mean *TLs* estimated from the active acoustics (Table 12). In addition, the passive acoustic recordings estimated mean *TLs* that were generally larger than the trawled Atlantic croaker (Table 12). When solely comparing the *TLs* from the passive acoustic recordings and the trawled Atlantic croaker, significant differences were observed in June ($p<0.001$) and October ($p=0.003$) but not in September ($p=0.783$) (Table 13). The same result occurred when comparing trawled individual Atlantic croaker *TL*s and predicted *TL*s from the passive recordings, rather than grouping in the *TL* bins. This indicates that the pooling that occurs when using *TL* bins did not affect the comparison results.

Next, the mean *TLs* of the fish community predicted from the active acoustics were compared to both the mean *TLs* from the trawled Atlantic croaker and the mean *TLs* of the Atlantic croaker predicted from the passive acoustic recordings. When comparing mean *TLs* between the active acoustics and the trawled Atlantic croaker, the analyses revealed that there was only a significant difference for the month of June ($p=0.001$) but not for September ($p=0.148$) and October ($p=0.953$) (Table 13). These data suggest that, except for June, the trawl generally represents the Atlantic croaker length-structure (Table 12). However, when comparing the *TL* estimates for the active and passive acoustics, there was a significant difference in all months ($p<0.05$) (Table 13). Thus, it is concluded that the *TL* estimates obtained from the passive acoustic recordings disagree with both the active acoustics and the trawled Atlantic croaker and therefore the predictions based on passive acoustics do not accurately represent the length-structure of the Atlantic croaker population in the Pamlico Sound estuary.

DISCUSSION

Atlantic Croaker Sound Production

Atlantic croaker make three sound types: a “scrape,” a “croak,” and a “pop”(Figure 6). The “scrape” has previously been noted only by Burkenroad (1931) who hypothesized that the “scrape” sound, which he called a “croak,” was produced by pharyngeal stridulation. In this study it was noted that the sound was produced in the anterior-end of the fish. In addition, the frequencies of this sound were between 5000 – 9000 Hz (Figure 7). For *Haemulon sciurus* (Family Haemulidae) which stridulates its pharyngeal teeth, the frequency of sound-production spans between 0 – 8000 Hz with dominance between 1500 – 4000 Hz (Moulton 1958). While the F_0s for the “scrape” sound noted in this study for Atlantic croaker were higher than those noted by Moulton (1958) for *Haemulon sciurus*, it is believed that the resonance frequency (4528 Hz) of the bucket may have led to some of the discrepancies. Thus, the resonance of the bucket may have dominated the frequency signal; over that of the F_0 of the “scrape” sound. I agree with Burkenroad’s (1931) observation and hypothesize that pharyngeal stridulation is a possible mechanism of sound-production for the Atlantic croaker “scrape” sound. However, the sound needs to be explored further in a tank where the resonance frequency does not overlap the F_0s of the sound. The “croak” sound has previously been described by Smith (1905) and Tower (1908) who showed that the sound was made by the swimbladder-sonic muscle mechanism. Additionally, I hypothesize that the “pop” sound, which has never before been described in the literature, is produced by the same swimbladder-sonic muscle mechanism. The frequencies produced from the “pop” were similar to the frequencies produced by the “croak” and they have similar wave forms. More research needs to be conducted to prove that these two sounds have the same mechanisms.

It is also necessary to understand the behavioral relationships that are expressed with each sound type. The "scrape" sound seems to be produced when the fish is approached from the anterior end and is commonly associated with flared opercules. Thus, this sound may be associated with a defense mechanism. The "croak" sound is produced when a fish is disturbed (e.g. held by a human). This elongated, multi-pulsated sound may serve as a warning signal to conspecifics. Lastly, is that of the "pop" sound, which has been observed as a possible aggressive response to conspecifics when competing for food. Here it is hypothesized that Atlantic croaker are territorial, especially during feeding. This mechanism may be a signal used to warn conspecifics prior to a more aggressive encounter.

The F_0 of the "croak" sound for this project ranged from 323 – 936 Hz. Previous work has shown that the Atlantic croaker F_0 for the "croak" sound ranged from 300 – 1000 Hz (Fish and Mowbay 1970). In addition, Gannon (2007) found that Atlantic croaker produced "croak" sounds between 600 – 1200 Hz. Therefore, the results shown in this study generally agree with previous findings. However, Gannon (2007) reported Atlantic croaker sound frequencies that were 200 Hz higher than the findings of this study and Fish and Mowbray (1971). In addition, Gannon's (2007) results showed that Atlantic croaker did not make the "croak" sound at frequencies below 600 Hz, but others have discovered that Atlantic croaker do produce sounds below 600 Hz (Fish and Mowbray 1970, Fine et al. 2004). The disagreement in F_0 in these studies may relate to the limitations (i.e. cutoff frequency) of the 500-m^2 concrete pond with depths ranging from 0.5 – 1.2 m (Gannon 2007). In order to determine the cutoff frequency, which is the point at that sound can no longer travel within the water column (Urick 1983), of the concrete pond used in the Gannon (2007) study, the formula for cutoff frequency for shallow water was used (Urick 1983):

$$f_0/Hz = \frac{c_1/m/s}{4h/m}\sqrt{\frac{1}{1-(\frac{c_1/m/s}{c_2/m/s})^2}}\,, \qquad \textbf{(Eq. 13)}$$

where f_0 is the cutoff frequency, c_1 is the speed of sound in the water, h is the depth of the water, and c_2 is the speed of sound in the substrate. This allows me to estimate the cutoff frequency for the pond in Gannon's (2007) study as 443 Hz [assuming h = 0.85 m, a sandy bottom with c_2 = 1680 m/s (Simpson and Houston 2000), and saltwater with c_1 = 1500 m/s]. As a result a fish producing sounds with a F_0 <443 Hz will not be recorded on the hydrophone unless it is directly under the hydrophone because these frequencies cannot propagate more than 1 m away from the source through the water column. This is due to the maximum wavelength of attenuation in the pond, which is 3.4 m because:

$$h/m = \frac{\lambda/m}{4} = \frac{{}^{c/m/s}\!/_{f/s}}{4}, \qquad \textbf{(Eq. 13)}$$

where λ is the wavelength of a sound at frequency (f) and c is the speed of sound (Urick 1983). So for a 600-Hz sound in saltwater [the lowest F_o observed in Gannon's (2007) study], the wavelength is 2.5 m. If an Atlantic croaker in the Gannon (2007) study's pond was on the edge (i.e. at a depth of 0.5 m), then the maximum distance of propagation would be 2 m. A 600 Hz sound would not propagate unless the water depth was 0.6 m. Yet, the components of the sound that are higher frequencies than the F_0 would continue to propagate in the water column and be recorded on the hydrophone.

In these calculations, it was assumed that the pond used in the Gannon (2007) study had a sandy bottom however; this may not be the case. If the concrete pond contains sediments that are finer than sand and can trap gasses within the porewaters, then the cutoff frequency of the pond could be even higher then this estimate. Research has shown that shallow, sloping ponds with muddy sediments contain gases within their porewaters. The low-frequency components of sounds do not propagate well in this type of environment (Forrest et al. 1993). In fact, in a 200-m^2 clay pond with a depth that ranged from 2 – 6 cm and had a soft sediment composed of both clay silt and leaf litter, sounds below 4 kHz did not propagate well and that the bottom type led to even a higher frequency cutoff compared with a rigid bottom (Forrest et al. 1993). These authors hypothesized that this is due to the gasses trapped in the sediment, because the bubbles themselves are resonators with a specific F_0 (Forrest et al. 1993). This result could mean that the frequencies observed by Gannon (2007) for Atlantic croaker in the cement pond are most-likely the higher frequency components of the sound rather than the F_0. Gannon's (2007) pond would create a "high pass filter," which would lead to a higher F_0 estimate of Atlantic croaker sounds than observed in this and other studies (i.e. Fish and Mowbray 1970, Fine et al. 2004).

In the present study, it was evident that Atlantic croaker produce lower frequency "croak" sounds as they increase in size (Figure 9). Fish and Mowbray (1970) showed Atlantic croaker have F_0 ranges from 300 – 1000 Hz for fish that were 140 – 410 mm long. In addition, Gannon (2007) observed F_0 between 600 – 1200 Hz for fish that were between 69 – 221 mm *SL* (Figure 15). Here fundamental frequencies of Atlantic croaker were shown to be between 323 – 939 Hz for fishes between 66 – 250 mm *TL* and that fishes less than 60 mm *TL* (47 mm *SL*) are unable to make sound using the swimbladder-sonic muscle mechanism. These data suggest that it is then possible to use a linear regression equation to estimate the length (Figure 9) and weight (Figure 10) of individual fish in the Atlantic croaker population within a given area. This relationship is most-likely due to the size of the sonic muscle and swimbladder because both increase in size as the fish grows (Figure12).

Both the sonic muscles and the swimbladder seem to play an important role in the F_0 of the Atlantic croaker "croak" sound. Neither the argument for the swimbladder as the resonator (Sprague 2000) nor the argument for the sonic muscle as the resonator (Fine et al. 1997) that produces the F_0 is supported by these results. It is evident that both are highly correlated to the length of a fish and thus, the larger the fish, the longer the sonic muscles and swimbladder, and the lower the F_0 of sound it produces. It is highly probable that the F_0 component of a fish's vocal call may be caused by both the swimbladder resonance and the contraction rate of the sonic muscles because the vibrator (i.e. sonic muscles) is able to slightly modify the frequency of the bubble (i.e. swimbladder) (Bradbury and Vehrencamp 1998).

This research also suggests that there might be sexual differentiation in the Atlantic croaker "croak" sound ($p=0.001$) but this is based on only two adult females. When I limited the analysis to developing Atlantic croaker, no sexual dimorphism was evident ($p=0.391$). This is expected because Atlantic croaker do not show much sexual dimorphism of the internal structures until a body weight of 100 g (Hill et al. 1987). Of the 91 collected Atlantic croaker that were used for internal structure measurements in this study, only one was over the 100 g (Figure 10). Therefore, it is hypothesized that sexual dimorphism will become evident in the F_0 of the Atlantic croaker "croak" sound in fish that are around 150 mm *TL* or larger. Based on the results here, sexual dimorphism does not occur in the "croak" sound in fish that are smaller than 150 mm (Figure 11b).

Since *TL* is strongly associated with the F_0 of and Atlantic croaker's "croak" call (Table 5), the linear regression derived in Equation 11 may be useful to managers in order to devise a non-invasive management strategy for Atlantic croaker. This study additionally took field passive acoustic recordings of the Atlantic croaker “croak” sound and used them to estimate the mean *TL* of a fish using Equation 11. This step helped to explore the validity of a non-invasive management strategy suggest by Rountree et al. (2006).

Estimating the Lengths of Fishes in the Pamlico Estuary

In order to determine the validity of using passive acoustics in the field, first the legitimacy of using active acoustics to estimate the fish community length-structure using the *TS*/*TL* conversion developed by Love (1971) had to be established. Current literature shows both support (e.g. Foote 1983, Axenrot and Hansson 2004) and contest to this concept (e.g. Fabrizio et al. 1997, Boswell and Wilson 2008). The data in this study suggests that the active acoustic echosounder estimated a larger mean fish size than the trawl nets and a smaller mean fish size than the gillnets (Table 11). When the trawls and gillnets of all catches are combined, the mean fish size does not differ between the echosounder and the net estimates. These data indicate that there is a size-selectivity bias associated with each net type but that active acoustics is not size-selective. Because trawls typically catch small, slow-moving fishes (e.g. Huse et al. 2000, Lauth et al. 2004, Battaglia et al. 2006) while the gillnets collect large fishes with a specific body shape, which is dependent upon the net configuration (e.g. Huse et al. 2000, Millar 2000, Kurkilahti et al. 2002). Therefore, combining the nets should provide a reliable estimate for the community size-structure. When the nets were combined and compared to the estimated *TL* in the active acoustics, there was no significant difference for the fish community length-structure ($p>0.3$). This suggests that active acoustics is unbiased towards length and thus, should provide a good estimate of the fish community length-structure. Therefore, it can be utilized to compare with the trawl and passive acoustic data for Atlantic croaker; in order to determine if the size-selectivity issues observed in the trawl are affecting the comparisons between Atlantic croaker collected using passive acoustics and those collected in the trawls.

The passive acoustics approach did not estimate the length structure of Atlantic croaker acceptably, whereas the lengths of Atlantic croaker collected in the trawl did represent an appropriate mean length structure when compared to the active acoustic device (Table 13). However, it needs to be understood that size-selectivity is still a problem. The overall mean length structure from all the species combined was still significantly smaller than the predicted mean lengths on the active acoustics device (Table 10). The trawl showed that there was a continually growing population of Atlantic croaker (month by month) but a steady population length structure in the passive acoustics *TL* estimates (Figure 14). The active acoustic data followed a similar trend as that of the trawl but not the passive acoustics (Table 12). Therefore, this study suggests that directly using the F_0 recorded on the passive acoustic device is not an appropriate method for estimating Atlantic croaker *TL*. However, there may have been problems with the propagation of sound and thus it is important to model the sound propagation in the environment and use source localization to get a better F_0 estimate for the fish in the area.

Since cutoff frequency may have been a problem in the Gannon (2007) study, I decided to determine the cutoff frequencies at my field sites to decide if they were influencing the results for the passive acoustic length estimation. Using Equation 13, the cutoff frequency for PCS was estimated to be 291 Hz and for PMS it was 257 Hz. This indicates that any Atlantic croaker that was larger than 226 mm *TL* and 235 mm *TL*, respectively that was producing a “croak” or “pop” sound, would not have been recorded by the hydrophone, unless the fish was right next to the passive acoustic unit. This is because frequencies lower than ~250 Hz (PMS) and ~300 Hz

(PCS) would not propagate in the environments. Additionally, the bottom at both sites does contain packed, muddy sediments that may hold bubbles. This could further limit the cutoff frequencies of the environments (Forrest et al. 1993) but the higher frequency components of the acoustic signal would propagate through the water column. When measuring the F_0 of recorded Atlantic croaker on the passive acoustic devices, some of these higher frequency components from distant Atlantic croaker may have been included in the analyses rather than the direct signals from nearby Atlantic croaker. This means that the *TLs* estimated using Equation 11 would have underestimated the length of the fish and thus, predicted smaller fish than were actually in the area. This relationship is certainly evident in September and October when the Atlantic croaker collected in the trawls were over 150 mm *TL* (Figure 14) but were predicted to be over a wider size range when using the *TLs* predicted from the passive acoustic recordings. However, in June, the opposite relationship may have been true; the passive acoustic device estimated a wider range of lengths when compared to the trawl (Figure 14). This phenomenon may be explained by the lengths at which Atlantic croaker begin to produce the "croak" sound. I showed that Atlantic croaker are unable to produce "croak" sounds until 60-mm *TL* (47 mm *SL*). This is in agreement with Hill et al. (1987) that showed that sonic muscle development did not occur until after 45 mm *SL*. In June, several of the Atlantic croaker collected in the trawl were small individuals (<60 mm *TL*) (Figure 14) and unable to produce the "croak" sound. These individuals were caught in the trawl but could not have been recorded on the passive acoustic device. Indeed, small Atlantic croaker (<60 mm *TL*) were predicted from the passive acoustic recordings. These predictions may have been due to fish larger than 60 mm *TL* but were too far away from the recording device causing the propagation of the higher frequency components of their call through the water column since the cutoff frequency of the environments were between

~ 250 and 300 Hz. Therefore, the *TL* predictions for the passive acoustics should be higher than the *TL* estimates in the trawl in months when there are very small (<60 mm *TL*) Atlantic croaker in the area.

There are two methods with which to solve the issue of estimating only direct-on F_0 signals. The first method is to create a computer algorithm, like that of Tiemann et al. (2006) for the signals of sperm whales (*Physeter macrocephalus*), that is able to identify echoes and direct signals from a single omni-directional hydrophone. A second option is using a hydrophone array. This later approach would allow triangulation of the location of a single Atlantic croaker that is recorded simultaneously on at least three hydrophones. This would help to only select nearby fish for *TL* estimates and to determine the distance the fish is from the hydrophones, the closest hydrophone to the fish, and the environmental parameters that may be influencing the F_0 of his call as it propagates throughout the water column (e.g. Tiemann et al. 2004). Either of these methods should improve the *TL* estimates at a specific time.

Another issue that may influence the passive acoustic estimate is that spot (*Leiostomus xanthurus*) also produce sounds using their swimbladder and the sonic muscles (Fish and Mowbray 1970). Their sounds are similar to that of the Atlantic croaker "pop" sound and may have been mistaken for Atlantic croaker sounds from the field recordings. Additionally, the F_0s of the sounds they produce are higher than that of Atlantic croaker (Fish and Mowbray 1970). The trawl collected a higher proportion of spot for June, September, and October (0.58, 0.84, 0.50, respectively), when compared to Atlantic croaker (0.24, 0.02. 0.12, respectively). The spot "knock" call may have been misidentified for that of an Atlantic croaker "pop" call. The only in-lab recordings of spot that are available are those collected by Fish and Mowbray in the 1960s (available at: http://macaulaylibrary.org, Cornell University). An in-depth study looking at the acoustic structure of captured spot needs to be completed in order for researchers to clearly differentiate between the spot and the Atlantic croaker call. Then a computer algorithm that is able to distinguish between the calls of these two species needs to be derived in order to avoid this issue in the future.

The last concern addressed in this paper that may be hampering the estimation of Atlantic croaker *TL* from its F_0 is that of the size-distribution that this study was able to collect in the Pamlico Estuary. Atlantic croaker can grow to a *TL* of over 500 mm (Ross 1988). However, I only collected Atlantic croaker up to 250 mm. This was probably due to the size selectivity of the trawls and possibly selecting gillnets with the wrong size mesh. This means that size/F_0 information is available for fish only up to 250 mm. Larger Atlantic croaker may not follow the same F_0:*TL* relationship as those that are YOY. I used Fish and Mowbray's recordings of Atlantic croaker (available at: http://macaulaylibrary.org, Cornell University) from the 1960s to determine if the linear regression in this study is still applicable for larger Atlantic croaker. Because the acoustics library only had a range of lengths of Atlantic croaker recorded in a single recording, I had to use both mean *TL* and mean F_0 to determine the relationship. Their Atlantic croaker recordings for fish with the mean *TL* of 150 mm have a predicted mean F_0 of 554 Hz, which falls within my estimates for fish of a similar length (Figure 16). When I looked at the F_0 of Atlantic croaker from Fish and Mowbray's 1960s recordings, that had a mean *TL* of 400 mm, the mean F_0 was 277 Hz. This F_0 does not agree with the linear prediction reported in this study. Instead, this point seems to fall on the possible logarithmic regression, similar to what has previously been observed in the Whitemouth croaker (*Micropogonias furnieri*) (Tellechea et al. in press). Therefore, the relationship between F_0 and *TL* may be different from that of Equation 8. It may look more like:

$$F_0/Hz = 2358.71 - 810.34(Log_{10}TL/mm)$$

(Eq.14)

Therefore, it is important to explore the F_0:*TL* relationship for larger Atlantic croaker since the relationship between F_0 and *TL* may change as the fish matures (Figure 17).

CONCLUSION

This thesis explored the relationships between the F_0 of an Atlantic croaker's "croak" call and the size of the fish. It is evident that there is a linear, inverse relationship between Atlantic croaker size and F_0 of the produced sound for fish between the lengths of 50 and 250 mm. However caution should be taken because the relationship may be logarithmic rather than linear when fish larger than 250 mm are included in the analysis. Additionally, both the sonic muscle and the swimbladder size play important roles in the F_0 produced by a given fish. The data reported here support both parameters as drivers of F_0. There were also no observed differences between sexes in the produced F_0 of developing Atlantic croaker. There may be a more defined sexual dimorphism at the mature stage (>100 g) but there were too few adult females in this study to fully explore this hypothesis. The data currently represented here show that *TL* can be estimated from F_0 by using Equation 11.

When conducting the field tests for estimating *TL* from F_0, it became evident that the *TLs* estimated from the passive acoustic devices did not accurately represent Atlantic croaker population length-structure. This difference may have been due to 1) cutoff frequencies of the environment, 2) prediction of *TL* from passive acoustics using echos and indirect sounds rather than direct recordings to obtain F_0*s*, 3) using spot "knock" recordings in the analysis rather than Atlantic croaker "croak"/"pop" sounds, and 4) predicting the *TL* of fish outside the size range of the laboratory derived linear regression estimate (60 and 250 mm). This equation may be inaccurate for Atlantic croaker larger than 250 mm.

While there are sampling design problems and data collection limitations apparent within this research, it is important to understand the implications of a non-invasive passive acoustic method for estimating the *TL* of Atlantic croaker and possibly other sound-producing species. If the acoustic environment can be modeled and the limitations of the passive acoustic approach understood prior to using passive acoustic methodology to estimate the lengths of Atlantic croaker, then the use of this technology to monitor the population is promising. However, more research is needed to clearly define the environmental and technological limitations prior to applying the concept to a field situation.

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FIGURES

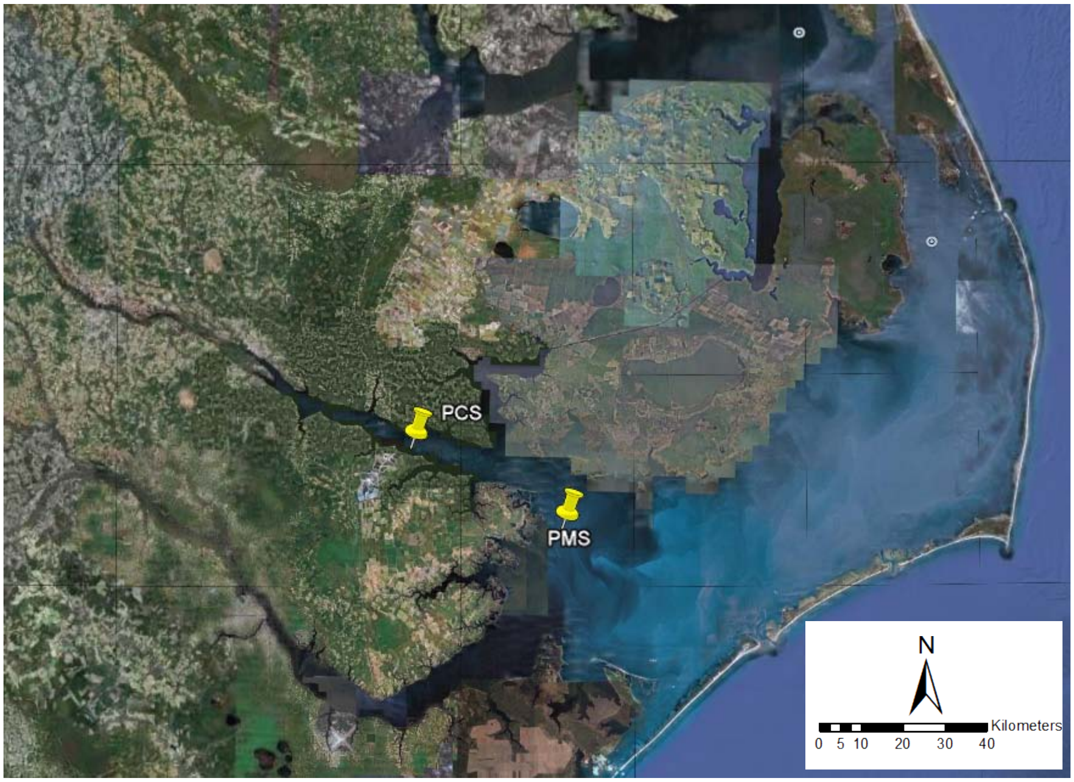

Figure 1: Site locations for the instrumented tripod (ITPod) deployment and fish collections. PMS is located in the Pamlico Sound while PCS is in Pamlico River. Both locations are part of the Albemarle-Pamlico Estuarine System in North Carolina (generated in Google Earth 2010).

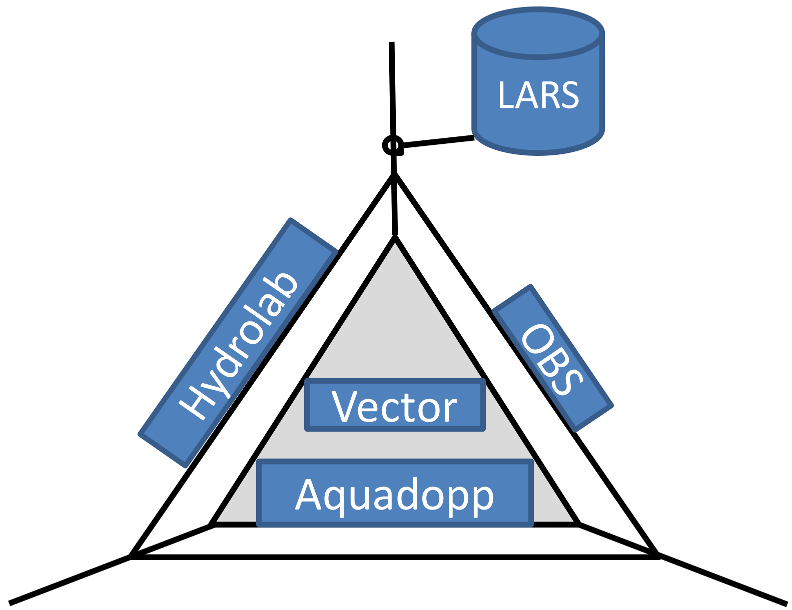

Figure 2: Instrumented tripod (ITPod) set-up. All equipment was deployed on a metal tripod and placed on the bottom of the Pamlico, except the long-term acoustic recording system (LARS), which was not directly attached to but placed near the tripod. The LARS recorded ambient sounds. The Hydrolab Sonde recorded water temperature, salinity, dissolved oxygen, conductivity, and turbidity. An Aquadopp measured the speed and the direction of currents throughout the water column. The Optical Backscatter Sensor (OBS) measured turbidity using acoustic backscatter calculations. Lastly, the Vector used acoustics to measure current velocity and wave movement at a fixed depth.

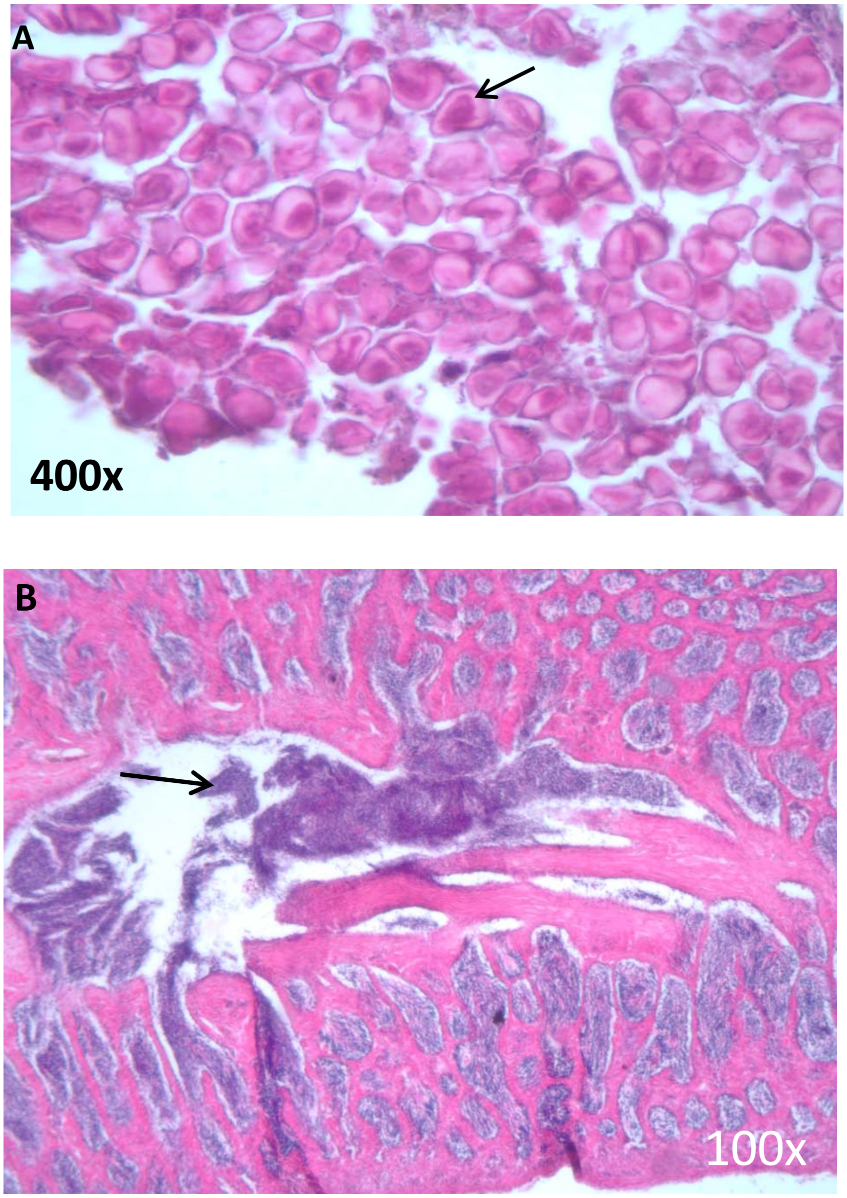

Figure 3: Microscopic examination of the histology of Atlantic croaker gonads dyed with hematoxylin and eosin. A) An 82-mm *TL* Atlantic croaker (Fish ID #271) containing oocytes (arrow) (400X). B) A 158-mm *TL* male Atlantic croaker (Fish ID #507) with sperm (arrow) (100X).

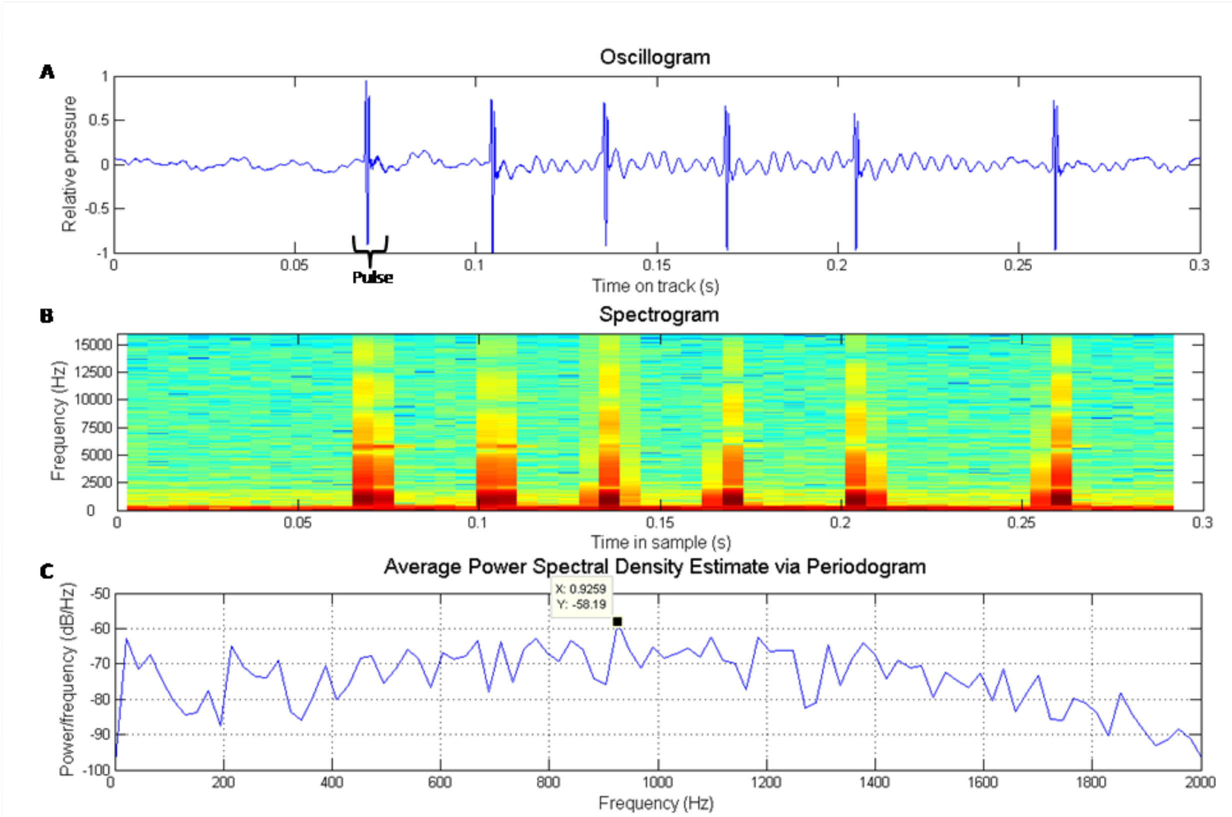

Figure 4: Acoustic signature of Atlantic croaker ID #280, which is 77 mm *TL*. A) Shows an oscillogram of a 0.3-s acoustic response. Note that there are a total of 6 pulses within this acoustic response. B) A spectrogram of the same acoustic response that shows the majority of energy lies below 1000 Hz but some of the harmonics do produce energy at higher frequencies. C) The interpretation of the F_0 using an average power spectral density curve after a Fast Fourier Transform (FFT) for the six pulses produced in this example. The peak power occurs at ~926 Hz.

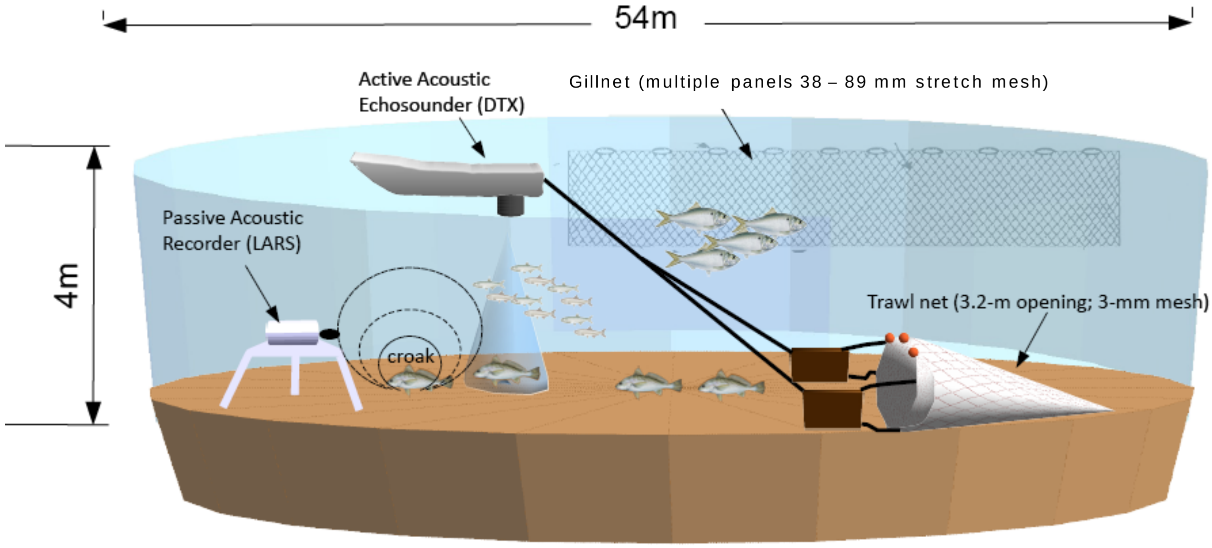

Figure 5: A representation of the sampling design used in this project. All sampling gear was deployed near the location of the ITPod that contained the passive acoustic recording device for recording Atlantic croaker sounds. The gillnet with multiple panels (38.1, 50.8, 63.5, 76.2, 88.9-mm stretch mesh) was set around 1830-hrs and picked-up around 1230-hrs. The trawl and the BioSonics DT-X echosounder were simultaneously deployed between 2000-hrs and 0400-hrs in 3 replicates for 120-s each. Note that the echosounder transducer was actually located on the port side of the vessel, instead of in the center of the vessel. The mean trawl length was 54-m at an average depth of 4-m (image created by J.J. Luczkovich).

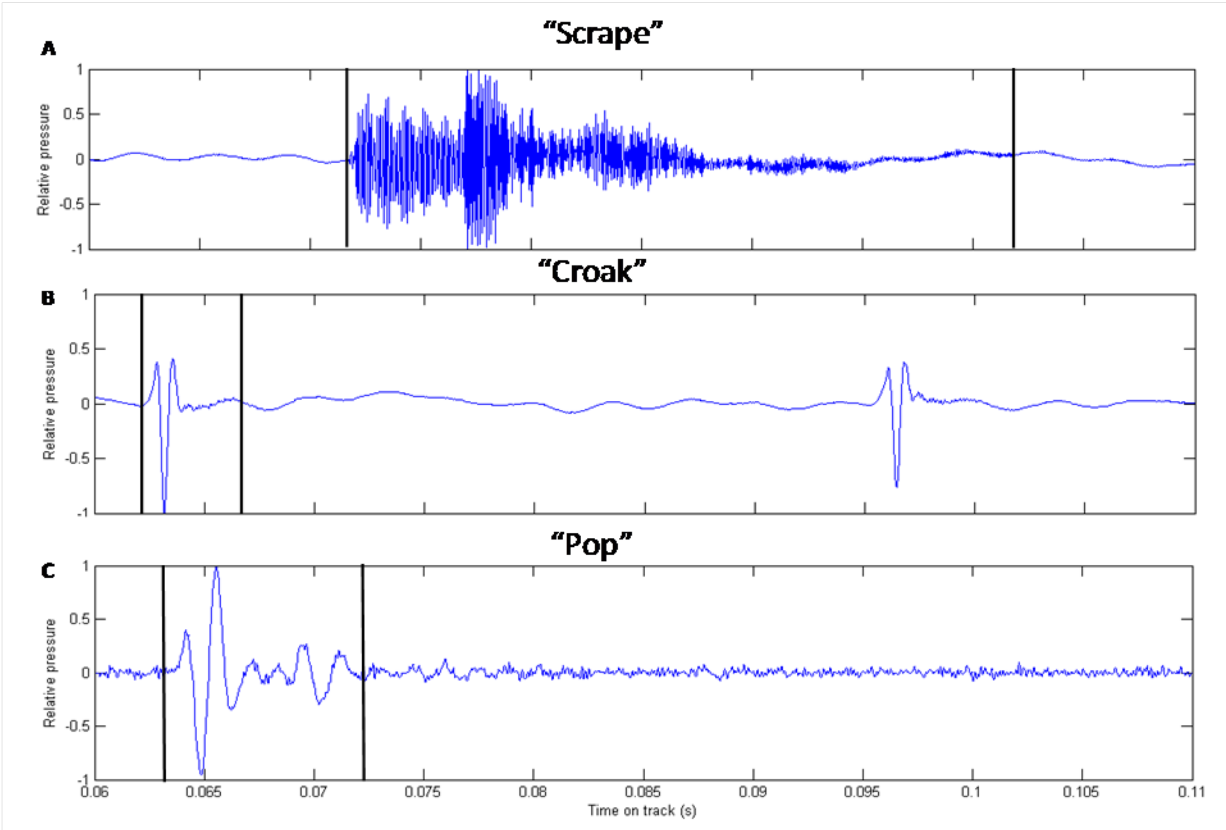

Figure 6: Atlantic croaker have three sound types. A and B were both produced in the same recording session from a single Atlantic croaker (Fish ID #70), which is 162 mm *TL*. A) An example of the "scrape" sound produced by an unknown mechanism; it has a F_0 of ~7900 Hz. B) An example of the "croak" sound produced by the swimbladder-sonic muscle mechanism and has a F_0 of ~660 Hz. C) An example of the "pop" sound produced by an Atlantic croaker. The F_0 of this sound is ~710 Hz. The black lines indicate a single pulse. Note that the "scrape" sound has a much longer pulse length when compared to both the "croak" and the "pop".

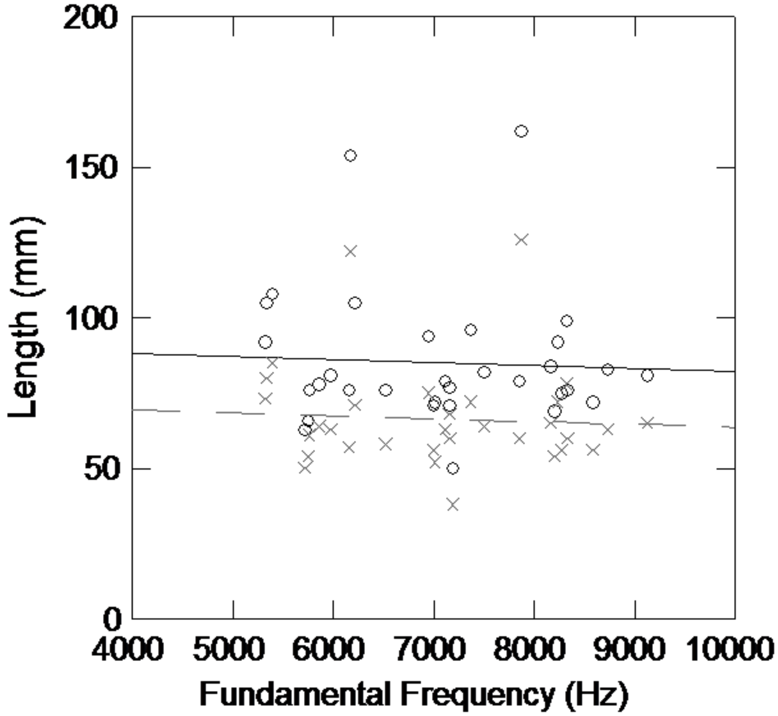

Figure 7: Fundamental frequency (F_0, Hz) for the Atlantic croaker "scrape" sound (N=33) by *TL* (mm). A linear regression analysis indicates that there is no relationship between the F_0 of the scrape sound and the *TL* (o, —) and *SL* (x,- - -) (R^2=0.051 and R^2=0.060, respectively) of the fish. The F_0s produced by this sound type are between 5000-9000 Hz.

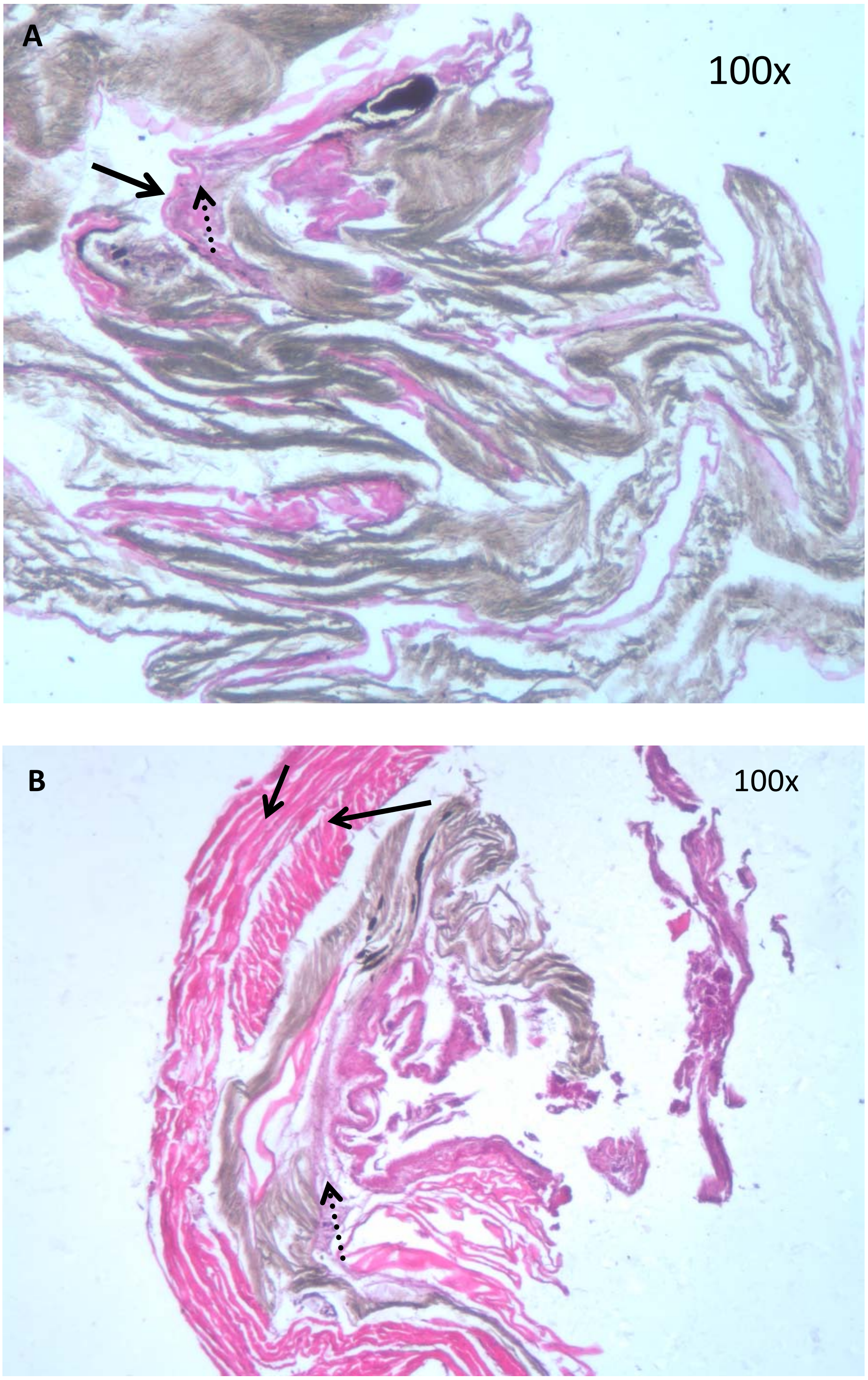

Figure 8: Microscopic examination of Atlantic croaker's sonic muscle (→) and the associated connective tissue (⇢) stained with hematoxylin and eosin. A) Atlantic croaker (Fish ID #201) is 50-mm *TL* with early sonic muscle development (100X). B) Atlantic croaker (Fish ID #208) that is 84-mm *TL*. The sonic muscle of this fish is well-developed; note the well-defined striations that are characteristic of skeletal musculature**.** The unstained material around the sonic muscle is connective tissue that attaches to the abdominal cavity of the Atlantic croaker.

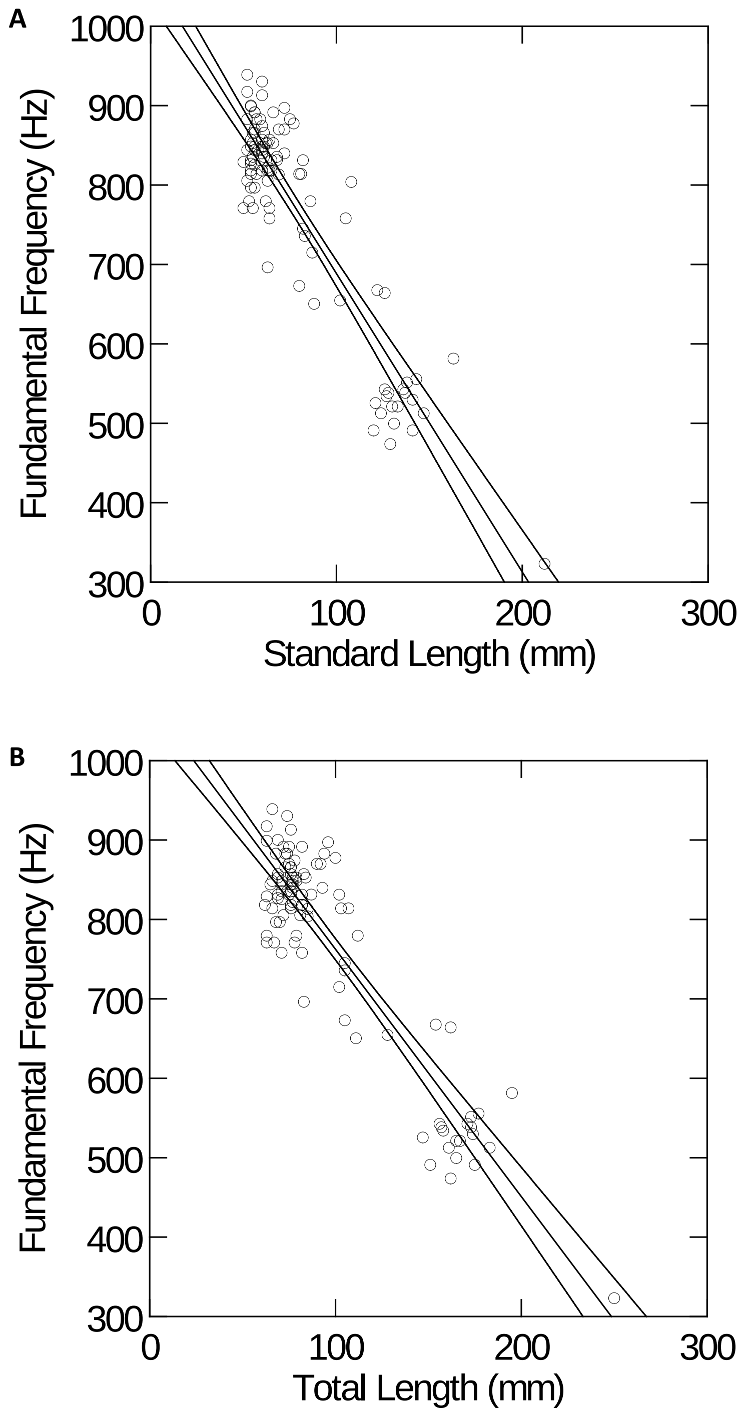

Figure 9: A linear comparison between the F_0 (Hz) and length (mm) of the Atlantic croaker's "croak" sound type. Length can be predicted using F_0 where A) SL = 248.7 – 0.2 (F_0) (R^2= 0.83) and from B) TL = 305.3 – 0.3 (F_0) (R^2=0.84). Note that upper and lower confidence intervals are displayed of the figures for each linear regression equation.

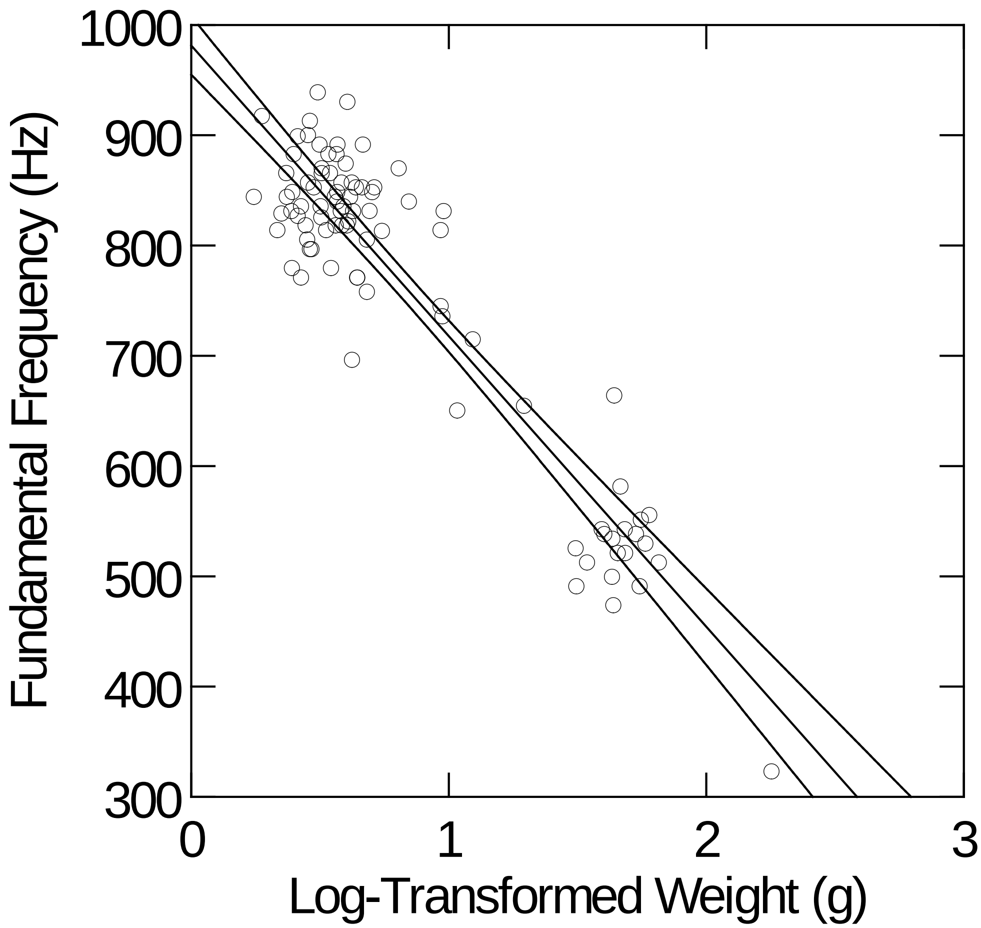

Figure 10: Linear regression comparison between F_0 (Hz) and the $\log_{10}$ of weight (*Wt*) of the "croak" sound produced by Atlantic croaker. Weight can be predicted from F_0 where, $\text{Log}_{10}\ Wt = 7.692 - 0.008(F_0)$ (R^2=0.868). The 95% confidence interval is displayed on this figure for the linear regression equation.

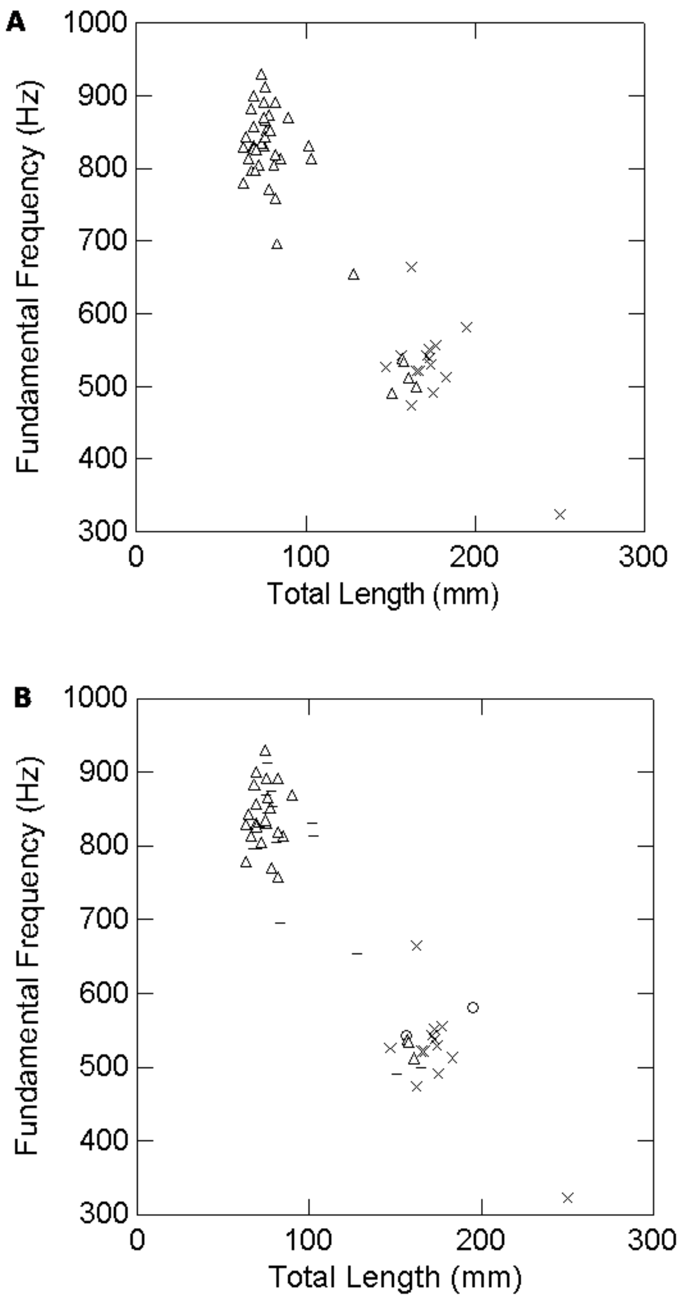

Figure 11: A comparison between *TL* (mm) and F_0 (Hz) for the Atlantic croaker "croak" call by maturity and sex. A) Developing (Δ) and mature (x) Atlantic croaker generally predicted F_0 (R^2=0.56) but the F_0 is strongly correlated with *TL* (R = -0.818). B) Developing male (-), developing female (Δ), adult male (x), and adult female (o) Atlantic croaker *TL*s with their associated F_0. Note, there is no difference between sexes (p=0.618) at the developing stage and there are too few adult females (N=2) to compare sexes during the adult stage.

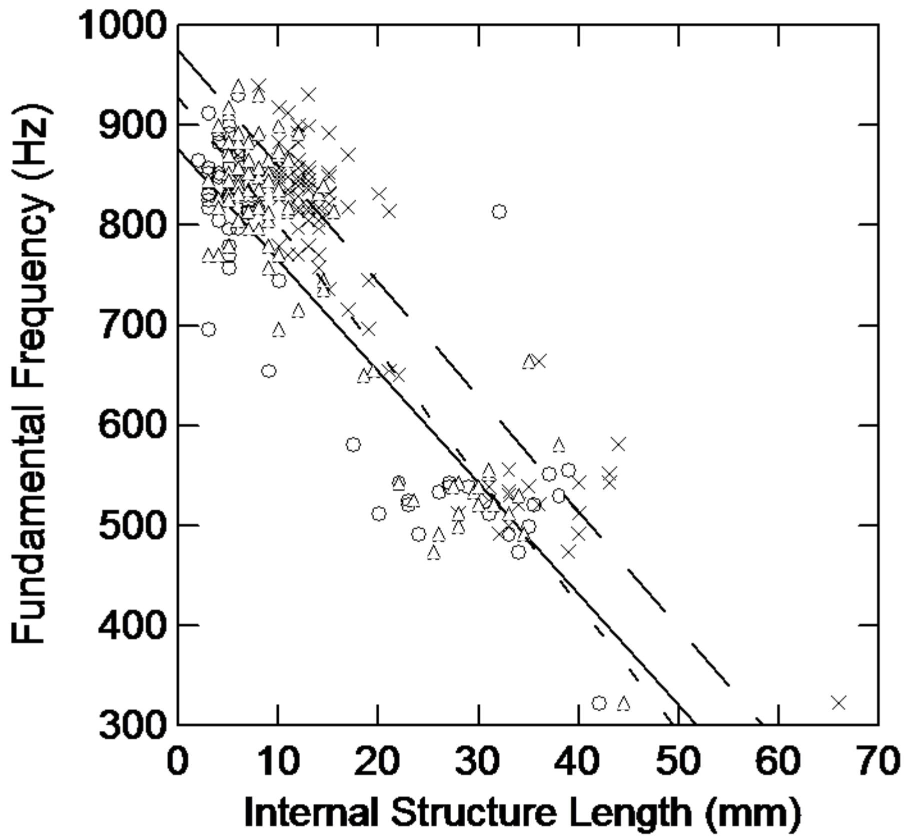

Figure 12: A linear comparison between length (mm) of the internal structures [swimbladder (x, ___), sonic muscles (Δ, ...) and gonads (o, - - -)] and the produced F_0 (Hz) for all Atlantic croaker recorded in the laboratory. Sonic muscle length was the strongest predictor of F_0 when specifically looking at the length/F_0 (R^2=0.832) relationship for the internal structures.

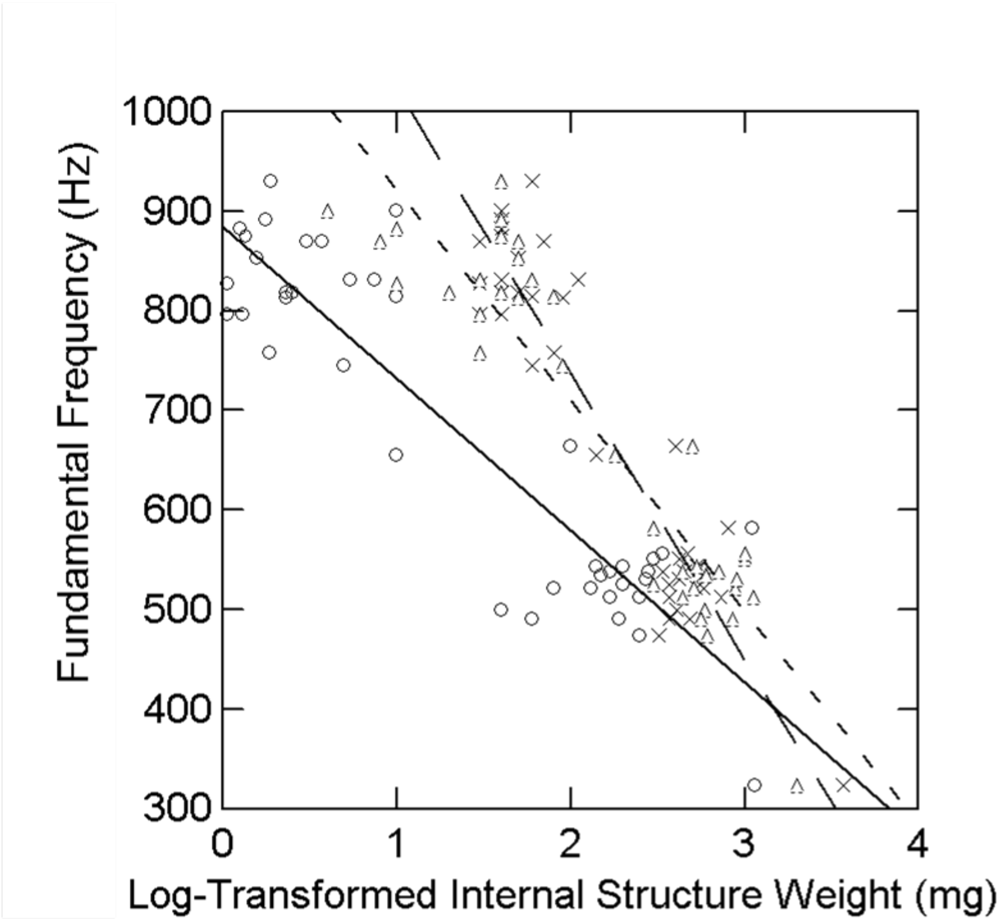

Figure 13: A linear comparison between the $\log_{10}$-transformed weights (mg) of the internal structures [swimbladder (x, - - -), sonic muscles (Δ, ...) and gonads (o, —)] and the produced F_0 (Hz) for all Atlantic croaker recorded in the laboratory. All three internal structures had highly significant ($p \leq 0.001$) linear regressions with R^2 values between 0.826 and 0.891 (Table 5).

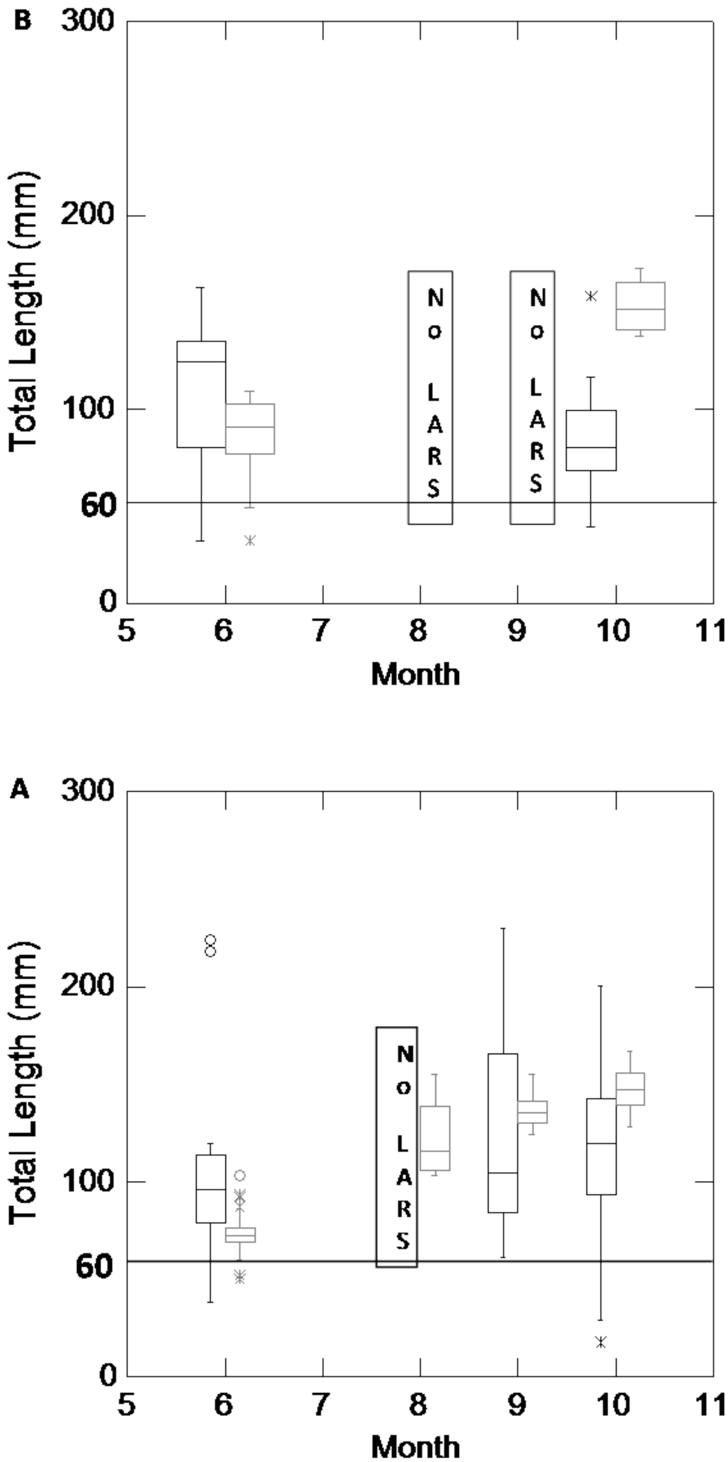

Figure 14: A monthly comparison for the *TLs* of Atlantic croaker collected in the trawl (grey) and estimated from the passive acoustic device (black) at site A) PCS and B) PMS in the Pamlico Estuary. The black line labeled 60 mm *TL* represents the smallest size at which Atlantic croaker are able to produce sounds.

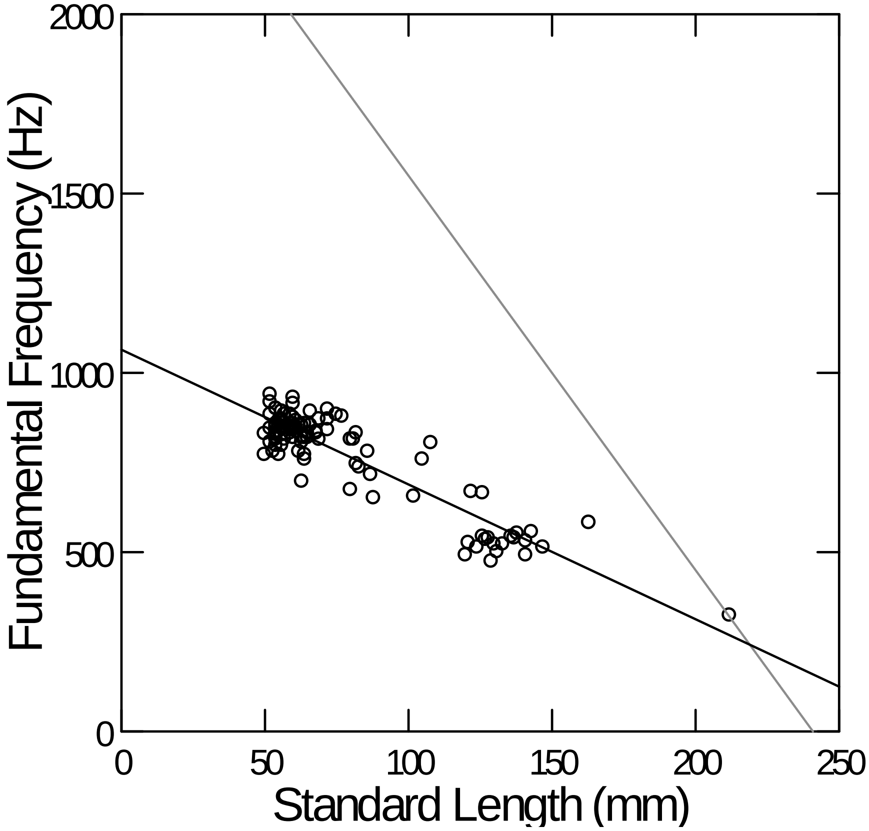

Figure 15: The fundamental frequencies (Hz) of Atlantic croaker by *SL* (mm) for data obtained in both this study (o,—), and the Gannon (2007) pond study (—); the line for the Gannon study is estimated using his published figure that relates peak frequency to that of median *SL* (Gannon 2007, Figure 4). Gannon's estimates were based on aggregates of Atlantic croaker within specified length groups; whereas my F_0 data are obtained from individual fishes.

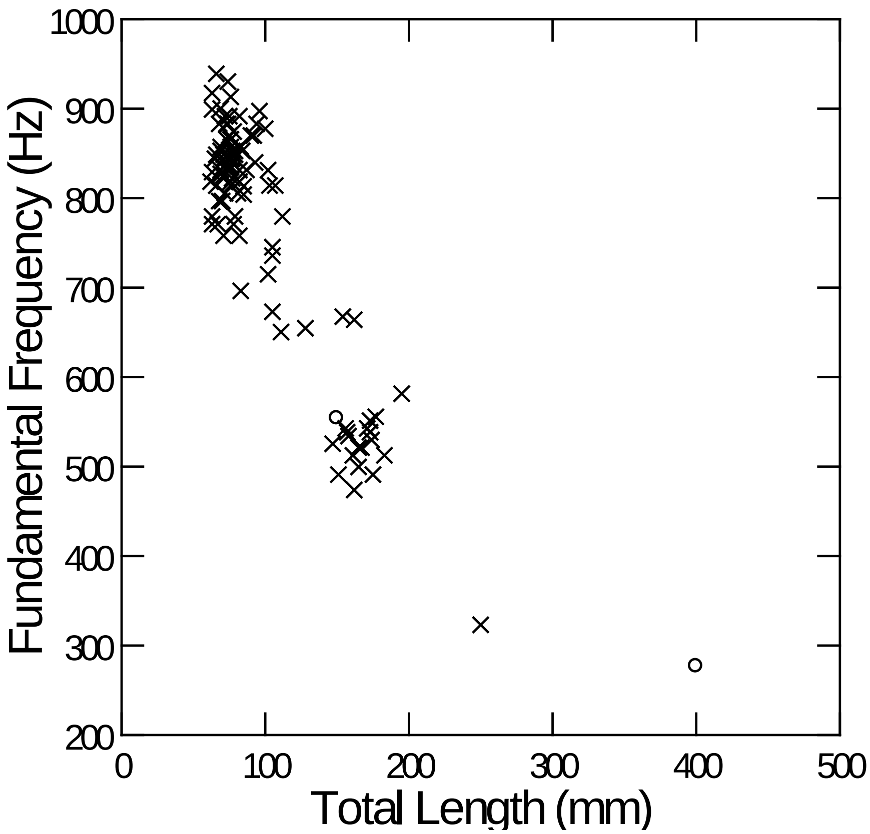

Figure 16: The F_0 (Hz) of the Atlantic croaker "croak" sound by *TL* (mm) for data obtained in this study (x) and in Fish and Mowbray (available the Macaulay Library) (o). For Fish and Mowbray, five individual Atlantic croaker were recorded between the lengths of 140-160 mm and five more were recorded between 390-410 mm. Since individual lengths were unknown, each of the five in both size categories were grouped with a mean length and a mean F_0 and plotted on this figure. All Fish and Mowbray recordings were analyzed using the same method as this study to obtain F_0.

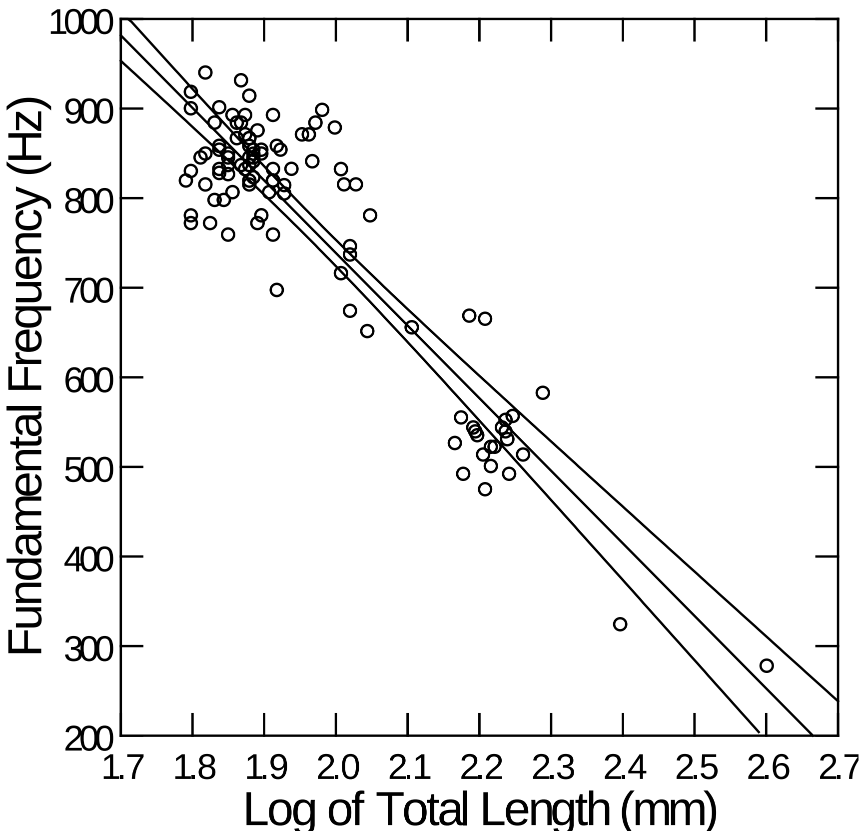

Figure 17: A linear regression comparison for the combined F_0 (Hz) of the Atlantic croaker "croak" sound with the $\log_{10}$-transformed *TL* (mm) for data obtained in this study and in Fish and Mowbray (available the Macaulay Library). F_0 can be predicted using $F_0 = 2358.710 - 810.037(\text{Log}_{10}TL)$ ($R^2=0.83$, $p<0.001$). Note that upper and lower confidence intervals are displayed for the regression equation.

TABLES

Table 1: Measured water quality parameters using a Hydrolab DS5X on the ITPod from May – Dec. 2008 for both PCS and PMS. Average temperature (°C) ± standard deviation (SD), salinity (ppt) ± SD, depth (m), and bottom type are reported at PMS and PCS in the Pamlico estuary.

Site	**PMS**	**PCS**
Average Temperature (°C)	22.0 ± 9.8 SD	21.9 ± 9.3 SD
Average Salinity (ppt)	21.1 ± 2.1 SD	15.7 ± 1.6 SD
Depth (m)	4.2	3.7
Bottom Type	Mud	Mud

Table 2: The instrument sampling specifications for the bottom-mounted ITPods deployed at two sites (PCS and PMS) the Pamlico Sound, North Carolina between April and December 2008.

Instrument	Sampling Rate	Function
LARS	4 or 6 per hour	Recorded 10s of ambient sound (<10kHz) and sound pressure level (dB) in a wav file.
Hydrolab	1 or 2 per hour	Measures dissolved oxygen (mg/l, %), temperature (°C), salinity (ppt), conductivity (mS/cm), and turbidity (NTU) within the water column.
Aquadopp	1 or 2 per hour	Measures the speed (m/s) and direction of the current within the water column using sound pressure level (dB) (1Hz sampling rate).
OBS	1 per hour	Measurement of suspended solids (NTU) within the water column (8 Hz sampling rate).
Vector	1 to 3 hour interval	Measures the temperature, pressure, tilt, and direction using sound pressure level (dB) for currents and waves (8 Hz sampling rate).

Table 3: Trawled Atlantic croaker collection times and dates by site with corresponding passive acoustic analysis of croaker recordings in local standard time. These data were pooled into monthly comparisons by site because the passive acoustic sampling dates did not always correspond with trawling dates due to equipment failure or environmental noise.

Site	Trawl Date	Trawl Times	LARS Dates	LARS Times
PMS	06/18/08	2000 - 2200	06/17/08 – 06/18/08	0108 – 0053
PCS	06/27/08	2200 - 2300	06/23/08 – 06/24/08	1845 – 1830
PCS	08/31/08, 09/13/08	2100 – 2300 2130 – 2300	08/29/08 – 08/30/08	1958 – 1943
PMS	10/20/08	2145 - 2230	10/18/08 – 10/19/08	1600 – 1550
PCS	10/25/08	2100 - 2200	10/26/08 – 10/27/08	2015 - 2000

Table 4: Total length (*TL*, mm) bins estimated using Love's (1971) equation that converts target strength (*TS*, dB) to *TL* to prepare active acoustics data for length comparisons.

Bin	**Total Length Range (mm)**	**Bin**	**Total Length Range (mm)**
1	<67.5	40	182.2-186.9
2	67.5-69.1	41	187.0-191.7
3	69.2-71.0	42	191.8-196.9
4	71.1-72.8	43	197.0-202.2
5	72.9-74.8	44	202.3-207.5
6	74.9-76.8	45	207.6-213.0
7	76.9-78.8	46	213.1-218.7
8	78.9-80.9	47	218.8-224.5
9	81.0-83.0	48	224.6-230.4
10	83.1-85.2	49	230.5-236.4
11	85.3-87.4	50	236.5-242.6
12	87.5-89.8	51	242.7-249.1
13	89.9-92.2	52	249.2-255.7
14	92.3-94.6	53	255.8-262.4
15	94.7-97.1	54	262.5-269.4
16	97.2-99.7	55	269.5-276.6
17	99.8-102.4	56	276.7-283.9
18	102.5-105.1	57	284.0-291.4
19	105.2-107.9	58	291.5-299.1
20	108.0-110.7	59	299.2-307.1
21	110.8-113.7	60	307.2-315.2
22	113.8-116.7	61	315.3-323.6
23	116.8-119.8	62	323.7-332.2
24	119.9-122.9	63	332.3-341.0
25	123.0-126.2	64	341.1-350.0
26	126.3-129.9	65	350.1-359.3
27	130.0-133.0	66	359.4-368.8
28	133.1-136.5	67	368.9-378.6
29	136.6-140.1	68	378.7-388.6
30	140.2-143.8	69	388.7-399.0
31	143.9-147.7	70	399.1-409.6
32	147.8-151.6	71	409.7-420.4
33	151.7-155.6	72	420.5-431.6
34	155.7-159.7	73	431.7-443.0
35	159.8-164.0	74	443.1-454.8
36	164.1-168.2	75	454.9-466.8
37	168.3-172.8	76	466.9-479.2
38	172.9-177.4	77	479.3-491.9
39	177.5-182.1		

Table 5: The R^2 prediction for the GLMs created using the measured structures that may influence F_0. Each predictor was plotted against the F_0 of the Atlantic croaker that was obtained from laboratory recordings. The total number (N) in each model, resulting F-statistic, degrees of freedom (df), and associated p-values are provided for each GLM.

Predictor of Fundamental Frequency	**R^2**	**N**	**F-statistic**	**df**	**p-value**
Total Length (mm)	0.840	102	524.33	1	<0.001
Standard Length (mm)	0.829	102	492.07	1	<0.001
Log_{10}-Transformed Weight (g)	0.868	90	577.13	1	<0.001
Sonic Muscle Length (mm)	0.832	92	447.04	1	<0.001
Log_{10}-Transformed Swimbladder Area (mm^2)	0.858	81	476.82	1	<0.001
Log_{10}-Transformed Gonad Area (mm^2)	0.817	49	209.36	1	<0.001
Log_{10}-Transformed Sonic Muscle Weight (mg)	0.891	92	348.42	1	<0.001
Log_{10}-Transformed Swimbladder Weight (mg)	0.838	92	465.54	1	<0.001
Log_{10}-Transformed Gonad Weight (mg)	0.826	40	179.847	1	<0.001
Sex	0.203	55	13.538	1	0.001
Sex-Juveniles only	0.019	41	0.754	1	0.391
Maturity	0.561	56	68.957	1	<0.001

Table 6: Pearson correlation matrix for *TL* (mm), sonic muscle length (mm), $\log_{10}$-transformed gonad area, and $\log_{10}$-transformed swimbladder area, which were the most influential to the F_0 of the Atlantic croaker "croak" call. All internal structures are highly correlated with the *TL* of the fish. This indicates that the GLMs for the internal structures are highly influenced by *TL*.

	Total Length (mm)	**Sonic Muscle Length (mm)**	**$\log_{10}$ of Gonad Area (mm^2)**	**$\log_{10}$ of Swimbladder Area(mm^2)**
Total Length (mm)	1.000			
Sonic Muscle Length (mm)	0.961	1.000		
$\log_{10}$ of Gonad Area (mm^2)	0.944	0.885	1.000	
$\log_{10}$ of Swimbladder Area(mm^2)	0.973	0.933	0.919	1.000

Table 7: Pearson correlation matrix for *Wt* (g), $\log_{10}$-transformed sonic muscle weight (mg), $\log_{10}$-transformed gonad weight (mg), and $\log_{10}$-transformed swimbladder weight (mg), which were influential to the F_0 of the Atlantic croaker "croak" call. The weights of all internal structures are highly correlated with the *Wt* of the fish. This indicates that the general linear models for the internal structures are highly influenced by the overall *Wt* of the fish.

	Weight (g)	**Log_{10} of Sonic Muscle Weight (mg)**	**Log_{10} of Gonad Weight (mg)**	**Log_{10} of Swimbladder Weight (mg)**
Weight (g)	1.000			
Log_{10} of Sonic Muscle Weight (mg)	0.982	1.000		
Log_{10} of Gonad Weight (mg)	0.918	0.911	1.000	
Log_{10} of Swimbladder Weight (mg)	0.976	0.964	0.898	1.000

Table 8: Descriptive statistics for the *TLs* collected in the trawl and estimated from the passive acoustic device (LARS) using Equation 11. They are separated by month of collection and site of collection. The total number (N) of Atlantic croaker collected by each gear-type, the minimum *TL*, maximum *TL*, and the mean *TL* (± SD) are reported for each gear type by month and over the entire sampling period. NaN indicates that the passive acoustic device failed or that the descriptive statistics are not applicable because Atlantic croaker were not collected in the trawl during that month.

Month	**Site**	**Statistic**	**LARS**	**Trawl**
6	PCS	N	17	165
		Min (mm)	37.9	50.0
		Max (mm)	223.9	103.0
		Mean (±SD)	103.2 ± 50.0	72.8 ± 6.8
	PMS	N	67	18
		Min (mm)	32.1	32.0
		Max (mm)	162.9	109.0
		Mean (±SD)	11.5 ± 34.2	84.9 ± 23.2
8	PCS	N	NaN	4
		Min (mm)	NaN	103.0
		Max (mm)	NaN	155.0
		Mean (±SD)	NaN	122.3 ± 23.2
	PMS	N	NaN	0
		Min (mm)	NaN	NaN
		Max (mm)	NaN	NaN
		Mean (±SD)	NaN	NaN
9	PCS	N	96	12
		Min (mm)	61.1	124.0
		Max (mm)	229.8	155.0
		Mean (±SD)	123.6 ± 46.9	135.9 ± 8.4
	PMS	N	NaN	0
		Min (mm)	NaN	NaN
		Max (mm)	NaN	NaN
		Mean (±SD)	NaN	NaN
10	PCS	N	349	16
		Min (mm)	17.5	128.0
		Max (mm)	200.7	167.0
		Mean (±SD)	116.2 ± 34.9	147.3 ± 10.4
	PMS	N	42	24
		Min (mm)	39.6	138.0
		Max (mm)	158.2	173.0
		Mean (±SD)	84.2 ± 22.6	152.3 ± 11.9

Table 8: continued

Month	Site	Statistic	LARS	Trawl
Overall	PCS	N	462	197
		Min (mm)	17.5	50.0
		Max (mm)	229.8	167.0
		Mean (±SD)	117.3 ± 38.4	83.7 ± 26.2
	PMS	N	109	42
		Min (mm)	32.1	32.0
		Max (mm)	162.9	173.0
		Mean (±SD)	101.0 ± 33.0	123.5 ± 38.1
	Combined	N	571	239
		Min (mm)	17.5	32.0
		Max (mm)	229.8	173.0
		Mean (±SD)	114.2 ± 37.9	90.7 ± 32.4

Table 9: An Analysis of Variance (ANOVA) comparing the predicted *TLs* of Atlantic croaker from the passive acoustic device (LARS) and the *TLs* of Atlantic croaker collected in the trawl by month and by site. In each case, gear was the factor variable and *TL* was the dependent variable. The total number (N) in each model, resulting F-statistic, degrees of freedom (df), and associated p-values are provided for each test.

Predictor of Total Length		**N**	**F-statistic**	**df**	**p-value**
Month	6	267	142.83	1	<0.001
	9	108	0.82	1	0.368
	10	431	45.25	1	<0.001
Site	PCS	659	125.74	1	<0.001
	PMS	151	12.99	1	<0.001

Table 10: An Analysis of Variance (ANOVA) that compares the *TL* of the fishes collected in the active acoustic device (DT-X) to that of the trawl, gillnet, and nets (trawl and gillnet combined) by month. Each net (gillnet and trawl net) was compared individually to the active acoustics but also combined and compared to the active acoustics. In each case, gear was the factor variable and *TL* was the dependent variable. The total number (N) in each model, resulting F-statistic, degrees of freedom (df), and associated p-values are provided for each test.

Predictor of Total Length			**N**	**F-statistic**	**df**	**p-value**
DT-X	Month	6	83	12.64	1	0.001
vs.		9	63	1.30	1	0.258
Trawl		10	84	3.77	1	0.066
		11	62	4.39	1	0.040
DT-X	Month	6	82	0.91	1	0.344
vs.		9	40	28.54	1	<0.001
Gillnet		10	63	3.31	1	0.074
		11	59	0.21	1	0.648
DT-X	Month	6	97	6.14	1	0.015
vs		9	70	1.08	1	0.303
Nets		10	92	0.82	1	0.368
		11	68	<0.01	1	0.974

Table 11: Descriptive statistics for the *TLs* of the fishes collected in the active acoustics (DT-X), trawl, gillnet, and combined gillnet and trawl (i.e. nets), separated by month of collection. The total number (N) of fish collected by each gear-type, the minimum *TL*, maximum *TL*, and the mean *TL* (± SD) are reported for each gear type by month and over the entire sampling period.

Month	**Statistics**	**DT-X**	**Trawl**	**Gillnet**	**Nets**
6	N	4388	1065	93	1158
	Min (mm)	67.4	67.4	83.0	67.4
	Max (mm)	491.9	177.4	269.4	269.4
	Mean (±SD)	174.2 ± 103.2	99.4 ± 28.3	152.9 ± 55.7	129.5 ± 54.4
9	N	273	754	18	772
	Min (mm)	67.4	67.4	119.8	67.4
	Max (mm)	218.7	155.6	409.6	409.6
	Mean (±SD)	116.7 ± 33.9	102.2 ± 25.0	223.2 ± 88.7	125.1 ± 64.2
10	N	519	361	51	412
	Min (mm)	67.4	67.4	177.4	67.4
	Max (mm)	378.6	255.7	218.7	255.7
	Mean (±SD)	152.7 ± 73.0	124.6 ± 40.6	197 ± 14.1	140.5 ± 47.9
11	N	1985	339	51	390
	Min (mm)	67.4	67.4	83.0	67.4
	Max (mm)	230.4	196.9	479.2	479.2
	Mean (±SD)	127.7 ± 44.8	102.8 ± 31.6	136.5 ± 102.6	127.2 ± 83.6
Overall	N	7165	2519	213	2732
	Min (mm)	67.4	67.4	83.0	67.4
	Max (mm)	491.9	255.7	479.2	479.2
	Mean (±SD)	146.2 ± 77.3	108.2 ± 33.4	165.2 ± 75.9	131.0 ± 60.9

Table 12: Descriptive statistics for the *TL* of the fishes analyzed from the active acoustics (DT-X), Atlantic croaker collected in the trawl, and predicted *TLs* of Atlantic croaker from the passive acoustic recordings (LARS). They are separated by month of collection. The total number (N) of fish collected by each gear-type, the minimum *TL*, maximum *TL*, and the mean *TL* (± SD) are reported for each gear type by month and over the entire sampling period.

Month	**Statistics**	**DT-X**	**Trawl**	**LARS**
6	N	4388	283	84
	Min (mm)	67.4	67.4	67.4
	Max (mm)	491.9	110.7	224.5
	Mean (±SD)	174.2 ± 103.2	86.1 ± 13.7	123.3 ± 40.0
9	N	273	15	96
	Min (mm)	67.4	105.1	67.4
	Max (mm)	218.7	155.6	230.4
	Mean (±SD)	116.7 ± 33.9	130.4 ± 15.1	134.7 ± 48.1
10	N	519	40	391
	Min (mm)	67.4	129.9	67.4
	Max (mm)	378.6	177.4	202.2
	Mean (±SD)	152.7 ± 73.0	153.9 ± 15.0	119.3 ± 37.0
Overall	N	5180	339	571
	Min (mm)	67.4	67.4	67.4
	Max (mm)	491.9	177.4	230.4
	Mean (±SD)	151.9 ± 84.2	118.5 ± 33.3	125.5 ± 41.9

Table 13: An Analysis of Variance (ANOVA) that compares the *TL* of the fishes collected in the active acoustics (DT-X), Atlantic croaker collected in the trawl, and predicted *TLs* of Atlantic croaker from the passive acoustic recordings (LARS) by month. In each case, gear was the factor variable and *TL* was the dependent variable. The total number (N) in each model, resulting F-statistic, degrees of freedom (df), and associated p-values are provided for each test.

Predictor of Total Length			**N**	**F-statistic**	**Df**	**p-value**
Trawl	Month	6	46	14.35	1	<0.001
vs.		9	43	0.08	1	0.783
LARS		10	51	9.95	1	0.003
DT-X	Month	6	76	12.92	1	0.001
vs.		9	43	2.18	1	0.148
Trawl		10	66	0.004	1	0.953
DT-X	Month	6	86	6.31	1	0.014
vs		9	66	4.57	1	0.036
LARS		10	93	6.89	1	0.01

APPENDIX – ANIMAL USE PROTOCOL

Animal Care and Use Committee
East Carolina University
212 Ed Warren Life Sciences Building
Greenville, NC 27834
252-744-2436 office • 252-744-2355 fax

September 23, 2008

Joseph Luczkovich, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Luczkovich:

Your Animal Use Protocol entitled, "Using Sound Recordings to Determine the Size, Sex, and Age of an Atlantic Croaker (*Micropogonias undulates*) Population," (AUP #D222) was reviewed by this institution's Animal Care and Use Committee on 9/23/08. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Robert G. Carroll, Ph.D.

Robert G. Carroll, Ph.D.
Chairman, Animal Care and Use Committee

RGC/jd

enclosure