STUDENT FINAL REPORT TO THE UNIVERSITY OF HAWAIʻI MARINE OPTION PROGRAM

Bioimpedance and Condition of Reef Fish Across a Landscape Gradient

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Abstract: Accurately assessing the health and reproductive potential of reef fish populations is essential to fisheries management. Traditional length-weight based techniques may offer general conclusions about the overall growth of a fish stock, but fail to identify specific compositional information about fish, such as cellular health and lipid content. Bioimpedance, measured as phase angle, has been experimentally applied to answer the need for a cost-effective and efficient method to determine the overall health of a fish species. However, little is known whether phase angle may be sensitive to habitat quality and may ultimately signal energy available for allocation to reproduction. Our goal was to evaluate the potential of phase angle as an indicator of reproductive allocation. As part of a larger study of habitat utilization, two Gulf of Mexico species of reef fish with relatively high site fidelity and similar spawning season yet different reproductive strategies were collected via standardized fishing: gray triggerfish (*Balistes capriscus)* and vermilion snapper (*Rhomboplites aurorubens).* We made comparisons of phase angle and condition across two seasons, spring and winter, and two habitat designations, high relief and low relief reef structure. We found evidence for differences in energy storage and allocation. Gray triggerfish exhibited much higher values of the hepatosomatic index (% HSI) in comparison to the vermillion snapper, indicating the liver to be a potentially important energy storage mechanism for this marine nest building species. As well, gray triggerfish showed a seasonal phase angle response with phase angle increasing in the spring with values ranging from 22-24, compared to the winter phase angle range of 16-18. Vermilion snapper, a pelagic spawner, showed no obvious energy storage/allocation pattern in either phase angle or the hepatosomatic index. The differences between species may be indicative of a variation in energy usage between them. Vermilion snapper exhibit characteristics of having their energy directly utilized from the environment. In contrast, gray triggerfish appeared to exhibit a pattern of energy storage and later utilization. However, neither species exhibited phase angle differences related to reef habitat relief, albeit seasonal sample sizes related to location and habitat differences were relatively small. It is apparent that effectively measuring reproductive potential depends on understanding how energy is stored and allocated. We conclude that phase angle may be a promising tool for species with long-term energy storage patterns, but more research is needed in order to cost-effectively measure allocation in species with a pattern of direct energy utilization.

Key words: Bioimpedance, reproductive potential, phase angle

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Introduction

Successful fisheries management relies on accurate estimates of fish stock abundance and condition in order to sustainably manage a species (Wuenschel et al. 2012). Surveys are completed with the goal of estimating the length, size, growth rate, and reproductive potential of a species (Cox 2009). The National Oceanographic and Atmospheric Administration (NOAA) oversees fisheries management in the United States, and the National Marine Fisheries Service (NMFS 2013) operates underneath NOAA to ensure that certain species identified as economically and ecologically important to an area are being fished at a sustainable rate.

The NMFS Southeast Fisheries Science Center in Panama City, FL manages several fish species within the Gulf of Mexico, and they are constantly working to develop new methods of evaluating fecundity in fish (NMFS 2013). The fecundity is the reproductive rate of a fish, and the reproductive potential is a measure of fecundity under optimal conditions (Wuenschel et al. 2012). When the reproductive potential of a species decreases significantly, it may signal an environmental or anthropogenic issue that is creating a limitation in the reproductive rate (Cooper et al. 2013).

One of the most common techniques of estimating reproductive potential is through a direct measurement of egg production, which involves removing the gonads, which are the reproductive organs of the fish, and directly counting the number of eggs being produced at various stages in the reproductive cycle. This method can be very costly, and it involves a significant amount of time spent in the laboratory. Other techniques such as histology and pathology can also provide details about the health of a fish stock, but the feasibility of these studies when placed within the time constraints of a fishing season often render them impractical for routine use (Cox and Heintz 2009). As a result, fisheries biologists are constantly looking for ways to bridge the gap between sensitive and highly accurate laboratory methods and quick, cost-efficient field methods for estimating variances in the health and reproductive potential of fish stocks from season to season (Cox and Heintz 2009).

The first order of egg production is energy allocated through the storage of lipids, and scientists have found a link between energy gained through food consumed and egg production (Woodhead 1960, Tyler and Dunn 1976, Hislop et al. 1978, Rideout and Morgan 2010, as cited in Wuenschel et al. 2012). Further investigating the lipid storage and body composition of a fish may be a solution towards an indirect measure of reproductive potential in fish species.

A relatively non-invasive and cost-efficient method for determining body composition and overall health of humans and animals is known as bioimpedance (Willis and Hobday 2008). Kumar et al. (2012). described bioimpedance as the response of a tissue to an externally applied electrical current. Water is the primary conductor of electricity within our bodies, and lipids are poor conductors of electricity. Based on these physical properties, the electrical current experiences resistance when it encounters cells within our bodies. These cells are enclosed in a lipid bilayer that forces the electrical current to act as a capacitor, therefore indirectly giving us information on the extra and intracellular water content of our cells. This information can then be translated to indicate cellular health and total body cell mass (Cox and Heintz 2009). This method is commonly used in the medical field as an indicator of disease, and athletes also use it to take a closer look at their body fat composition. Dr. Gary Fitzhugh (NMFS 2010) from the NMFS in Panama City, Fl has been studying the use of bioimpedance in fish for the purpose of determining body cell mass and fat-free mass in fish and linking this information to controlled feeding trials to determine if bioimpedance can predict the reproductive status of a fish.

This study will take Dr. Fitzhugh's (Fitzhugh et al. 2010) work one step further, and determine if a measure of bioimpedance known as *phase angle* will be able to indicate the reproductive potential of a species of fish. Phase angle is the arctangent of the ratio of body cell mass to fat-free mass of the tissues being analyzed (Cox and Heintz 2009).

$$Phase\ angle = tan^{-1}\left(\frac{X_c}{R}\right) * \frac{180°}{\pi}$$

When the electrical current is applied, two values are obtained and recorded in Ohms (applied voltage drop/current (amps) (Kumar et al. 2012). These values are *resistance* and *reactance*. Resistance (R) can be related to fast-free mass, and reactance (X_c) can be related to body cell mass through the use of chemical analysis that results in the development of calibration curves (Cox and Heintz 2009). Phase angle can range between 0° and 90°. A phase angle of 90° indicates that all cell membranes being analyzed have no extracellular fluid, and a phase angle of 0° indicates that within the system, the cell membranes are either non-existent or severely degraded (Cox and Heintz 2009). Low phase angles in both fish and humans appear to indicate cell death or a breakdown in the cell membrane, and higher phase angles indicate large quantities of intact cell membranes and body cell mass (Abu Khaled et al. 1988, Foster and Lukaski 1996, as cited in Cox and Heintz 2009).

This method is used to describe the results of bioimpedance in a linear fashion in which total cell mass is taken into account as well as the fat content of each cell. Knowledge of the fat content of a cell is important because reproduction is one of the most metabolically demanding activities in the life-history of a fish, and fish have been shown to store up energy for this activity in the form of lipids within their cells (Wuenschel et al. 2012). As a result, information on the cellular lipid content within a fish may be used as an indicator of the reproductive potential of the fish. Little is known about whether phase angle may be sensitive to habitat quality and may ultimately signal energy available for allocation to reproduction.

As part of a larger study of habitat utilization, bioimpedance was performed on two Gulf of Mexico species of reef fish: the gray triggerfish, *Balistes capriscus,* and the vermilion snapper, *Rhomboplites aurorubens.* Both species have relatively high site fidelity and a similar spawning season, yet very different reproductive strategies:

Methods and Materials

Study Sites

Fish were collected and processed in a four-year time span ranging from 2009-2013. Additional sampling throughout the summer was also performed. Fish were collected across 12 sites (Fig. 1) within the Gulf of Mexico, approximately 40-50 miles offshore. Sites were selected based on a landscape type of high or low coral cover.

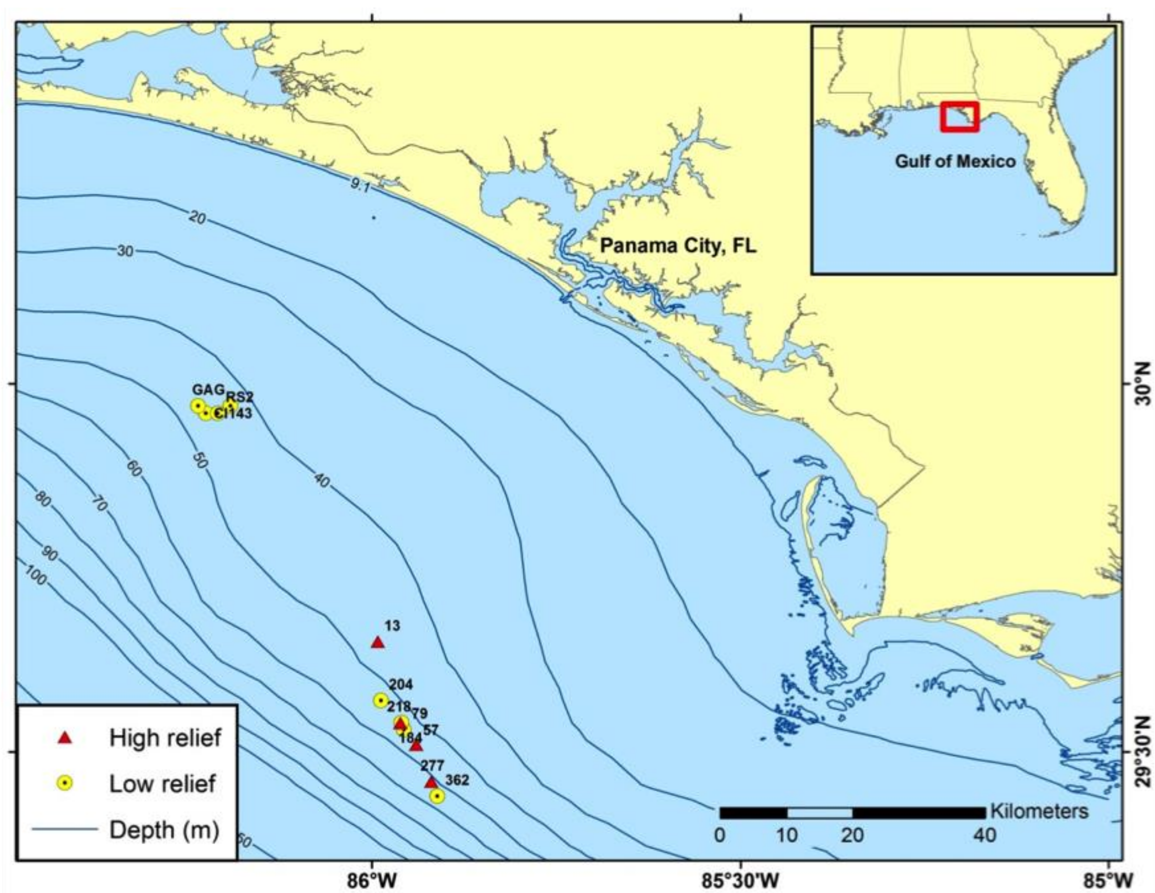

Fig. 1: Study Sites in the Gulf of Mexico. (NMFS 2013)

Landscape Type

High and low coral cover was determined though the use of side-scan sonar (Fig. 2). This type of sonar was used by the SEFSC in Panama City, FL for previous research projects. This is a type of acoustic sonar, and as the device is towed from a boat, waves are emitted that ping off of the seafloor. Images are obtained and spliced together (Fig. 3) to produce a picture of the geology of the seafloor, and from these images approximate coral cover can be determined.

Fig. 2: Side-scan sonar (NMFS 2013)

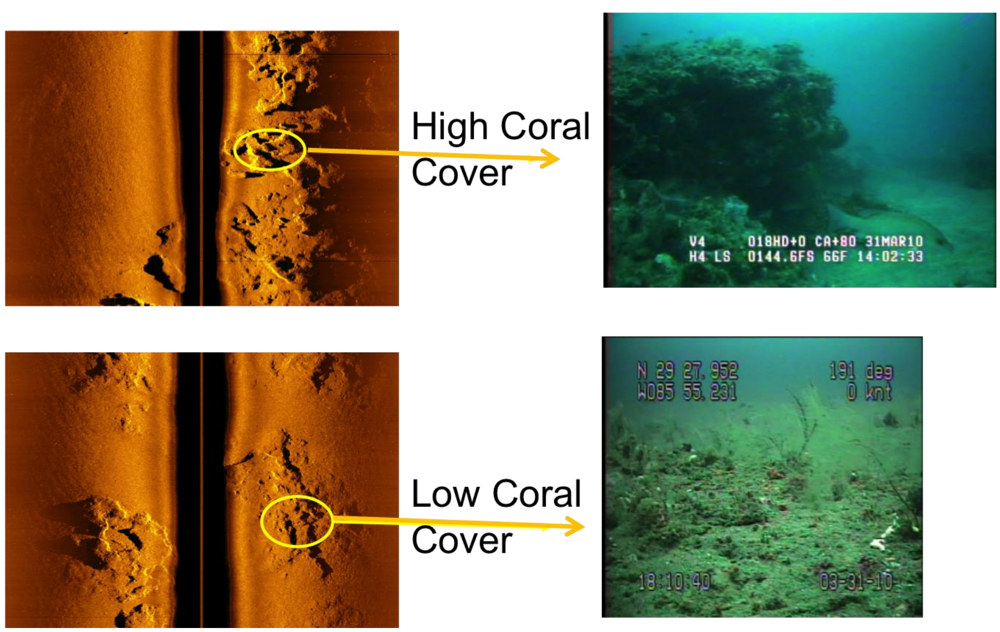

Fig. 3: Side-scan sonar images in comparison to coral cover (NMFS 2013)

Fish Collection and Processing

Standard hook and line fishing methods using a Sabiki or 5/0 hook type were used to catch the fish. The fish were placed on ice and processed within 48 hours of capture (Fig. 4). Each fish was sexed, and liver and gonads were removed and weighed.

Bioimpedance measurements were made following Cox and Hartman (2005), and all of the post-processing information was retained on data sheets.

Fig. 4: Bioimpedance and post-processing (NMFS 2013)

Data Analysis

Data sheets were entered into the word processing software Microsoft Excel. Within Excel, data was sorted by sampling day, location, species, coral cover, and season. Additional sorting involved breaking up the data into sections and identifying patterns for further analysis. Preliminary analysis was performed on the data by creating bar graphs of phase angle and sorting them by species, spawning season, and coral cover. Relationships were identified between phase angle and the hepatosomatic index of the fish.

Results

Phase angle

A difference was found in phase angle in the gray triggerfish between seasons, but phase angle of the vermilion snapper appeared to remain relatively stable across the seasons (Fig. 5). No obvious difference in phase angle was found between coral cover types for both species. Phase angle was higher in gray triggerfish during the pre-spawning season, spring, with a range of 22-24°. After spawning, during the winter, phase angle dropped, with a range of 16-18.5°. There were no apparent differences between the two coral types of high vs. low relief.

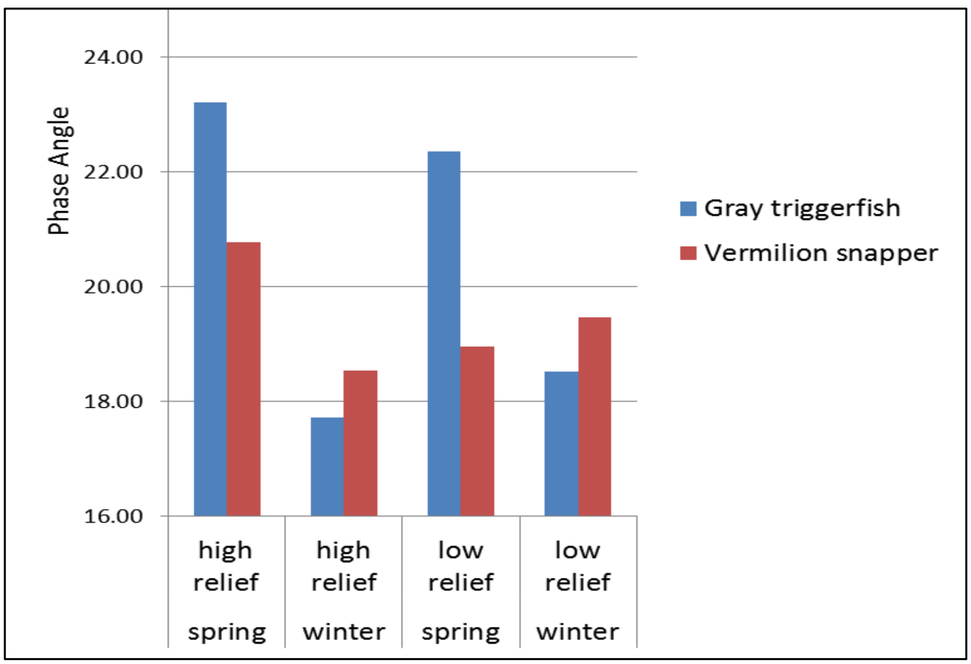

Fig. 5: Phase angle for gray triggerfish and vermillion snapper by season – spring and winter, and coral type -high vs. low relief (NMFS 2013)

Hepatosomatic Index

The hepatosomatic index (SHSI) of the gray triggerfish was much larger across all seasonal types and coral covers in comparison to the vermilion snapper (Fig. 6). The gray triggerfish %HSI ranged from 3.0-4.25%, while the vermillion snapper HSI ranged from 0.5-0.75%. Clear differences in liver size are visually demonstrated in Figure 7.

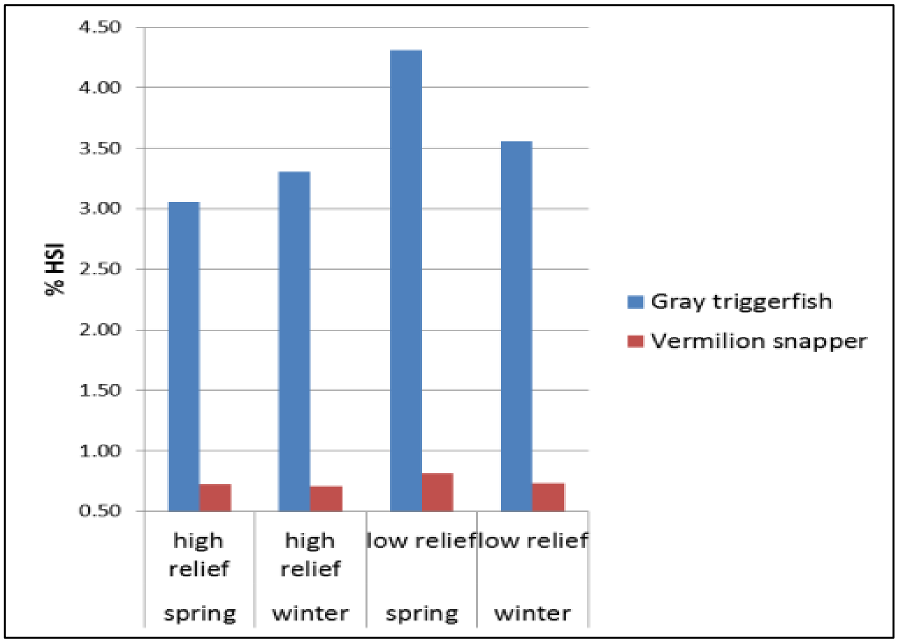

Fig. 6: Hepatosomatic index for gray triggerfish and vermillion snapper by season – spring and winter, and coral type - high vs. low relief (NMFS 2013)

A **B**

Fig. 7: Liver in comparison to body weight for gray triggerfish (A) and vermillion snapper (B) (NMFS 2013)

Discussion

Accurately assessing the health and reproductive potential of reef fish populations is essential to fisheries management. Traditional length-weight based techniques may offer general conclusions about the overall growth of a fish stock, but fail to identify specific compositional information about fish, such as cellular health and lipid content (Fitzhugh et al. 2010). Bioimpedance, measured as phase angle, was experimentally applied to answer the need for a cost-effective and efficient method to determine the overall health of a fish species.

I made comparisons of condition across two habitat designations, high relief and low relief reef structure. The catch per unit effort for vermilion snapper was much higher in low relief areas, indicating differences in habitat utilization between the two species. This species may be frequenting areas with lower coral cover in order to reduce prey competition with other species in areas with higher coral cover.

I also made comparisons of the hepatosomatic index (%HSI) across two seasons, spring and winter. The %HSI is the ratio of liver weight to body weight of a species. Spring and winter are the approximate pre and post-spawning seasons of both species, respectively (Ofori-Danson 1990, Allman 2007).

I found evidence for differences in energy storage and allocation, which may be a result of the varying reproductive strategies in both species. Gray triggerfish exhibited much higher values of the %HSI in the spring in comparison to the vermilion snapper, indicating the liver to be a potentially important energy storage mechanism for this species. The gray triggerfish is known as a marine nest-builder and this activity is estimated to require a high level of energy during the spawning season. The male will guard the nest until spawning, and both sexes will undergo parental care of the eggs until they hatch (Ofori-Danson 1990). In addition, very rarely is this species caught in a spawning condition, suggesting that it is not feeding during this time. Previous energy stores may be a primary source of energy during the spawning season for this species. Due to a low %HSI, the vermilion snapper did not appear to be exhibiting the same long-term energy storage pattern.

In addition, gray triggerfish showed a seasonal phase angle response with phase angle increasing in the spring, and decreasing in the winter. Vermilion snapper, a pelagic or broadcast spawner, showed notable seasonal reproductive development but no obvious energy storage/allocation pattern was detected. During spawning, the vermillion snapper is known to broadcast its eggs into the water column multiple times per season, with no parental care of the eggs whatsoever (Allman 2007). The differences in phase angle between the two fish may be indicative of a greater "income" response in vermilion snapper, characteristic of energy directly utilized from the environment as opposed to a "capital" pattern of energy storage and later utilization by gray triggerfish (Stephens et al. 2009). Finally, neither species exhibited phase angle differences related to reef habitat relief, albeit seasonal sample sizes related to location and habitat differences were relatively small.

It is apparent that effectively measuring reproductive potential depends on understanding how energy is stored and allocated in each species. I conclude that phase angle may be a promising tool for species with long-term energy storage patterns, but more work to cost-effectively measure allocation in species that fail to exhibit this pattern is needed.

Evaluation and Learning

The lessons I've learned form this project have reached much farther then the duration of my summer in 2013. While I was in Panama City, FL, for this research internship, I was exposed to the inner workings of a laboratory dedicated to marine fisheries management. I began to realize that being an intern is not about how much you know, but about how much you are willing to learn. I experienced the results of hard work and passion when I was invited to participate in a release of Loggerhead sea turtles into the Gulf Stream with NOAA Galveston. I focused not only on my research project, but also on what on the bigger picture of what it means to work in fisheries management, and the frustrations and red tape that follows behind every step of this kind of research.

Something else that I came away from this experience with is a realization of the importance of good communication. I have now presented on my summer research a total of four times, and I have been to three research conferences and received awards in two of them. The first presentation occurred at NOAA headquarters for the 2013 NOAA Science and Education Student Symposium in Silver Spring, MD, where I was very shaky, nervous, and my speech was read straight off of a piece of paper. That day, in my opinion I did not deliver a good presentation. I did not communicate well with my audience. I felt I had failed my own expectations, and I was terribly disappointed. However, I received another chance to present my research at the Fall 2013 ALEX Student Research Conference at UH Hilo. I attended this conference not to compete, but to "get back on the horse", so to speak. I practiced a lot and although I was still very nervous (that will never go away), I delivered a presentation that met my goal of good communication and zero notes. That day, I placed in the top 3 at that conference and received a research award. From then on, something clicked and I saw the results of hard work and critical thought on how to create a good presentation.

Improvement is always possible in my mind, and the day I will stop learning is the day I die (sadly). My work paid off again at the 31st Annual Marine Option Program Symposium in Oahu, where I took the award for best research out of all the UH campuses. I'm humbled to have been given all these opportunities starting with my entrance into the Marine Option Program in 2011. I strongly believe in encouraging the application of knowledge to real-world situations and experiences, and MOP has been exactly that in my life. Thank you!

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