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# Combined Gut Content-Stable Isotope Trophic Analysis and Satellite Tagging of the Pelagic Stingray Pteroplaytrygon violacea (Bonaparte, 1832) from the Western North Atlantic Ocean

Tiffany A. Weidner
*Nova Southeastern University*, tweidner@nova.edu

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NOVA SOUTHEASTERN UNIVERSITY OCEANOGRAPHIC CENTER

# Combined gut content-stable isotope trophic analysis and satellite tagging of the pelagic stingray *Pteroplaytrygon violacea* (Bonaparte, 1832) from the western North Atlantic Ocean

BY

Tiffany A. Weidner

Submitted to the faculty of
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in partial fulfillment of the requirements for the degree of
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# Thesis of
# Tiffany A. Weidner

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Approved:

Thesis Committee

Major Professor: ______________________________

David W. Kerstetter, Ph.D.

Committee Member: ___________________________

Amy C. Hirons, Ph.D.

Committee Member: ___________________________

Charles F. Cotton, Ph.D.

Florida State University Coastal and Marine
Laboratory

## Abstract

The pelagic stingray, *Pteroplatytrygon violacea*, is a bycatch species in the global pelagic longline fishery. However, little research has been conducted on its basic biology, including prey composition, trophic positioning, and habitat utilization. Descriptions of the habitat utilization have largely been through indirect analyses of catch rates in commercial fisheries, which also provided no information on actual behaviors. The first chapter of this thesis will describe the habitat utilization and behavior of four individual pelagic stingrays using electronic tagging technology. Prior diet descriptions were hampered, in part, by low sample sizes and accordingly provided little information on the ecological interactions of these animals. Similarly, the second chapter of this thesis will therefore provide a new diet description for the pelagic stingray using a combined analysis of traditional stomach contents with stable isotope values, thereby addressing both ingestion and assimilation. A more robust study of the trophic dynamics of the pelagic stingray, in conjunction with the description of its habitat utilization, will provide a better understanding of its role within the pelagic ecosystem. Ultimately, the goal is to obtain knowledge of the less economic species with good science so when management approaches shift from species-specific to ecosystem based, the transition will already have known information to change efficiently.

Key words: elasmobranch, telemetry, fisheries, diet, bycatch

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## Introduction

The pelagic stingray, *Pteroplaytrygon violacea* (Bonaparte, 1832) is a common bycatch species in commercial pelagic longline fisheries and purse seine fisheries around the world, and the only known dasyatid to commonly interact with pelagic fishing gear. However, very few studies on the life history and behavior of the pelagic stingray have been done to date. This thesis used stomach content descriptions, stable isotope ratio analyses, and electronic tagging to determine feeding ecology and vertical habitat utilization. Ultimately, the combination of all three methodologies will allow for the further understanding of the ecology and behavior of the pelagic stingray.

### *Pelagic Stingray Biology*

The pelagic stingray is distributed circumglobally in sub-tropical and tropical pelagic waters (Neer, 2008; Wilson and Beckett, 1970). The species is known to inhabit epipelagic waters; however, Neer (2008) hypothesized that the pelagic stingray is benthopelagic utilizing both benthic and pelagic waters based on commercial catch data. Using individuals caught as bycatch in the commercial pelagic longline vessels off Uruguay, Domingo et al. (2005) described the highest catch rates for pelagic stingray hooked on leaders that were targeting the 40-60 m depth range. The stingrays themselves are most commonly found only over deeper waters greater than approximately 4000 m deep; few catches were reported during pelagic longline sets in less than 600 m of water (Domingo et al., 2005).

Relatively little general biological research has been done on the pelagic stingray. There is a sexual dimorphism between females and males in reference to the size of the individuals, with females tending to reach adult size faster than males (Mollet et al., 2002). Maturation sizes for the pelagic stingray are 39 cm for females and 37 cm for males, respectively (Mollet et al., 2002). The average disk width of females was found to be 600 mm and males averaged to be about 500 mm in the western North Atlantic population (Wilson and Beckett, 1970); however, the specimens in Wilson and Beckett (1970) were caught using pelagic longline gear and purse seines, which could have resulted in a gear-based size bias to the samples. Potential predators include the oceanic whitetip shark *Carcharhinus longimanus* and the tiger shark *Galeocerdo cuvier* (Mollet et al., 2002; Simpfendorfer et al., 2001).

The pelagic stingray is viviparous, and the embryos are nourished from histotroph (commonly termed “uterine milk”). The gestation period is approximately two to three months (Neer, 2008), and the females are believed to migrate to warmer waters to parturate (Mollet, 2002). Pacific populations typically pup from November to March near the equator, the Mediterranean population pups prior to migrating to warmer waters, and finally the Atlantic populations differ depending on the hemisphere. The South Atlantic population breeds during the austral summer, whereas the North Atlantic population breeds during the summer months in the warmer southern waters (Mollet, 2002). Migration patterns for the pelagic stingray follow the warmer water temperatures of the different regions and were determined using by-catch records for drift gillnets and pelagic longline catch records (Mollet, 2002). Mollet (2002) also found that captive pelagic stingrays died in water below 13° C, suggesting a biological basis to the northern and southern boundaries of the species’ circumglobal distribution.

### *Previous Satellite Tag Studies*

Pop-up satellite archival tags (PSATs) were developed to assist in collecting data from organisms that generally do not surface often and who also are generally difficult to recapture (Sedberry and Loefer, 2001). Satellite transmitting technology was originally developed to give positioning of marine organisms who basked at the surface often (Block et al., 2001). More advanced tags allowed for the tags to be submerged during the deployment time with the use of archival data (Block et al., 2001). The pop-up satellite archival tags record data, such as light level intensity, temperature, depth, for a given period of time. The tag then detaches from organism, floats to the surface, and finally uses the ARGOS satellite system to transmit the stored data (Arnold and Dewar, 2001).

Data collected from PSAT deployments can be used to help determine movement relating to biophysical aspects of the environment. A study by Goodyear et al. (2006) tracked blue marlin *Makaira nigricans* using the archival pop-up tags. The study found that the marlin descended into the depths during the daytime hours and then ascended to shallower waters. The ascension into shallower waters was discussed to not only be to follow potential prey items but also to be in quiescent mode (Goodyear et al., 2006). Similar vertical migration patterns have been observed with swordfish *Xiphias gladius* (Abascal et al., 2010) and Atlantic sailfish *Istiophorus platypterus* (Mourate et al., 2010). The data can also aid in determining post-release mortality of bycatch species (e.g., Kerstetter et al., 2003). It is important to understand individual species' vertical habitat utilization when trying to assess a population who is either targeted or bycatch during commercial fishing operations (Luo et al., 2006). With the knowledge of vertical utilization of different species, hook depths and deployment times could be modified so not to overlap bycatch and targeted species' foraging habitats, thereby reducing the potential of bycatch (Luo et al., 2006).

### *Previous Diet and Trophic Studies*

Stomach content analysis, which has traditionally been the primary method, can help with studying food web interactions between different species and to help construct food webs (Trites, 2001). Problems with stomach content analyses include: high digestion rates, identification of partially digested material, mistakenly including the bait in the indices, and delays between the capture of the specimen and the chemical preservation of the stomach, thereby resulting in partial digestion of the contents prior to examination (Bowen, 1996).

Elasmobranchs can exist in many different trophic levels within marine communities. Stomach content analyses have been done for many species of elasmobranchs, but quantitative trophic level studies are a more recent development. Stomach content analyses in conjunction with Ecopath II modeling have been used for trophic estimation in sharks and rays (Wetherbee and Cortes, 2004). Some of the orders of elasmobranchs used in this trophic estimation method included Carcharhiniformes, Lamniformes, Hexanchiformes, and Squaliformes (Cortes, 1999). The combination of both stomach content and Ecopath II analyses estimated that sharks and rays (both coastal and estuarine species) averaged to be in the tertiary consumer level (Wetherbee and Cortes, 2004). Stable isotope analyses are widely becoming used more frequently along with stomach content analyses for more comprehensive descriptions of trophic interactions in aquatic systems; however, relatively few stable isotope studies have been done on elasmobranchs, especially pelagic species (Rau et al., 1983; MacNeil et al., 2005).

A Pacific study by Rau et al. (1983) used $\delta^{13}C$ stable isotopes to estimate the trophic positioning of several different marine organisms across several trophic levels, including five species of sharks.  Another trophic study was done in the northwest Atlantic Ocean by Estrada et al. (2003), although the study collected shark specimens from a local fishing tournament and therefore had a limited sample size.  Isotopic analysis included both $\delta^{13}C$ and $\delta^{15}N$ readings to determine trophic positioning of the different species.  However, the two studies both concluded that stomach content and stable isotope analyses worked together to create a better understanding of the trophic interactions for various ecosystems.

As fisheries management shifts from species specific to ecosystem based management plans, knowing how all species in the system interact with one another will become vital to make the transition smoother and easier.  Knowing the vertical habitat utilization of non-target species and target species can show interactions between the species and also with oceanographic parameters.  Diet and trophic studies of by-catch species help to understand where resources in the ocean are going to and overall dynamics of the pelagic realm.  While the pelagic stingray is currently of little economic importance, knowing and understanding all species life histories will be important for future management approaches.

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**Author’s Note:** This thesis is comprised of two chapters. With the agreement of my master’s committee, the two chapters are presented not in the traditional format but rather as two manuscripts to be submitted for peer-reviewed journal publication.

# **Manuscript 1:** Habitat utilization and vertical movements of the pelagic stingray, *Pteroplatytrygon violacea* (Bonaparte, 1832), in the western North Atlantic Ocean using pop-up archival satellite tags

Tiffany Weidner[1], Charles F. Cotton[2], David W. Kerstetter[1*]

[1] Nova Southeastern University Oceanographic Center, 8000 North Ocean Drive, Dania Beach, FL 33004 USA
[2] Florida State University Coastal and Marine Laboratory, 3618 Coastal Highway 98, St. Teresa, FL 32358 USA
*Corresponding author: kerstett@nova.edu; phone +1-804-854-9030

## Abstract

The pelagic stingray *Pteroplatytrygon violacea* is commonly encountered as bycatch in the pelagic longline fishery targeting swordfish and tunas. However, very little is known about its habitat utilization and whether depth or temperature differences between the pelagic stingray and pelagic longline fishing gear could be used to develop fisheries bycatch mitigation techniques. Four pop-up satellite archival tags (PSATs) with 13-day deployment durations were attached to pelagic stingrays in 2010 and 2011 in both the South Atlantic Bight (n=2) and the northern Gulf of Mexico (n=2). Analysis of the minimum straight-line distances from the first transmission locations showed that pelagic stingrays moved between 151-258 kilometers (km) from where each stingray was released (11.6-19.8 km/day). Data from these tags indicate significant diel difference in behavior, with all four animals utilizing deeper depth during daylight periods. All four stingrays appeared to follow a temperature regime above all other variables. All four animals also displayed frequent short-duration (*ca.* 5-minute lengths) movements of more than 50 m from the baseline depth of the diel period and a thermal range of approximately 8°C over 24-hour periods. Applying the known habitat utilization and behavior of less economically important species will help fisheries managers better understand both overall interactions with more economically valuable target species and the overall pelagic ecosystem.

Key words: behavior, elasmobranch, diel, fisheries, PSATs, bycatch

## 1.0 Introduction

The pelagic stingray *Pteroplatytrygon violacea* (Bonaparte, 1832) is distributed circumglobally in sub-tropical and tropical pelagic waters (Wilson and Beckett, 1970). Although the species is known to inhabit epipelagic waters, Neer (2008) used commercial fisheries data to hypothesize that the pelagic stingray is benthopelagic, utilizing both benthic and pelagic waters. Pelagic stingrays are primarily encountered as bycatch on pelagic longline fishing fleets targeting tunas (Family: Scombridae) and swordfish *Xiphias gladius* Linnaeus, 1758, but they have also been caught in the drift gillnets of the eastern Pacific (Mollet, 2002) and in both Atlantic and Pacific purse seine fisheries (Wilson and Beckett, 1970).

Analyzing bycatch data from commercial pelagic longline vessels off Uruguay, Domingo et al. (2005) described the highest catch rates for pelagic stingray hooked on leaders that were targeting the 40-60 m depth range for nighttime sets. These rays also were most commonly found only over waters greater than approximately 4000 m deep with few catches reported during pelagic longline sets in less than 600 m. Fishers in Brazil anecdotally observed female stingrays taking hooks set in shallower water (less than 60 m), whereas the males are predominantly captured on deeper (up to 800 m) set hooks (Ribeiro-Prado and Amorim, 2008). Based on analyses of bycatch in drift gillnet and pelagic longline fisheries, pelagic stingray migrations in the Pacific Ocean follow seasonally warm water temperatures (Mollet, 2002). Mollet (2002) also found that captive pelagic stingrays died in water below 13°C, suggesting a physiological basis to the northern and southern boundaries of the species' circumglobal distribution.

Satellite-based telemetry originally was developed to locate the position of marine organisms basking at the surface, however, more advanced tags allow for the tags to be submerged during the deployment time with the use of archival data (Block et al., 2001). Electronic pop-up satellite archival tags (PSATs) were developed to assist in collecting data from organisms that do not surface often and are generally difficult to recapture (Sedberry and Loefer, 2001). PSATs can record a series of data (e.g., light intensity, temperature, pressure) over the course of the programmed deployment, then detach from the organism, float to the surface, and transmit the stored data via the polar-orbiting Argos satellite system (Arnold and Dewar, 2001).

The use of PSAT tags has allowed for better understanding of the vertical habitat utilization and diving behavior of large open-ocean species (Sims, 2010). Goodyear et al. (2006) analyzed PSAT data from a blue marlin *Makaira nigricans* Lacépède, 1802 and found that the species descended into deep water during the daytime hours and then ascended to shallower waters at night. These vertical movements were not only presumed to follow potential prey items but also to be in quiescent mode (Goodyear et al., 2006). Similar patterns have been observed for swordfish (Abascal et al., 2010) and sailfish *Istiophorus platypterus* Latreille, 1804 (Kerstetter et al., 2011) tagged in the Atlantic. However, the specific movement pattern and behavior of an individual has been shown to be location and temperature dependent; i.e., not every individual of a species should be expected to exhibit similar spatial or thermal distribution patterns (Abascal et al., 2010).

The vertical habitat utilization of individual species can be used to assess potential interaction probabilities with fishing gear (e.g. Luo et al., 2006). With such knowledge of vertical migrations of different species, hook depths and deployment times could be modified to mitigate bycatch within other targeted species' foraging habitats (Sims, 2010). Alternatively, preferred depth ranges of a species could be used in combination with the actual depths of pelagic longline gear to standardize catch rates, thereby improving stock assessments (see Hinton and Nakano, 1996).

Historical fishing records and anecdotal information from commercial pelagic longline vessel captains suggest that the highest catch rates for the pelagic stingray occur when the gear targets 40-60 m depths at night (Domingo et al., 2005). Aside from the limited studies done in the South Atlantic Ocean and the Pacific Ocean with these fisheries-dependent data, little information exists about the depth distribution and movement patterns of the pelagic stingray. The present study aims to determine the vertical movements of the pelagic stingray and describe the species' habitat utilization using short-duration PSATs in the western North Atlantic Ocean.

## 2.0 Materials and Methods

### *2.1 Satellite tag deployment*

Four pelagic stingrays were selected aboard commercial pelagic longline vessels for their relatively large size and good health (i.e., level of activity and lack of visible damage). Each individual stingray was placed on the deck and the tail barb was clipped for safety by the captain's request on three of four rays (one stingray's barb was already absent). Each stingray was held down by two assistants: one holding down the main body and the other securing the tail. Tags were surgically tethered through the musculature on either side of the vertebrae on the tail as described by Le Port et al. (2008); the only change to the attachment method was the addition of a small chafing tube over the exposed tether on the ventral side of the tail (see Figure 1). The chafing tube was added because monofilament can cut through muscle tissue when repeatedly pulled taut, which would result in added physiological stress to the tagged individual and/or loss of the tag.

Four high-rate (HR) X-tag model pop-up satellite archival tags (Microwave Telemetry Inc., Columbia, MD, USA) were programmed to record and archive light intensity (unit-less relative scale of 0 to 244), water temperature (degrees Celsius), and pressure (converted to depth in meters) data in sampling intervals of 93 seconds for 13 days. Two tags were deployed in the eastern Gulf of Mexico and the other two tags were deployed in the western North Atlantic Ocean off the northeastern coast of Florida.

### *2.2 Data analysis*

Periods of day and night were delineated using predicted times of sunrise and sunset provided by the United States Naval Observatory (http://www.usno.navy.mil). Crepuscular periods were defined as 30 min before and after sunrise/sunset for each day and were excluded from subsequent analyses. An ANOVA test was used to analyze the data with SPSS (IBM SPSS Statistics, v.22), and a Tukey's Studentized range test was used after to find where differences between data sets were. Box and whisker plots were used to graphically represent the daytime and nighttime distributions of depth and temperature for all four PSATs. Depth differences between sequential 93-second period points were used to investigate the range and speed of vertical movements. The nature of the transmission of archival data created occasional discontinuous intervals in the data sets. All discontinuous intervals were eliminated from the analysis of individual dives. This model of tag did not provide light-level data sufficient for light-based geolocation estimation; all horizontal displacements were based on minimum straight-line distance (MSLD) between tagging location and location of first ARGOS system transmission.

To determine periodicity of diel migrations, fast Fourier transformations (FFTs) of depth data were analyzed using Igor Pro (WaveMetrics, v.6.2.2.2), thus inferring habitat utilization and potential foraging locations. FFT uses signal processing to detect periodic components within a time-series of the archived depth data and then reveals any sinusoidal patterns (Shepard et al., 2006; Graham et al., 2006). Frequencies of dominant patterns (e.g., diel periodicity) are shown as peaks within the frequency power spectrum (Meyer et al., 2007).

## 3.0 Results

### *3.1 PSAT Data Recovery*

Locations of tag deployment, tag pop-off, depth, temperature, and light level were obtained for four female pelagic stingrays (Table 1). All four tags remained attached to the stingrays for the full 13 days before detaching as pre-programmed and transmitting data to the ARGOS satellite system. Data recovery for the archived data ranged from 81-90% (Table 1).

### *3.2 Horizontal Movement*

MSLDs showed that pelagic stingrays moved between 151-258 kilometers (km) from the location of release (11.6-19.8 km/day). The two stingrays tagged and released in the western North Atlantic travelled 181 km and 258 km, and the two stingrays released in the Gulf of Mexico travelled 151 km and 173 km.

### *3.3 Depth and Temperature*

Overall diel differences in time-at-depth (F = 5943.924, $p < 0.001$) were observed and Tukey’s Studentized range test ($\alpha = 0.05$) showed that each fish followed unique time-at-depth patterns. All stingrays spent daytime hours in deep water and nighttime hours in shallower depths. Depth ranges for daytime and nighttime for individual tags ranged from -582.3 m to 0.00 m, respectively (Figure 2). FFT analyses indicated prominent peaks at the 0.4 to 0.48 values, representing the day and evening peaks combined as half-day cycles. These diel peaks, along with the corresponding depth profiles, are shown in Figure 4.

All four stingrays also exhibited significant diel differences in time-at-temperature (F = 5192.226, $p$ <0.001). Water temperatures recorded for all stingrays ranged from 10.2° C to 29.7° C during both daytime and nighttime, with mean values of 18.56° C and 22.03° C respectively (Figure 2).

## 4.0 Discussion

### *4.1 Depth and Temperature Utilization*

Few studies have examined vertical movements of any stingray species, likely because most species are demersal. The present study was the first to document the movement patterns of the pelagic stingray. A previous acoustic tracking study of round stingrays, *Urolophus halleri* Cooper, 1863, used active acoustic telemetry and automated acoustic receivers to record movement patterns, light levels, and temperatures (Vaudo and Lowe, 2006). PSAT technology allows for tagged animals to be studied without having them remain within the confines of a receiver array or requiring personnel and resources to actively track or recapture the tagged animals. PSATs are especially well-suited for pelagic species since recapture of specimens and/or the use of acoustic receiver arrays are not practical for studying their movements. Despite previous challenges of using PSATs to document movement patterns of batoid fishes (e.g., Grusha, 2005), all four tags remained attached in the present study for the full duration and data recovery was high.

The four stingrays displayed vertical differences between daytime and nighttime depths. They exhibited diel vertical migrations, spending daytime hours in deeper water and nighttime hours in shallower water (see Figure 3). Other pelagic fishes have been shown to display diel vertical migrations patterns, including swordfish (Sedberry and Loefer, 2001), escolar *Lepidocybium flavobrunneum* (Smith, 1843) (Kerstetter et al., 2008), and shortfin mako shark *Isurus oxyrinchus* Rafinesque, 1810 (Loefer et al., 2005).

Due to the overlapping depths associated with the stingrays' diel movements and other commercially important species (i.e., swordfish and shortfin mako shark) there is little potential for commercial fishing gear avoidance and reduction of stingray bycatch by simply altering fishing target depths.

With the use of FFT analysis, the periodicity of the observed pelagic stingray vertical movements was quantified to determine the diel pattern of the movements. Periodicity analyses have been previously used to determine diel movements of other pelagic species such as swordfish (Lerner et al., 2013) and whale sharks *Rhincodon typus* Smith, 1829 (Graham et al., 2006). The FFT analysis values were not exactly 0.5 and were probably skewed due to mid deployment changes in behavior and due to length of the daytime and nighttime periods varying. The days were not half daytime and half nighttime, so the rays were spending varying amounts of time at the surface and at depth. Previous diet studies suggest that the rays are feeding on prey items generally associated with surface waters or species that exhibit diel movements themselves (e.g., Ribeiro-Prado and Amorim, 2008; Wilson and Beckett, 1970). Our data suggest that pelagic rays are more likely migrating through the water column to follow vertically migrating prey such as squid and pelagic crustaceans during the nighttime.

All four animals in the present study also displayed frequent short-duration (*ca.* 5-minute lengths) movements of more than 50 m from the baseline depth over 24-hour periods. Other species have been shown to exhibit short-duration movements to depth, presumably for foraging activities, including sailfish (Kerstetter et al., 2011) and shortfin mako (Loefer et al., 2005). Similarly, the pelagic stingrays in the present study were probably making these short dives in pursuit of prey items such as squid or crustacean species (e.g., Wilson and Beckett, 1970; Veras et al., 2009).

The four study animals remained in water between 18.56° C to 22.03° C. Warmer water temperatures were most frequently recorded at night when the stingrays were more likely to be in shallower waters. Recorded temperatures were lower during the daytime hours as the stingrays moved to deeper depths. Pelagic stingrays' diel movement patterns appear to be driven by a temperature regime similar to other stingray species, such as the bat ray *Myliobatis californica* (Gill, 1865) (Matern et al., 2000). Other pelagic species of elasmobranch also occupy this temperature regime, such as bigeye thresher, *Alopias* *superciliosus* (Lowe, 1840) (Musyl et al., 2001), shortfin mako (Loefer et al., 2005) and blue sharks, *Prionace glauca*, (Linnaeus, 1758) (Queiroz et al., 2010). Oceanographic parameters (e.g., temperature) are known to affect habitat utilization in several pelagic teleosts, such as Pacific bluefin tuna *Thunnus orientalis* (Temminck and Schlegel, 1844) (Kitagawa et al., 2000) and yellowfin tuna *T. albacores* Bonnaterre, 1788 (Gulf of Mexico: Weng et al., 2009; Pacific: Schaefer et al., 2013). Overall migrating behavior of the pelagic stingrays appears to be driven by seasonal temperatures. Catch per unit effort (CPUE) from Uruguayan longline fisheries showed the lowest CPUEs in waters below 15.3° C (Forselledo et al., 2008; Ferrari and Kotas, 2013). While the stingrays remained in a specific temperature range, temperature is apparently not the dominant factor in the vertical diel migration (i.e., behavioral themoregulation). The pelagic stingray diel vertical migration apparently follows prey items up to the surface during nighttime hours and down to the depths during the day, with some short duration drives for prey pursuit.

Changes in the diel pattern midway through the deployment periods initially suggested predation of Tag 76998 and Tag 77000. These tags indicated the stingrays adopted much deeper depths than previous periods and archival light levels dropped to near zero. However, there was no gap between the archival and real-time depth/temperature data that would signify a predation event took place, as discussed in the tag ingestion events of Kerstetter et al. (2004). Kerstetter et al. (2004) also described a predation-mediated temporal delay in recorded temperature changes with depth changes, since predated tags are not directly exposed to ambient waters. Also recorded light levels were zero, consistent with being inside the alimentary canal before being egested or regurgitated. Based on the absence of any temporal delay in the recovered data, the two rays in the present study were likely not predated upon; rather, we attribute these mid-deployment changes in the diel pattern for these two rays simply to higher levels of particulates in the water column and movement patterns at increased overall depths. The mid-deployment reduction of light observed for Tag 76998 and Tag 77000 may have been caused by increased turbidity, for example, which decreases light transmission to depth. While increased turbidity could not be attributed to any storm events in 2010 or 2011 (Anon., 2013), Tag 77000 did move from offshore waters onto the continental shelf. Specifically, *chlorophyll a* levels were ~0.05 mg m-3 where the tag was deployed, but levels were ~0.5 mg m-3 where the tag popped-off over the continental shelf (http://www.aoml.noaa.gov). Tag 76998 exhibited a change in the depth pattern on 26 April 2011, where the stingray moved to much deeper depths at night than for the previous six days. The loss of light may also be due to the rays' diving deeper in the water column mid deployment period. Loss of light within the water column is attributed to different factors at different depths. In less than 10 m of water, light loss is mostly from phytoplankton, whereas 30 m to 40 m is attributed to the water itself (Lorenzen, 1972). The pelagic stingrays, to remain in temperatures between 11° C and 24° C, dove to deeper water depths and experienced the increased loss of ambient light.

While the present study was the first to attach PSATs to pelagic stingrays, there were some limitations. Only two PSATs were deployed in the two geographic locations due to budgetary limitations. However, pelagic stingrays are a circumglobal species, and different populations may utilize the vertical habitat differently or display different diel movement patterns in response to local oceanographic conditions. Such considerations should be considered in future population assessments based on fisheries-dependent data. Given the novelty of the attachment method, the temporal scope of this study was also limited, with tag deployments of relatively short duration (13 days). For future studies, longer tag deployments could yield a greater understanding of the impacts of lunar and seasonal cycles on the movements of pelagic stingrays.

### *4.2 Potential Fisheries Implications*

Knowing the vertical movements and habitat utilizations of bycatch species that are frequently caught on commercial fishing gear could help fishers deploy gear at depths less likely to interact with non-target species. Habitat-based stock assessment can be used to direct fishing activities to those depths being utilized by the target species or away from depths utilized by species of concern during specific times of the day (Hinton and Nakano, 1996; Maunder and Punt, 2004). Understanding the spatial distributions and movement patterns of marine fishes through PSAT data strengthens stock assessments and improves the overall effectiveness of fisheries management. In particular, the habitat utilization (depth and temperature distributions) of a species can help standardize catch and effort data from pelagic longline fisheries (e.g., Hinton and Nakano, 1996; Schaefer et al., 2013). By also incorporating other biological data with electronic tagging data, a greater understanding of population structure for the pelagic realm can be obtained (Abascal et al., 2010).

Due to the pelagic stingrays' documented interaction with commercial fishing gear (e.g., Piovano et al., 2005; Forselledo et al., 2008), information on the species' vertical habitat utilization is essential to fisheries management especially in regards to stock assessments and relative commercial fisheries catch and effort data. However, the pelagic stingray appears to utilize the same water depths (40-60 m) during the nighttime sets of the pelagic longline fishery targeting swordfish (Domingo et al., 2005). In order to reduce bycatch of pelagic stingrays, commercial pelagic longline operations could modify deployment strategies to target depths below 50 m; however, due to the overlapping of depths and temperature regimes used by the targeted species and the pelagic stingray, it is highly unlikely that this would be a successful modification to deployment strategies. A study by Piovano et al. (2010) suggests the usage of larger-sized circle hooks as a means of reducing the bycatch of pelagic stingrays and thereby reducing haul back time and potential harmful interaction with the species. Further investigation would be necessary to determine if modifying gear or fishing depth would be economically feasible to reduce the bycatch of pelagic stingray.

Studying the movements of non-target species improves the overall knowledge of resource and vertical habitat utilization while also providing insight into their interactions with more economically important species (Kohler and Turner, 2001). Incorporating the vertical habitat utilization of non-target species with those of target species can reveal interactions occurring between species, which are often correlated with oceanographic parameters (e.g., temperature). Knowing the habitat utilization of the non-target species provides a better comprehension of the pelagic ecosystem by fisheries managers and science as a whole. While the pelagic stingray is currently of little economic importance, as management shifts from species-specific to ecosystem-based approaches, documenting species' movement patterns will be important parameters for ecosystem management.

## 5.0 Acknowledgements

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Table 1. Tagging date and location, sex, percent data recovery, and distanced travelled (minimum straight-line distance between tagging and tag pop-up locations) for four pop-up satellite archival tags deployed on four female pelagic stingrays (*Pteroplatytrygon violacea*) in the western North Atlantic Ocean during 2010. Deployment period of all four stingrays was 13 days.

| **Tag Number** | **Tagging Date** | **Tag Deployment Locations** | **Pop-off Date** | **Tag Pop-off Locations** | **Disc Width** | **Data Recovery** | **Minimum Straight Line Distance** |
|---|---|---|---|---|---|---|---|
| **76996** | 8/19/2010 | 23°14’50.06”N,<br>78°24’29.05”W | 9/1/2010 | 29°23’27.60”N,<br>77°8’31.20”W | 55 cm | 90% | 181 km |
| **77000** | 11/8/2010 | 27°25’1.20”N,<br>85°47’60.00”W | 11/21/2010 | 28°46’44.40”N,<br>86°1’44.40”W | 61 cm | 81% | 151 km |
| **76995** | 11/10/2010 | 27°41’60.00”N,<br>85°24’0.00”W | 11/23/2010 | 28°46’55.20”N,<br>86°40’55.20”W | 76 cm | 88% | 173 km |
| **76998** | 4/18/2011 | 30°9’50.40”N,<br>77°22’58.80”W | 5/1/2011 | 27°57’10.80”N,<br>78°9’14.40”W | 64 cm | 88% | 258 km |

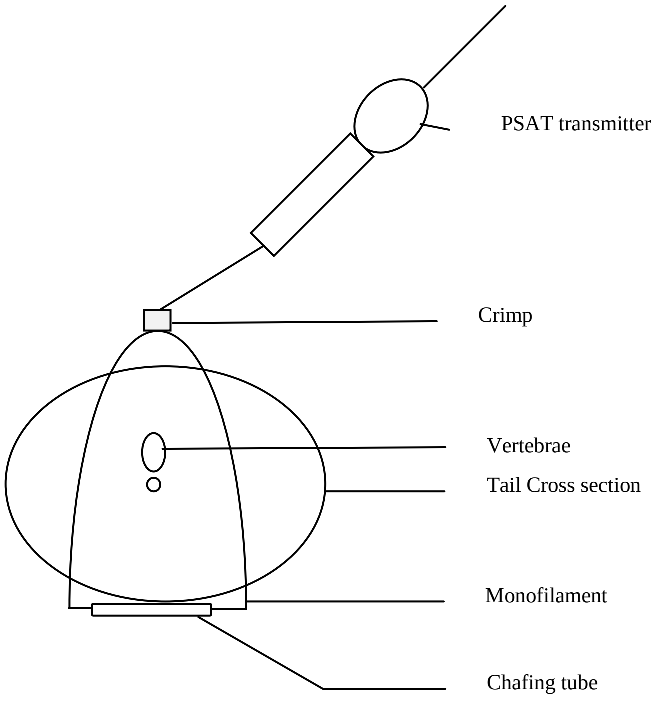
Figure 1. Diagrammatic cross-section of the surgical attachment of the pop-up satellite tag in the base of the tail of the stingray adapted from Le Port et al. (2009). The tags were attached anterior to the barb. The tags were tethered through musculature on either side of the tail vertebrae, with a chafing tube at the base to reduce friction of the tag on the animals.

Figure 2. Box-and-whisker plots showing the depth (m) and temperature (°C) data for the four pelagic stingrays monitored by pop-off satellite archival tags for 13-day deployment durations. The depth distributions are separated into day and night periods for both depth and temperature for a. Tag 76998, b. Tag 76996, c. Tag 76995, and d. Tag 77000. Open circles indicate outliers in the samples, and asterisks are extreme outliers (three times the box).

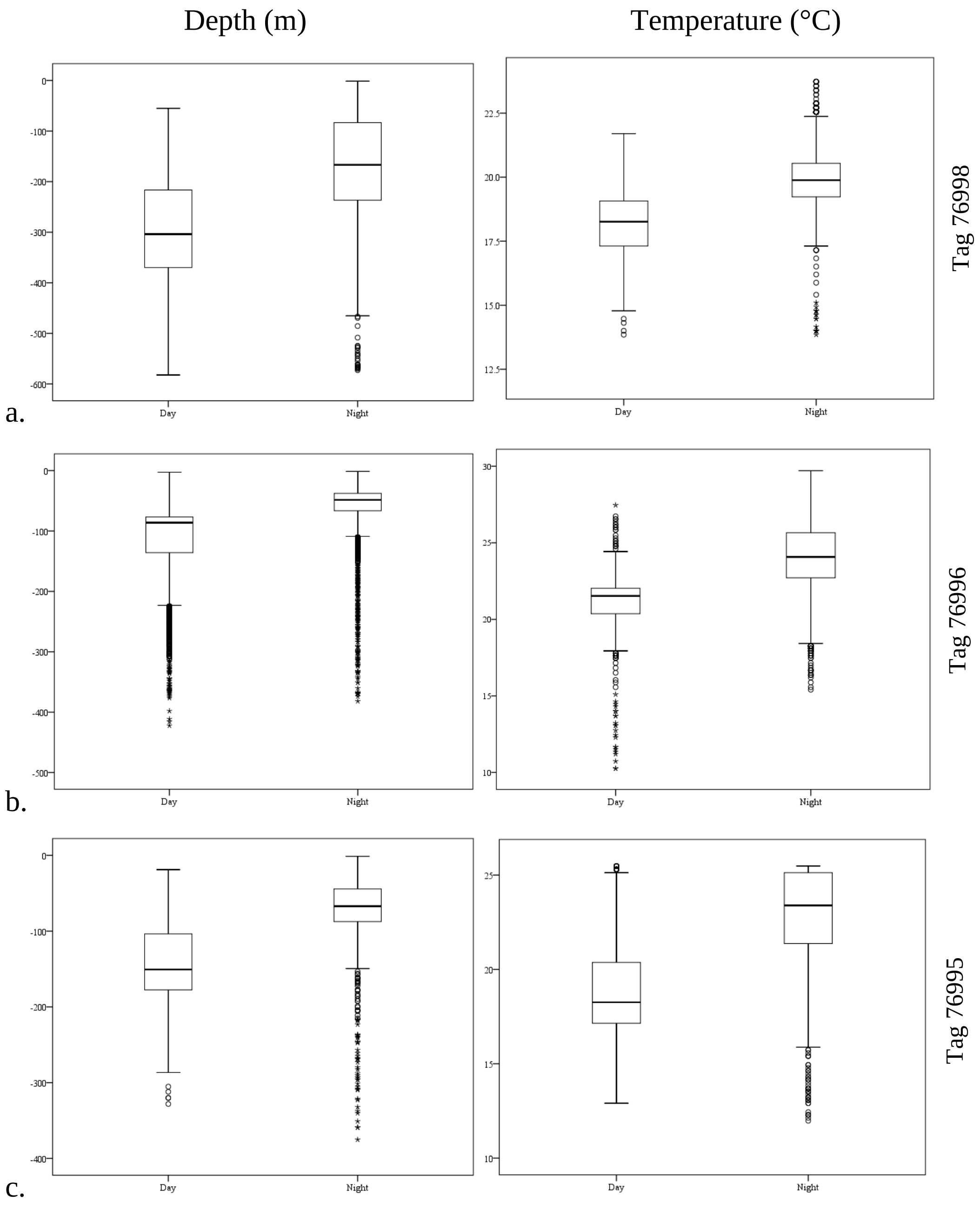
Figure 3. Line graphs depicting the mean depths (m) for daytime and nighttime per day for the 13-day deployment durations for all four tags deployed in a. the South Atlantic Bight and b. the Gulf of Mexico.

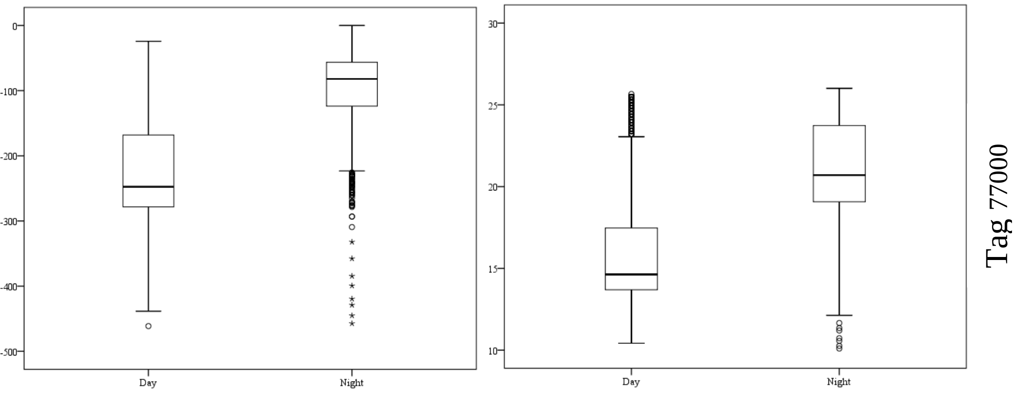
Figure 4. Fast Fourier Transformation distributions displaying dominate peaks (in hertz) and corresponding depth graphs (in meters) for a. Tag 76998, b. Tag 76996, c. Tag 76995 and d. Tag 77000.

[Figure 1]


PSAT transmitter
Crimp
Vertebrae
Tail Cross section
Monofilament
Chafing tube

[Figure 2]

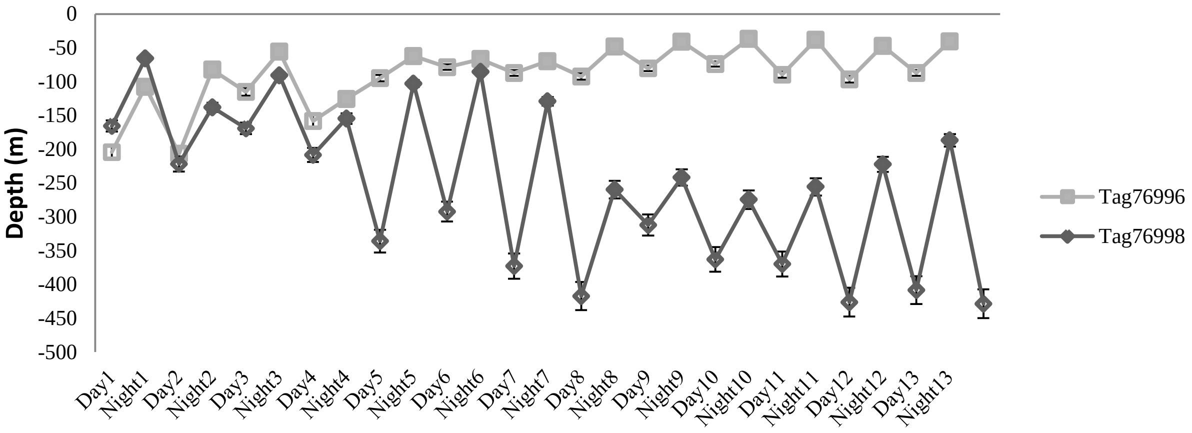

Depth (m)
Temperature (°C)
a.
0
-100
-200
-300
-400
-500
-600
Day
Night
22.5
20.0
17.5
15.0
12.5
Day
Night
Tag 76998
b.
0
-100
-200
-300
-400
-500
Day
Night
30
25
20
15
10
Day
Night
Tag 76996
c.
0
-100
-200
-300
-400
Day
Night
25
20
15
10
Day
Night
Tag 76995


0
-100
-200
-300
-400
-500
Day
Night
30
25
20
15
10
Day
Night
Tag 77000

d.

[Figure 3]

a.


0
-50
-100
-150
-200
-250
-300
-350
-400
-450
-500
Depth (m)
Day1
Night1
Day2
Night2
Day3
Night3
Day4
Night4
Day5
Night5
Day6
Night6
Day7
Night7
Day8
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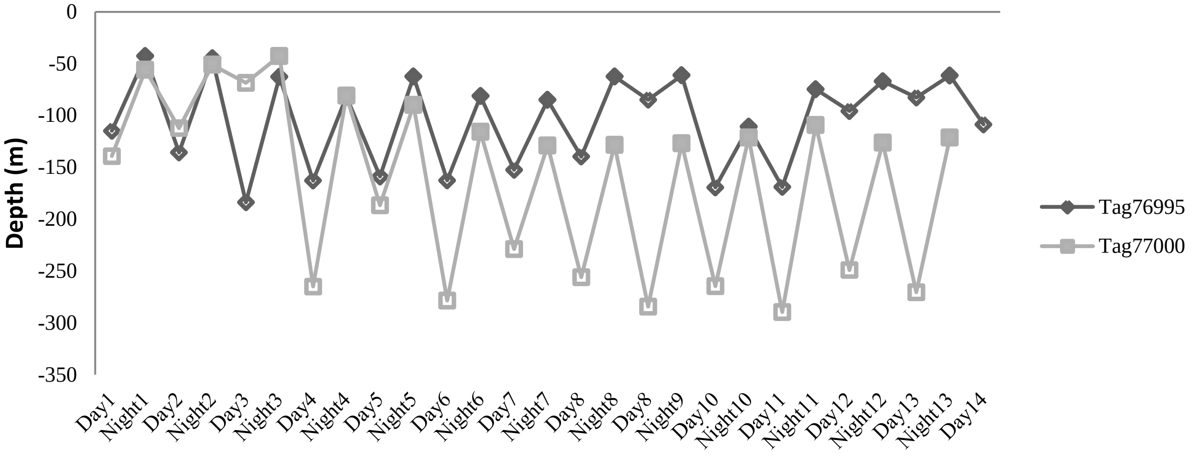
Day12
Night12
Day13
Night13
Tag76996
Tag76998

b.


0
-50
-100
-150
-200
-250
-300
-350
Depth (m)
Day1
Night1
Day2
Night2
Day3
Night3
Day4
Night4
Day5
Night5
Day6
Night6
Day7
Night7
Day8
Night8
Day8
Night9
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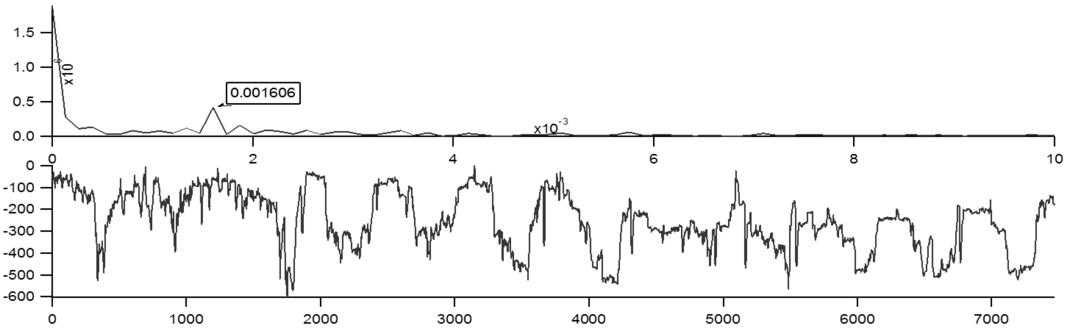
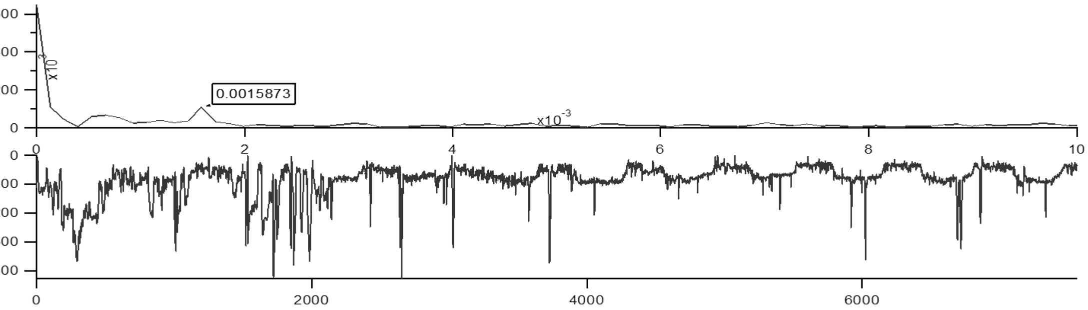
Night10
Day11
Night11
Day12
Night12
Day13
Night13
Day14
Tag76995
Tag77000

[Figure 4]

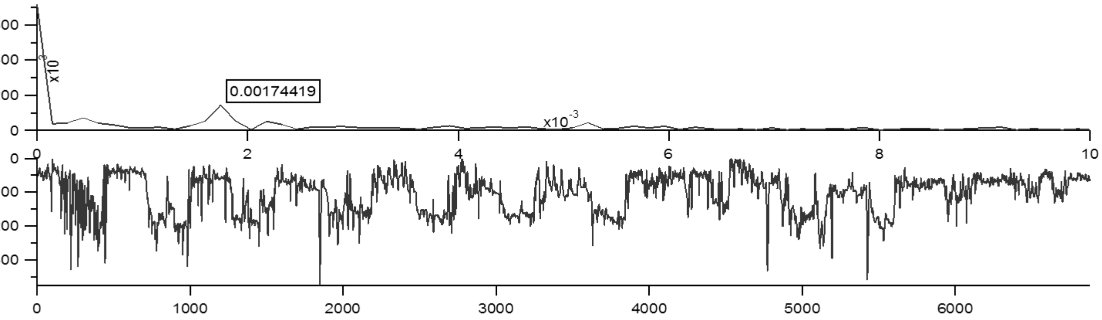


a.

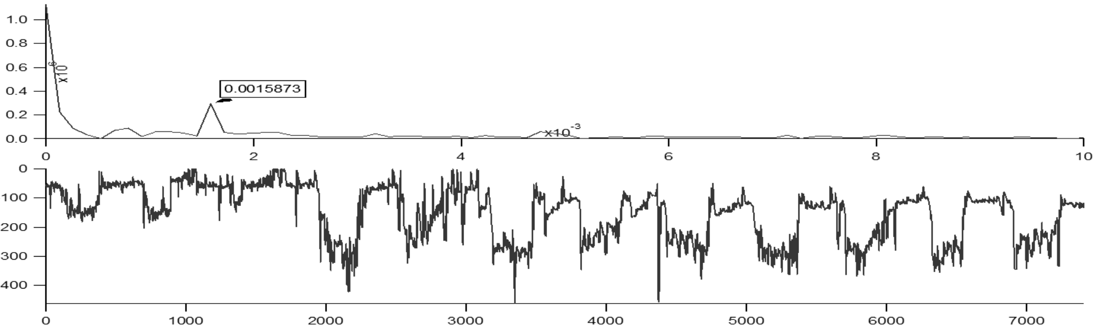
b.



c.



d.



# **Manuscript 2:** Combined gut-content and stable isotope trophic analysis of the pelagic stingray *Pteroplaytrygon violacea* from the western North Atlantic Ocean

Tiffany A. Weidner[1], Amy C. Hirons[2], and David W. Kerstetter[1,*]

[1] Nova Southeastern University Oceanographic Center, 8000 North Ocean Drive, Dania Beach, FL 33004 USA

[2] Farquhar College of Arts and Sciences, Nova Southeastern University, Davie, FL 33004 USA

[*] Corresponding author: kerstett@nova.edu

## Abstract

The understanding of trophic relationships is vital for correctly modeling ecosystems and ecosystem effects of fisheries removals. The pelagic stingray is found in epipelagic sub-tropical and tropical waters worldwide and individuals are a common bycatch in pelagic longline fisheries. For this work, 156 specimens (81 males and 75 females) were collected during pelagic longline fishing operations in the U.S. South Atlantic Bight and Gulf of Mexico between August 2008 and November 2011. Stomach content analyses found that the major prey items were cephalopod mollusks (59.18%), followed by actinopterygiian fishes (37.75%), and decapod crustaceans (35.71%). These rates of prey items found in the stomachs coincided with previous studies done in the Pacific Ocean. In contrast to previous studies that found high percentages of empty stomachs (63%), the current percentage of empty stomachs was much lower (25.6%). In addition, stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ was performed on white muscle to correlate trophic level data from the gut-content analysis. The $\delta^{13}C$ values ranged from -18.81 to -16.70‰ while the $\delta^{15}N$ ranged from 6.11 to 11.88‰. The stingrays were feeding on a different carbon source than other pelagic elasmobranchs, but were feeding within two trophic levels. The understanding of the pelagic stingray trophic position can help fisheries management as it begins to transition into ecosystem-based management.

Key Words: elasmobranch, diet, trophic, pelagic, stingray, *Pteroplaytrygon*

## Introduction

Pelagic waters have vast areas of oligotrophic deep water away from the more turbid, nutrient-rich waters of the coastal zone. Species found in the pelagic realm are typically generalist in their prey selection or successful scavengers; food, when it becomes available, is quickly consumed. The lower productivity of oligotrophic pelagic waters result in potential overlap of prey items for predators and feeding competition compared with areas of higher productivity. Species found in the pelagic realm are often difficult to study due to the high mobility of the animals and lack of access to study specimens. Many studies have enlisted the help of the pelagic longline fishing fleets in cooperative research programs to collect specimens, such as billfishes, marine mammals, and elasmobranchs, which are occasionally caught as bycatch. Many organisms that are part of the bycatch complex in the pelagic longline fishery have little known about their life histories (Cortes, et al., 2010; Simpfendorfer, et al., 2008).

Many bycatch organisms in commercial pelagic longline fisheries are relatively understudied, which potentially could lead to the depletion of an ecologically vital species. The International Union for Conservation of Nature (IUCN) lists approximately 47% of all pelagic elasmobranchs as 'data deficient'; however, the pelagic stingray was recently moved from 'data deficient' to 'least concern' as long as the stock continues to be monitored through the pelagic observer data (Forselledo et al., 2008). The combining of both stomach content and stable isotopes analyses will provide a better understanding of the food web interactions of the stingray. There is a lack of information on pelagic food webs, specifically for those species that can both impact the larger predatory fishes of economic import and alter the overall structure of the pelagic food web (Rooker et al., 2006).

The pelagic stingray *Pteroplaytrygon violacea* is distributed circumglobally in sub-tropical and tropical pelagic waters, although there is a distinct population found only within the Mediterranean Sea (Wilson and Beckett, 1970; Neer, 2008). Pelagic stingrays are primarily encountered as bycatch on pelagic longline fishing fleets targeting thunnid tunas and swordfish *Xiphias gladius* in the Pacific and Atlantic oceans. However, specimens have also been caught in the Pacific Ocean by drift gillnets of the swordfish fishery (Mollet, 2002) and the purse seine nets used to target tunas (Wilson and Beckett, 1970). The population of pelagic stingray is currently not considered to be greatly impacted by these fisheries because the species is speculated to have high reproductive output of the live-bearing elasmobranchs (Camhi et al., 2009). Limited data on feeding behaviors by the pelagic stingray contribute to the unknown effect that fisheries are exerting on the species.

Quantitative studies of food webs and trophic positioning include several different techniques. There is the traditional stomach content analysis, which includes physical removal of the stomach and subsequent examination of the stomach contents. There is also the potential to use stomach lavage techniques that allow for the live release of the individual animal, although these studies have been extremely rare in pelagic fishes. This method is, unfortunately, also pragmatically impractical for many pelagic species due to limited space during capture, safety concerns, and time limitations while collecting at sea. Finally, there are captive diet studies in aquaria; however, problems arise because the full variety and seasonal abundance changes of potential prey items cannot be fully replicated within an aquaria setting (Bowen, 1996).

Stomach content analysis, which has traditionally been the primary method, can help assess food web interactions between different species and to help construct food webs with comparative diet studies (Preti et al., 2001; Trites, 2001). Problems with stomach content analyses include: high digestion rates, identification of partially digested material, mistakenly including the bait in the indices, and delays between the capture of the specimen and the chemical preservation of the stomach, thereby resulting in partial digestion of the contents prior to examination (Bowen, 1996).

Stable isotope analysis has become a widely used technique in combination with stomach content analysis to estimate trophic position. Use of biochemical techniques, such as stable isotope ratios, helps to alleviate biases such as unrecognizable prey items, stomach content “snapshots,” and insufficient sampling numbers to provide adequate conclusions to trophic interactions (MacNeil et al., 2005). Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are transferred from prey to predator in a predictable way with $\delta^{13}C$ increasing 0.5-1‰ per trophic position and $\delta^{15}N$ increasing by 3-4‰ (DeNiro and Epstein, 1978; 1981; Vander Zanden et al., 1999).

Relatively few studies have analyzed the diets of the pelagic stingray. Prior stomach content analysis studies demonstrated a variety in their feeding selections. The items that were found in the stomach of 16 specimens by Wilson and Beckett (1970) included six small squid or squid beaks, four sygnathid seahorses, two monacanthid filefish, one coelenterate medusae, a single barracudina *Paralepis* sp.; one stomach had some unidentifiable teleost fish remains, and nine stomachs were either empty or only contained bait. A single pelagic stingray within this same study had parts of both a demersal thalassinid decapod and a holoplanktonic heteropod snail. The items that were found in the stomachs reflected the pelagic habitat that the stingray was known to utilize for predation. Davalos-Dehullu and Gonzalez-Navarro (2003) looked at the stomach contents of a single female pelagic stingray caught in the Gulf of California and identified two skulls of the teleost chub mackerel *Scomber japonicus*. A more robust dietary study was done on pelagic stingray by Ribero-Prado and Amorim (2008) using a sample size of 157 individuals collected in Brazilian waters of the South Atlantic by commercial pelagic longline fishing vessels. Ninety-nine stomachs (63%) were empty, but within the remaining stomachs, cephalopods (predominately *Loligo* spp. squid) were the dominant prey item (50%), followed by actinopterygiian fishes (19%) and crustaceans (17%). A study by Veras et al. (2009) found dominant prey items to be hyperiid amphipods, teleosts, brachyuran megalopae and pteropods in the southwestern equatorial Atlantic Ocean. Feeding seasonality was associated with sexual dimorphism, but no specifics were described (Mollet et al., 2002). The sample sizes or shorter sampling periods of these studies did not allow for assessment of seasonal or age-related diet shifts.

In contrast, the current study on the pelagic stingray diet composition and trophic position used a larger sample size from the western North Atlantic population. The previous stomach content studies did not utilize stable isotope techniques to help evaluate trophic positioning of the species. The combination of a larger sample size of stomachs and the usage of stable isotope analysis helped create a better overall, comprehensive picture of the trophic interaction of the pelagic stingray.

## Materials and Methods

### *Specimen Collection*

Pelagic stingrays were collected opportunistically by fisheries observers aboard U.S.-based commercial pelagic longline vessels targeting tunas (Family: Scombridae) and swordfish *Xiphias gladius* in the western North Atlantic Ocean and Gulf of Mexico between approximately 25°N and 35°N and westward of 75°W. The stingrays were brought on board, and the disk width (DW) measured and sex determined. Specimens were then retained whole in the fish hold on ice for the remainder of the trip (*ca.* 5 days). Other specimens were caught incidentally by other commercial pelagic longline vessels and retained frozen in the bait freezer until collected.

### *Section 1A: Stomach content analyses*

Once landed, weights and DWs were recorded in the laboratory. Per the methods of Bowen (1996), the stomach was removed, weighed, and placed in 10% buffered formalin for approximately one month until thoroughly preserved. The stomach was then transferred to 70% isopropyl or 70% ethanol for long-term storage prior to content analysis.

During content analysis, the stomach was weighed, opened, and the contents emptied into a petri dish. The empty stomach was weighed and the contents sorted. Any identifiable material was recorded and placed into small vials to be later identified to lowest taxon. Stomach contents were presented in the following indices: percentage by number, percentage by volume or weight, percentage of occurrence, and by the index of relative importance (Cortes, 1997). Percentage by number (%N) is determined by number of prey items of each prey type. The number of each prey type was then calculated to a percentage of the total number of prey items counted. Percentage by volume or weight (%V or %W) analyzes the weight of each prey item as a percentage of the total weight of prey items in an individual stomach. Percentage of volume or weight suggests the relative importance of a given prey item to the overall ingested diet of the individual consumer. Percentage of occurrence (%O) quantifies the diet by compiling a total list of prey items found and then compared to the presence or absence of the prey item. High percentages of occurrence indicate that the given prey item is found in many individual specimens (Bowen, 1996). The index of relative importance (IRI) is calculated as:

$$IRI= \%O(\%W + \%N)$$

The IRI is then converted to a percentage (per Cortez, 1997):

$$\%IRI= 100*IRI / \Sigma IRI$$

*Section 1B: Stable isotope analyses*

Muscle tissue samples were collected from the dorsal pectoral wing of the stingray during October and November 2008, 2009, and 2011. The white muscle tissue of the pectoral wing was chosen due to the lack of skeletal muscle generally available on the rays; the dorsal section of the wing allowed for a substantial amount of tissue to be taken. A total of 49 samples were collected (25 females and 24 males) and the samples were frozen in a -20°C standard freezer until processed. White muscle tissues were analyzed for $\delta^{13}C$ and $\delta^{15}N$ by dehydrating the samples at 60°C for 48-72 hours, ground and homogenized with a Wig-L-Bug alamagamator, and pelletized before analysis using an isotope ratio mass spectrometer (Estrada et al., 2003). Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ were used to determine dietary assimilation of prey items and also to help predict the potential trophic feeding level of the pelagic stingray (McCutchan et al, 2003; Vander Zander and Rasmussen, 2001). T-tests were used to assess statistical differences between seasonality and sexes. Significance was assessed at $\alpha=0.05$.

Literature values for potential prey and additional pelagic fish species' $\delta^{13}C$ and $\delta^{15}N$ were used due to the expense and time required to collect additional organisms from the study area. Collection of all prey items would also not be logistically feasible due to time constraints, as well as the logistical difficulty associated with physically collecting some specimens (e.g., small pelagic shrimp, squid species, isopods) while aboard the cooperating commercial fishing vessels. Several studies have already reported values for some prey items such as Atlantic herring, pelagic squids, and Atlantic flying fishes (e.g., Estrada et al., 2003; Rau et al., 1983).

## Results

### *Section 1A: Stomach Content Analysis*

A total of 156 stomachs were analyzed (males: n=81, DW mean=48.7 SD± 3.8; females: n=75, DW mean=52.4 SD± 6.5). In contrast to previous studies, the present study had only 25.6% empty stomachs and 11.54% had unidentifiable digested material. Of the empty stomachs, 55% belonged to male stingrays and 45% were female stomachs. Macroalgae, predominantly *Sargassum* sp., was found in 4.09% of the stomachs. Parasitic nematodes were found in 1.36% of the stomachs. However, they were assumed to be incidental or resident parasites versus an actively consumed prey item and thus excluded from subsequent analyses. The stomach content data is represented in Table 1 as percent occurrence, percent number, percent weight, and IRI (Bowen, 1996) for each prey item found in the stomachs. The actual weights of prey items were used rather than reconstituted weights, in large part because length-weight morphometric relationships are unknown for most of the recovered prey items.

Prey items found in the stingray stomachs included mollusks, teleosts, and crustaceans. The IRI and percent frequency of prey items can be graphically seen in Figure 1a and b. Mollusks (cephalopods) comprised the largest portion of the diet by %O: 59.2%, %N: 43.3%, %W: 14.9%, and %IRI: 70.3%. Squid species had values of %O: 8.3%, %N: 42.7%, %W: 14.5%, and %IRI: 73.5%. However, of the 149 individual squids identified in the stomach contents, only 12 were including the soft bodies. The rest of the percent occurrence was determined from beaks found in the stomachs.

Teleost fishes followed with values of %O: 37.8%, %N: 1.9%, %W: 5.5%, and %IRI: 5.7%. Unknown teleosts index values of %O: 22.5%, %N: 12.0%, %W: 4.3%, and %IRI: 8.1%. Due to advanced stages of digestion, *Hippocampus* sp. seahorses and monocanthid filefish were the only identifiable subcategory of teleosts. *Hippocampus* sp. has values of %O: 6.1%, %N: 2.0%, %W: 0.3%, and %IRI: 0.3%, while the filefish had values of %O: 9.1%, %N: 5.2%, %W: 0.9%, and %IRI: 1.2%.

Crustaceans were comprised the smallest observed portion of the pelagic stingray diet. The index values were %O: 35.7%, %N: 31.0%, %W: 2.0%, and %IRI: 24.0%. Shrimp species were the only identifiable crustacean prey, with values of %O: 24.5%, %N: 27.8%, %W: 2.0%, and %IRI: 16.1%.

### *Stable Isotope Analysis*

Stingray disk widths ranged from 41-70 cm, which indicated all the sampled were from reproductively mature adults (Neer, 2008). The $\delta^{13}$C ranged from -18.81 to -16.70‰ with a mean of -17.85±0.437‰ and $\delta^{15}$N ranged from 6.11 to 11.88‰ with a mean of 8.57±1.25‰. The dorsal muscle tissue was collected during various seasons over a four year time period, but samples were collected opportunistically, and therefore not consistently across seasons. While seasonality could not be tested, inter-annual variability indicated no significant difference in either stable isotope between any of the years (ANOVA: $\delta^{15}$N f= 2.41, p-value <0.05; $\delta^{13}$C f=3.06, p-value<0.05). Both stable isotope ratios indicated the stingrays were foraging across two trophic levels based on the fractionation values for both carbon and nitrogen. The percent C/N ranged from 2.37 to 3.13 with a mean of 2.77, indicating a diet not overtly rich in lipids. The $\delta^{13}$C values were similar between females versus males with values ranging from -18.81 to -16.70 and -18.59 to -17.18, respectively. Female stingrays had a comparable range in $\delta^{15}$N to males, 6.11 to 11.88‰ vs 6.47 to 10.95‰. There were no significant differences between male and female individuals for either $\delta^{13}$C (t-test: t=0.22, $\alpha>0.05$) or $\delta^{15}$N (t-test: t=0.25, $\alpha>0.05$) (Figure 2). Squid beaks and tissue, followed by shrimps, were the most common representatives in the stomach contents. While the sampling of potential prey items was not included in this study, stable isotope values of potential prey, related elasmobranch species, and pelagic teleosts found in western North Atlantic pelagic waters were gathered from published literature for trophic comparisons (Figure 3).

## Discussion

### *Stomach Content Analysis*

Stomach content analysis supported previous findings from other stomach content studies on the pelagic stingray. However, the present study had a significantly lower rate of empty stomachs (25.6%) compared to 56.25% in Wilson and Beckett (1970) and 63% in Ribero-Prado and Amorim (2008). The difference in percentage of empty stomachs between the current study and previous studies could be due to the shorter time between capture and fixation of the stomachs in this study. Similar to Ribero-Prado and Amorim

(2008), cephalopoda were the dominant prey item in the stingray diet (Figure 1a and b). The cephalopods were identified as belonging to the families Loliginidae and the majority from Ommastrephidae. Members of the class Crustacea were the next dominant prey items in percent number and percent index of relative importance. Crustaceans, specifically the deepwater shrimp *Heterocarpus ensife*, were a prominent prey item found in the stomachs of pelagic stingrays caught in the southwestern equatorial Atlantic Ocean (Veras et al., 2009). Teleost fishes were the third most prevalent prey item, with unknown teleosts occurring in the highest abundance followed by Monacanthidae then *Hippocampus* spp. Similar prey items were found in all stomach content studies done on the pelagic stingray. Studies involving quantitative stomach content studies on blue sharks, *Prionace glauca*, showed a strong prey preference for teleosts and cephalopods (Kohler, 1987).

While there was no statistical difference between seasons in prey items, squids appeared more often in the stomach contents during the spring and summer months while crustaceans were more prominent prey items during the winter months. Squid activity in offshore waters is pronounced during the summer months due to stratification of the water column (Straudinger, 2006). The shift could be due to the availability of prey items at the different times of the year. The rays have been known to seasonally target schools of mating squids (Neer, 2008). Ontogenetic shifts in diet have been observed in aquaria with the pelagic stingray; younger age classes fed predominately on crustaceans and as they grew in size, shifted to a cephalopoda based diet (Mollet et al., 2002). The study suggested the shift from a crustacean- to a squid-based diet may have been for caloric intake, where larger rays were opting to eat squids over the crustaceans (Mollet et al., 2002). However, due to the gear selectivity of specimen capture, this study could not confirm an ontogenetic diet shift with the western North Atlantic ray population. There is a potential bias for the IRI of prey items due to the probably accumulation of squid beaks found in the stingray stomachs. The beaks of squids are known to digest slower than the rest of its soft body parts. Some studies looked at classifying the overall digestive stage of the beaks and only consider a “type A” beak which was only ingested no more than six days prior and had the two parts intact with some cartilage still present (Piatkowski and Pütz, 1994).

### *Pelagic Stingray Diets*

Both stable isotope ratios indicated the stingrays were foraging across two trophic levels based on the fractionation values for both carbon and nitrogen. Female and male stingrays had similar $\delta^{13}$C and $\delta^{15}$N throughout the study, indicating no trophic differences between genders as well as amongst years. Based on fractionation factors of 0.5-1‰ and 3-4‰ for $\delta^{13}$C and $\delta^{15}$N, respectively, potential prey likely had $\delta^{13}$C of ~-19‰ and 5.5-6‰ in $\delta^{15}$N. The *Illex argentinus* closely resembles these values. No shrimp stable isotope values were located for the Mid-Atlantic Bight region for prey comparison, however stable isotope values from the Gulf of Mexico shrimp species caught around Florida were $\delta^{13}$C of -17.5‰ to -14.5‰ and 8.3‰ in $\delta^{15}$N (Fry, 1983). The organisms in Figure 3 with $\delta^{13}$C values less than -19‰ were all lipid-rich species. If they were incorporated in the stingrays' diet, they would have significantly increased the C/N ratio that the analyses of pelagic stingray tissues did not exhibit. The majority of elasmobranch species values taken from the literature appeared to have relied upon prey that utilized a similar carbon source (inshore vs. offshore) but were one to two trophic levels more enriched than the stingrays themselves (Rau et al., 1983; Estrada et al., 2003). Three nearshore elasmobranch species were incorporated into the graph to give reference the pelagic stingrays were likely utilizing an offshore carbon source (Tilley et al., 2013). The teleost species with a similar carbon source to the pelagic stingrays were all more enriched trophically and could not have contributed to their diets significantly. *Hippocampus* sp. were found in nearly one-third of all stingray stomachs and the lone stable isotope value from the literature (Logan et al., 2011) suggests that they may be a possible contributor to overall diet.

Sargassum was also found in the stomachs of the stingrays (4.1%). The stingrays were likely not feeding on the sargassum directly, but ingesting it incidentally while preying upon organisms living in the aggregated mats, such as seahorses and small fishes. Rooker et al. (2006) looked at sargassum as the primary producer in pelagic systems, and observed stable isotope ratios of sargassum, wahoo *Acanthocybium solandri*, dolphinfish *Coryphaena hippurus*, king mackerel *Scomberomorus cavalla*, yellowfin tuna *Thunnus albacores*, and blackfin tuna *Thunnus atlanticus*. The stable isotope ratios confirmed that sargassum is not the base for the pelagic food web; instead, the largest fraction of organic matter in the pelagic system was from particulate organic matter rather than from the sargassum itself (Rooker et al., 2006). Pelagic stingrays are likely to be opportunistically feeding on the small fish species associated with the floating sargassum mats of the pelagic waters.

Analysis of stomach contents has traditionally been the way to study the diet of an organism. However, stomach contents only provide a “snapshot” picture of what an animal has recently ingested. Since pelagic elasmobranchs are assumed to be intermittent feeders and typically found with prey items in advance digestion stages (Joyce, et al., 2002; Wetherbee and Cortes, 2004), determination of an exact diet can prove to be difficult. By incorporating additional techniques, such as stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$ values, the understanding of the trophic interactions by a species in a given ecosystem can be better interpreted. Both techniques in combination provided a greater understanding of pelagic stingray diet composition, and confirmed previous studies of the opportunistic feeding style in the pelagic food web. As fisheries management shifts to a more ecosystem-based framework, understanding and having the trophic dynamics of middle-level predators like the pelagic stingray will become vital.

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Table 1. Percent occurrence (%O), percent number (%N), percent weight (%W) and index of relative importance (%IRI) of prey items from 156 pelagic stingrays from the Gulf of Mexico and western North Atlantic Ocean. The three taxa of prey items were calculated along with the more specific families. Also included in the table is the percent occurrence for partially digested material, macroalgae (sargassum), and empty stomachs.

| **Prey Item** | **% O** | **% N** | **% W** | **%IRI** |
|---|---|---|---|---|
| **Cephalopoda** | **59.18** | **43.27** | **14.88** | **70.27** |
| Teuthida | 59.18 | 43.26 | 14.51 | 74.00 |
| **Crustacean** | **35.71** | **30.95** | **1.98** | **24.01** |
| Shrimp | 24.49 | 27.79 | 1.97 | 16.10 |
| Unknown crustacean | 11.22 | 3.15 | 0.01 | 0.78 |
| **Teleost** | **37.76** | **1.92** | **5.50** | **5.72** |
| *Hippocampus* sp. | 6.12 | 2.01 | 0.25 | 0.31 |
| Monocanthid filefish | 9.18 | 5.16 | 0.92 | 1.23 |
| Unknown teleost | 22.45 | 12.03 | 4.32 | 8.11 |
| | | | | |
| Partially Digested Material | 11.54 | | | |
| Macroalgae (*Sargassum* sp.) | 4.09 | | | |
| Empty | 25.64 | | | |

Figure 1. a. A graphical representation of the percent occurrence of the different items ingested by pelagic stingrays collected in the western North Atlantic Ocean. Squid, shrimp, and “unknown teleosts” were the dominant prey items found in the collected stomachs. b. The index of relative importance (IRI) values calculated for the items that were found in the stingrays’ stomachs.  Squid had the highest IRI values, followed by shrimp and “unknown teleosts.”

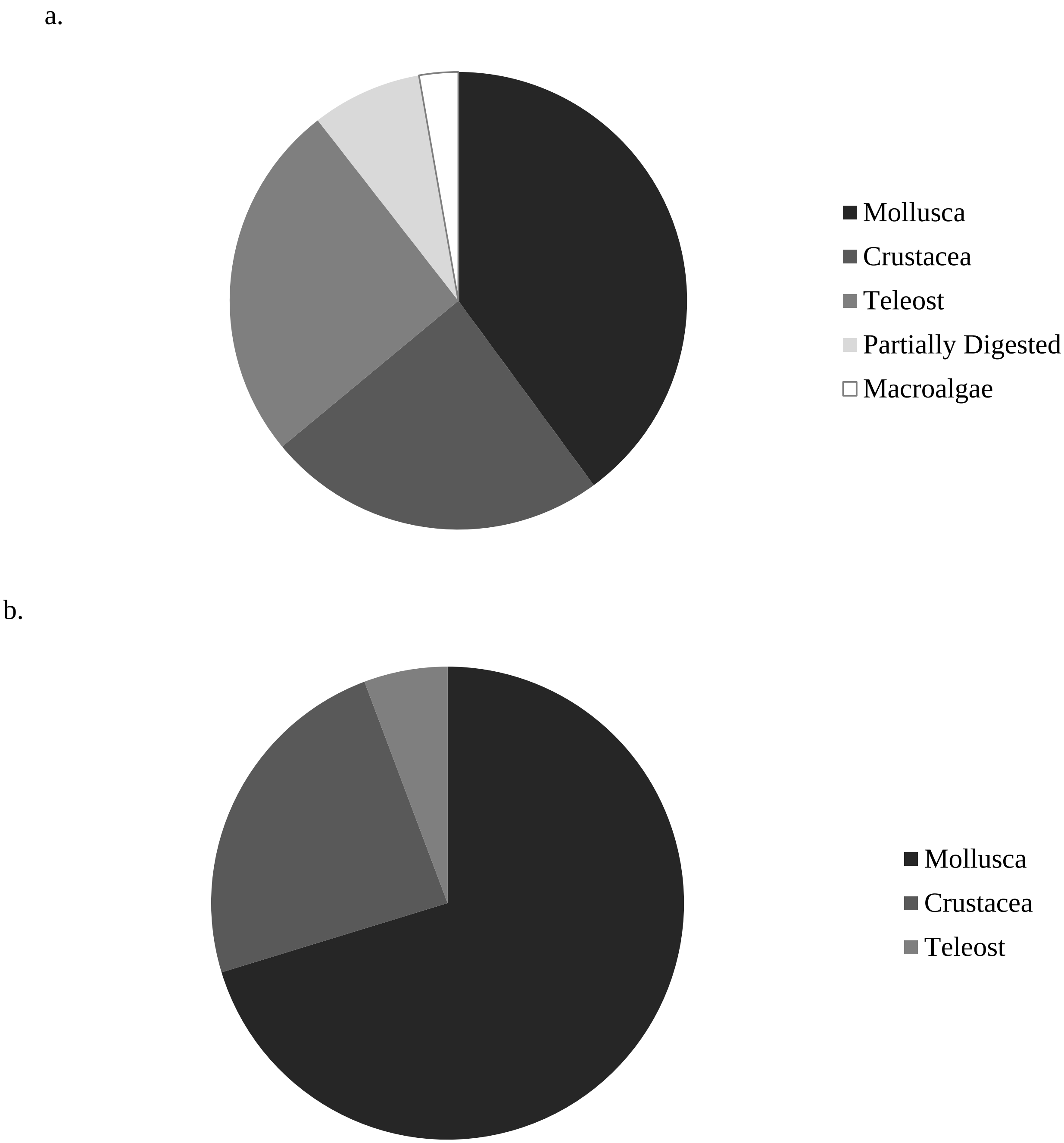


Figure 2. A comparison of $\delta^{13}C$ to $\delta^{15}N$ between male and female pelagic stingrays collected in the western North Atlantic Ocean.

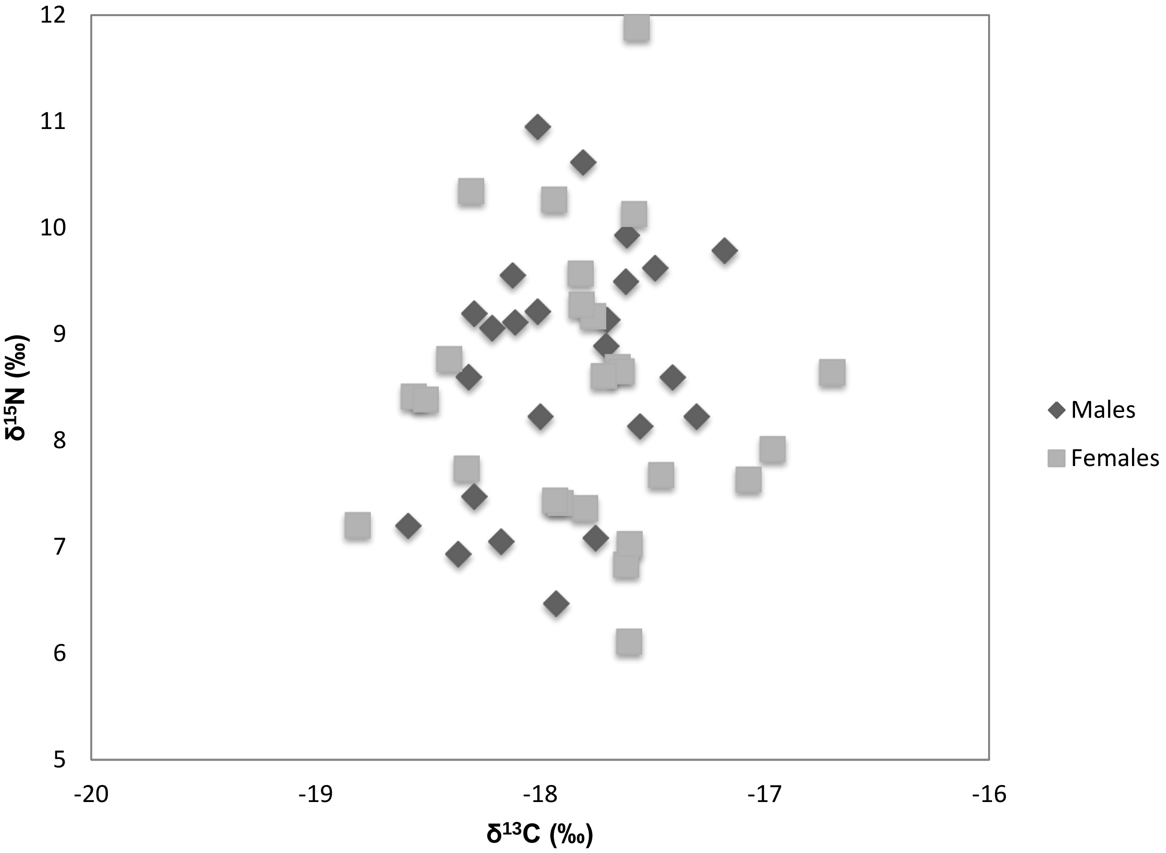
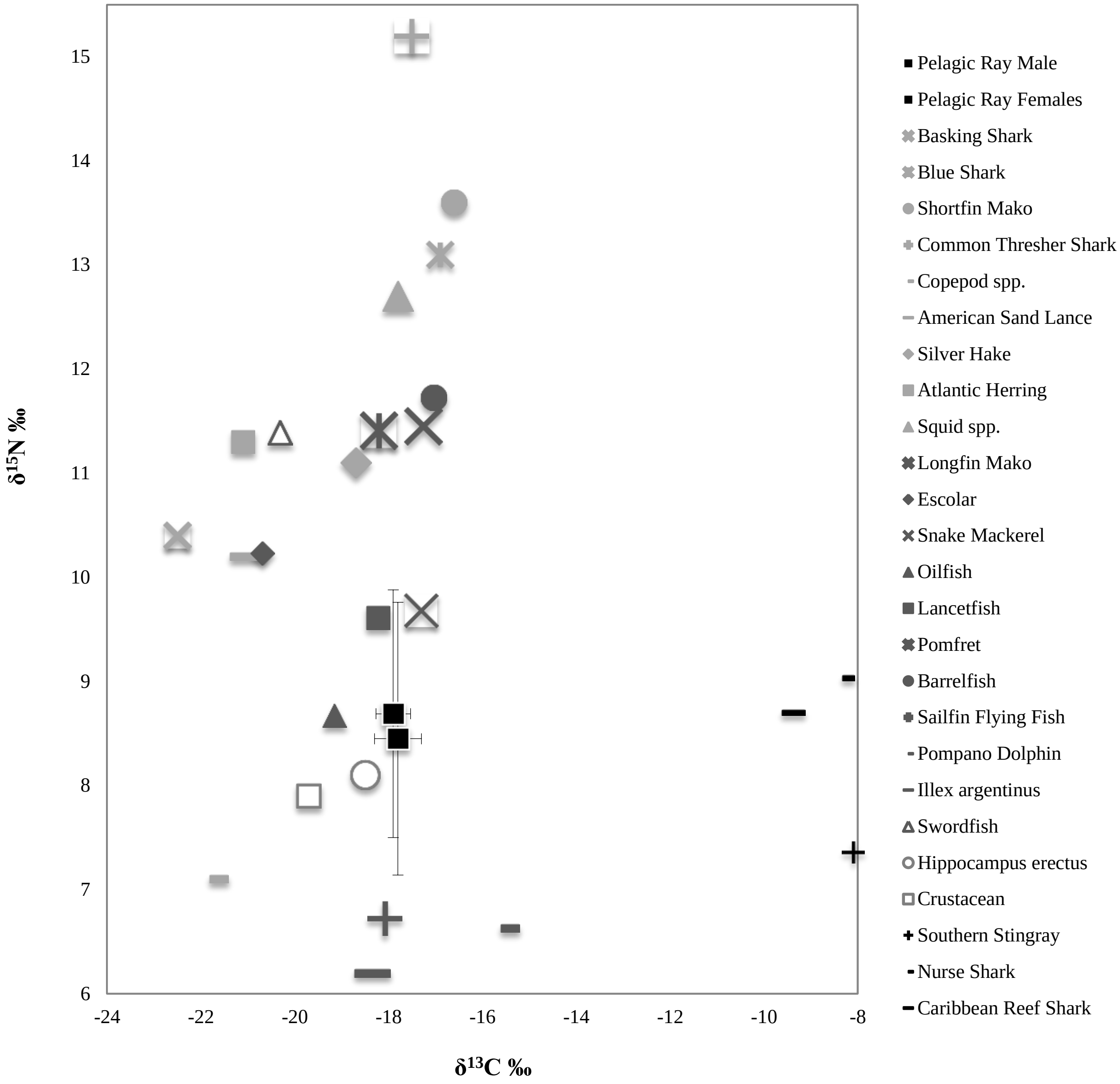


Figure 3. The stable isotope values of $\delta^{15}$Nand $\delta^{13}$C for pelagic stingray (males and females) and additional species of fish, elasmobranchs, and prey items from the western North Atlantic Ocean.  Data from other pelagic and mesopelagic species was taken from Estrada et al. (2003), Rau et al. (1983), Logan et al. (2001), Tilly et al. (2013), Keller (2009), and Heemsoth (2009).



## Conclusion

Understanding the life history and habitat utilization of a non-targeted species can tell science a great amount about an ecosystem. Fisheries managers can use the habitat utilization of a bycatch species to reduce the interactions between commercial fishing gear and non-target species, which would ultimately lead to lower economic impacts on the fishers themselves. The PSAT data can also reinforce the habitat utilization and the prey items found in the stomach content of a species. The PSAT data shows the pelagic stingrays vertically migrating from depth to shallower waters, potentially in pursuit of prey items. The combination of the stomach content and stable isotopes confirm the stingray's in the pelagic food web. Using both the tradition stomach content with the chemical analysis of the stable isotope shows what is not only consumed by the pelagic stingray, but what is ultimately assimilated into the diet. Continued research into non-targeted, less economically important species can eventually lead to ecosystem based management and better science in the pelagic environment.

The pelagic stingray is following a diel movement. They are at depth during daytime hours and in shallower waters during nighttime hours. They appear to be following prey items up to the surface for foraging activity. Also, knowing the vertical utilization can help decrease species interaction with commercial fishing gear and ultimately reduce bycatch. However, the pelagic stingray appears to be occupying the same water depths as the pelagic longline fishery targeting swordfish (40-60m). To reduce bycatch numbers of pelagic stingray, gear modification would be the best alternative.

The diet of the pelagic stingray was found to be similar to previous studies, except with a lower percentage of empty stomachs. The stingrays were primarly feeding on cephalopod mollusks, actinopterygiian fishes, and decapods crustaceans. The stable isotope analysis helped to confirm the pelagic stingrays were feeding in the pelagic ecosystem, along with snake mackerel, oilfish, and lancetfish, which are also bycatch species caught on pelagic longline fishing gear. Stomach content and stable isotope analysis help to confirm the trophic positioning of the stingray in the pelagic food web.

The research presented could lead to further investigations into the pelagic stingray's life history. Longer deployments of PSATs with more geographical variation would show how different populations of the stingray interact with oceanographic parameters. The longer deployments can also help to verify migration patterns of the stingrays which have been observed in the species.  Obtaining more samples of gut content and stable isotopes will also help to strength the conclusions from this study. Having data from several years and sampled throughout the year would begin to show variation in the diet and any potential feeding cycles going on throughout different seasons. A more diverse age class would help to confirm the observed ontogenetic shifts of the species, which due to gear selectivity, this study could not observe.

Continuing to learn and study life histories from bycatch species will help understand the ecosystem of the species that are economically important. As fisheries management is starting to open up to the idea of an ecosystem based management approach, having the knowledge of bycatch species will help managers make stronger arguments and management plans for the future of the fisheries.  The more science understanding of life histories of pelagic species can only allow for strong science and management decisions in the future.