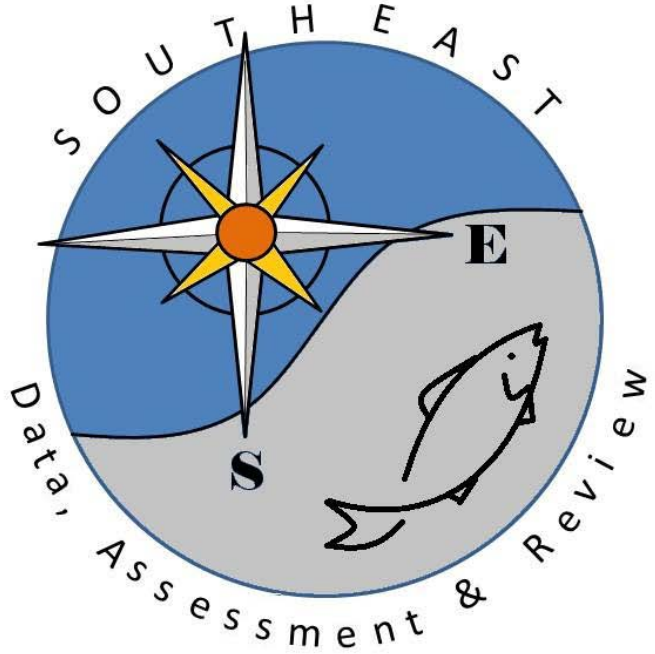
Working Paper for Red Snapper Data Workshop (SEDAR 31)

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Methods

Red snapper were collected from seven different sites comprising three distinct habitat types, including two standing oil and gas platforms (hereafter standing), two oil and gas platforms that were cut and toppled in place to create artificial reefs (hereafter toppled) and three natural rock reefs on the shelf edge bank (hereafter bank). Locations of sampling sites appear in Figure 1. Sampling was conducted during June and July in the summers of 2009 and 2010.

Red snapper were collected with Chevron traps (hereafter traps) and vertical longlines (hereafter longline). Traps were baited and left to soak for two hours at each location. After soaking, the traps were hauled on board and all red snapper were numbered and put on ice until processed on board the research vessel. Those that were not processed at sea were kept on ice until returned to land and analyzed in the laboratory. Longlines contained four baited hooks and were fished for between one and two hours, until fifty fish were collected at each site, or as time and weather allowed. All other species were counted and discarded overboard. For all fish collected, morphometric measurements were recorded (total length [TL] in millimeters for each fish; total weight [TW] in kilograms when possible), sex was determined by macroscopic examination of gonads, when possible, and sagittal otoliths were removed, rinsed, and stored in coin envelopes until processed.

Age and growth

The left sagittal otolith of each fish was sectioned in a transverse plane following the methods of Cowan et al. (1995) using the Hillquist model 800 thin-sectioning machine equipped with a diamond embedded wafering blade and precision grinder. Otolith sections were read under a dissecting microscope with transmitted light and a polarized light filter at 20x to 64x magnification. Counts of opaque annuli were made along the ventral margin of the sulcus acousticus from the core to the proximal edge (Wilson and Nieland, 2001). Edge condition of the otolith's margin was coded according to Beckman et al. (1989). Annulus counts were performed by two independent readers without knowledge of date and location of capture, and morphometric data. When initial counts disagreed, annuli were counted a second time. When a consensus between readers could not be reached, annulus counts from the more experienced reader were reported. Precision between readers was evaluated with the coefficient of variation (CV), index of precision (D) (Chang, 1982), and average percent error (APE) (Beamish and Fournier, 1981). Ages of red snapper were estimated from the number of opaque annuli, assumed birthdate, and capture date, following the equation described by Wilson and Nieland (2001):

$$\text{Age (days)} = -182 + (\textit{annulus count} \times 365) + ((m-1) \times 30) + d, \quad (1)$$

where m = ordinal number (1-12) of month of capture; and d = ordinal number (1-31) of the day of the month of capture. It was assumed that annulus formation begins on 1 January. To account for the uniform birthdate of 1 July, 182 days were subtracted from each age estimate.

Reproductive biology

For all female red snapper collected, ovaries were excised and extraneous visceral and adipose tissues were trimmed away. Gonads were placed in labeled plastic freezer bags, frozen and transported to the LSU Fisheries Science Laboratory. In the laboratory, gonad tissues were thawed, blotted with a paper towel and weighed (nearest 0.01 gram) and fixed in a 10% formalin solution (37% formaldehyde diluted with deionized water) for a minimum of 2 weeks. Post-fixation, a cross-section of ovary tissue was removed at random from one of six subsections comprising each pair of ovaries. Subsamples were secured in labeled histology cassettes, and deposited into histology jars filled with 10% formalin. Subsample jars were topped with 10% formalin to prevent desiccation, sealed tightly and transported to the LSU School of Veterinary Medicine histology laboratory for slide preparation.

Ovarian tissue subsamples were processed at the LSU School of Veterinary Medicine by histology laboratory staff and myself. Histology cassettes containing tissue samples were vacuum infiltrated and embedded in paraffin wax. A microtome was used to cut embedded samples to 4 μm thickness. Embedded tissue slices were mounted on labeled slides in a warm water bath, allowed to dry, then stained and counterstained with hematoxylin and eosin (H and E), respectively. Coverslips were affixed with Permount.

Ovary tissues were microscopically examined (Olympus BX41 microscope, 40x magnification) and 4 stages of oocyte development for heterochronal fishes were identified. These 4 stages were defined by Wallace and Selman (1981): primary growth (PG), cortical alveoli (CA), vitellogenic (V) and hydrated (H) (Figures 6A and 6B).

The immature ovary contains only un-yolked oocytes: primary growth oocytes and cortical alveoli (Hunter and Macewicz 1985a), while an ovary containing vitellogenic oocytes (i.e., coalesced yolk) is capable of reproduction (Brown-Peterson et al. 1988). Therefore, the benchmark for maturity in this study was vitellogenesis (Hunter and Goldberg 1980). The presence of hydrated oocytes indicated imminent (within the next 24 hours) spawning.

Two post-developmental oocyte stages were also identified: post-ovulatory follicles (POF) and atretic oocytes. The occurrence of fresh POF (Figure 6C) indicated spawning had recently occurred (within the past 24 hours) (Hunter and Macewicz 1985a). Atresia (Figure 6D) marks the degeneration and resorption of oocytes in the ovary prior to being spawned (Hunter and Macewicz 1985a).

Gonadosomatic index was estimated by using methods described by Hunter and Macewicz (1985a). Spawning phase was determined by using methods described by Brown-Peterson et al. (2011). Sizes- and ages-at-50%, -75% and -100% maturity were determined by sorting reproductively mature females into nearest-50-mm TL size classes and annual age classes and compared among habitat types. Batch fecundity was determined gravimetrically using methods described by Hunter et al. (1983). Spawning frequency was estimated using three standard methods: 1) the hydrated oocyte method (H method) described by Hunter and Macewicz (1985a), 2) the post-ovulatory follicle method (POF method) described by Hunter and Macewicz (1985a) and 3) the time-calibrated method (TC method) described by Wilson and Nieland (1994). Annual fecundity was estimated according to methods described by Nieland and Wilson (1993).

Dietary analysis

Stomachs of red snapper were removed and weighed to the nearest 0.1g to determine full stomach wet weight, fixed in 10% formalin for 24 to 48 hours and subsequently transferred to ethanol for storage until analysis. Contents of the stomach and esophagus were removed, sorted under a dissecting microscope, and identified to the lowest taxonomic level possible. Stomach contents were then separated and grouped by taxon and dried at 60°C for 24 to 48 hours in a DX 600 drying oven. When possible, individual organisms were counted and recorded. Once dried, contents were weighed to 0.0001g to determine dry weight of each taxonomic grouping of prey (hereafter prey items).

Cumulative prey curves were constructed for each habitat to determine whether a sufficient number of stomachs were collected to accurately describe the diets of red snapper at each habitat (Ferry and Cailliet 1996). When the cumulative prey curve reaches a stable asymptote there exists a sufficient number of samples for analysis of dietary habits.

Stable isotope analysis was conducted on a sample of muscle tissue removed from each red snapper. Due to the large number of fishes collected, stable isotope samples were limited to include only fish that had viable guts in order to make a broader assessment of the diets through the use of both techniques. Tissue samples were dried at 60°C for 24 hours in a DX 600 drying oven and then pulverized. A sample of ground tissue weighing between 5.0 – 7.0mg dry weight was placed in an aluminum capsule and mixed with approximately 10mg of Vanadium pentoxide (V_2O_5). Samples then were analyzed for isotopic composition of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ with a Finningan MAT

DeltaPlus continuous-flow stable isotope mass spectrometer.

A sample of red snapper muscle tissue was also used to test for energy density to determine if the different habitats were contributing disproportionately to caloric intake of red snapper. Tissue samples were dried at 60°C for 24 hours in a DX 600 drying oven and then pulverized, and subsequently pressed into a 1g pellet for analysis. Caloric density was determined with a Parr 6200 oxygen bomb calorimeter.

Hydroacoustic sampling

Acoustic backscattering data was collected using a BioSonics echosounder equipped with three downward-looking split-beam transducers of frequencies 70, 120, and 200 kHz, calibrated by the standard sphere method (Foote et al. 1987). Data was collected at a threshold of -100dB, with a pulse duration of 0.4ms. Transducers were mounted on a pivoting boom along the side of the research vessel, and the boom was lowered when on site. The vessel was equipped with a bulbous bow and kort nozzle propellers, which were designed to provide a smoother ride and potentially reduce the level of acoustic background noise produced by the ship.

Ten acoustic transects were completed at each site at a cruise speed of approximately five knots. At the artificial reef sites, transects were approximately 2 km in length, extending 1 km from the site in all directions. Transects began due north or south of the site, and continued in a circular pattern, with the structure as the center point (Figure 2), expanding on the pattern used by Soldal et al (2002). At the bank sites, transects varied in length depending on the size and shape of the bank. For each bank site the rough center point was determined, and transects were centered on that point, marked

with GPS for future surveys. Ten transects were completed at the bank sites as well, using the same circular pattern.

Acoustic data was catalogued and post processed using Echoview (Version 5.2; SonarData Pty. Ltd.) to obtain values of nautical area scattering coefficient (NASC), volume backscatter (S_v), and target strength (TS). Both NASC and volume backscatter are considered to be a proxy for fish biomass (Simmonds and MacLennan, 2005) and are based on the summation of area backscattering per unit area (NASC) or volume (S_v) (MacLennan et al. 2002). Target strength is an acoustic estimate of fish length, and can be used to determine length frequency distributions in a sampled volume of water. Taken together, these values are used to determine relative fish density in a given sampling area.

Statistical Analyses

Age and growth

Analysis of variance (ANOVA) was used to compare mean TL, TW, and age between habitats (SAS Institute, 2008). Means were first ln-transformed to meet the assumptions of normality and homogeneity of variance. Tukey's Studentized Range (HSD) test was used for pair-wise comparisons. Size and age frequency distributions were compared by region with the Kolmogorov-Smirnov two-sample test. A chi-squared (χ^2) test was used to determine if sex ratios differed from a 1:1 ratio. Total weight—TL relationships were fitted with linear regression to the model $TW = aTL^b$ from ln-transformed data by region. Analysis of Covariance (ANCOVA) was used to compare the linearized slopes and intercepts.

Weighted mean size-at-age was compared for the most common ages (3-7 yrs)

using ANOVA with a Tukey's Studentized (HSD) Adjustment for post-hoc comparisons. Growth was modeled for observed TL and TW at age using von Bertalanffy growth equations (VB), and tested with likelihood ratios (Haddon, 2001). Models were fitted with nonlinear regression by least squares (SAS Institute, 2008) in the forms:

$$TL_t=L_\infty(1-e^{-k(t)}) , \quad (2)$$

$$TW_t=W_\infty(1-e^{-k(t)})^b , \quad (3)$$

where: TL_t = TL at age t; TW_t = TW at age t; L_∞ = TL asymptote; W_∞ = TW asymptote; k = growth coefficient; t = age in yr; b = exponent derived from TW-TL regressions. Models were forced through 0 for comparison purposes due to of a lack of small, young individuals in all sample populations. For all statistical tests, significance was measured at an alpha level of 0.05.

Reproductive biology

ANOVA and the Mann-Whitney U-test (used for mean frequencies of non-parametric data) were used to evaluate equality of sample means for age, total length and total weight between habitat types and sampling years. Linear regression (GLM) was used to evaluate the significance of length-weight relationships, and analysis of covariance (ANCOVA) was used to compare length-weight regression parameters between habitats. Chi-square analyses were used to test male-to-female ratio and spawning frequency between habitat types. All tests were considered significant if $p<0.05$.

Dietary analyses

Three different methods were used to analyze the results of the gut content analysis, including percent composition by weight, percent composition by number, and frequency of occurrence. Percent composition by dry weight (%W) is used for the majority of statistical analysis because it is believed to provide the best assessment of the nutritional contribution of individual prey items (Bowen 1996; McCawley 2003; Wells et al 2008a). As such, an index of relative importance (IRI) was constructed using the %W values for all prey items at each site with the formulas in McCawley and Cowan (2007). First the frequency of occurrence is calculated as:

$$FO = \frac{Number \text{ of stomachs containing one prey category}}{\text{Number of stomachs containing prey}}$$

The IRI is then calculated as:

$$IRI = (\%N + \%W) \times FO$$

where N is the number each prey item found, W is the total dry weight of each prey item and FO is the frequency of occurrence. Finally, a percent IRI (%IRI) is calculated as:

$$\%IRI = \frac{\text{IRI for each prey category}}{\text{Sum of all IRI values}} \times 100$$

The IRI is used to examine the overall composition of diets for each species at each habitat type (standing, toppled, and shelf edge). The IRI is useful because it describes the diets based on the contribution of each prey item by weight.

Gut content data were analyzed using PRIMER (Plymouth Routine in Multivariate Ecological Research; Warwick, 1990), using percent composition by dry weight following a log(x+1) transformation to normalize the data, and reduce the importance of abundant prey items. A Bray-Curtis similarity index was constructed from the transformed data, and a permutation analysis of variance (PERMANOVA) was run

using this matrix to compare each stomach to every other stomach. A two-way PERMANOVA was used to compare prey items between habitat types and study years, and *a posteriori* tests were applied. Following PERMANOVA, the original transformed data was analyzed using the similarity percentages (SIMPER) option, which examines the within group (habitat) similarity as well as the between group dissimilarity. This method allows the identification of prey items that contribute to the differences in diets between sites.

Stable isotope data were assessed using an ANCOVA (SAS Institute, 2009), with total length as a covariate, to determine if there is a difference in mean values of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ for each species between habitat types. Values of stable isotope ratios also were used to analyze the niche breadth of each species at each site, following Layman et al. (2007 a, b). Stable isotope ratios are known to be affected by ontogeny, especially in red snapper (Wells et al. 2007) and therefore all stable isotope values were first corrected for standard length using regression analysis. Adjusted values were then individually plotted in their $\delta^{13}C$- $\delta^{15}N$ niche space for comparisons of dietary breadth between sites, using two different metrics. Total area (TA) is a measure of overall niche space and is determined by calculating the area associated with the smallest polygon that contains all individuals (Layman et al. 2007 a, b). Centroid distance (CD) is a measure of the overall trophic diversity and is determined by recording the distance of each individual from the mean $\delta^{13}C$- $\delta^{15}N$ value for the population (Layman et al. 2007 a, b). Mean centroid distances were compared between sites using an ANOVA and Tukey HSD post ANOVA tests for significant results at the $p = 0.05$ level. Calculation of TA and CD were completed using MATLAB (2007).

Caloric density data were compared among sites and habitats, first with a 2-way ANCOVA to test if length was a significant covariate (SAS Institute, 2009). Following the ANCOVA, a 2-way ANOVA was conducted to test for differences in caloric density between year and habitat (SAS Institute, 2009). When necessary, Tukey HSD post ANOVA tests were applied for significant results at the $p = 0.05$ level.

Hydroacoustic sampling

Acoustic backscatter data was binned into 10m x 10m cells for the artificial reefs and 10m x 50m (depth x distance) cells for the natural reefs. The background noise removal and spike filter operators in Echoview were applied to remove noise and any interference caused from the vessels movement. Data was analyzed statistically as a function of the distance from the geographic center point of each sample area. For the artificial reef sites, the geographic center point was at the rig. Therefore, data was analyzed as a function of distance from the rig and depth at the sites. In this way, we are attempting to classify the spatial extent to which reef fishes associate with large artificial reefs. For the natural reef sites, the same analysis was run to compare distribution and abundance of reef fishes over a similar spatial extent and similar depth profile.

Data was analyzed in SAS using the GLIMMIX procedure, which is appropriate for dealing with large data sets and deals appropriately with non-normal data. Both NASC and S_v were modeled among sites and between habitats as a function of depth and distance from the center point of the study area.

Results

During the summers of 2009-2011, 317 red snapper were collected from natural

habitats on Louisiana's shelf-edge banks (Jakkula, Alderdice, Rezak and Bouma) and 219 red snapper from standing platforms in the Eugene Island artificial reef planning area (EI 325 and EI 346; Table 1).

Age and growth

The red snapper from the shelf-edge banks ranged from 244 to 807 millimeters total length (Figure 3A) and from 0.346 to 7.071 kilograms total weight (Figure 3B). The red snapper from the standing platforms ranged from 196 to 818 mm total length (Figure 3A) and from 0.108 to 8.71 kg total weight (Figure 3B). The male-to-female ratio of the red snapper collected from the shelf-edge banks was 0.85:1 and was 0.94:1 for the red snapper collected from the standing platforms. Ages for 261 red snapper from the shelf edge banks and from 222 red snapper from standing platforms (483 aged fish in total, Figure 3C) have been estimated.

Across all three years, the red snapper collected from the standing platforms were significantly larger than the red snapper from the shelf edge banks (mean TL Tukey's test: $p=0.0012$, Figure 3A; mean TW Tukey's test: $p<0.0001$, Figure 3B). The TL and TW frequency distributions were significantly different between the two habitats (P>KSa: $p<0.0001$) with the shelf edge banks having a lower proportion of larger individuals; 27.3% of the red snapper from the shelf edge banks were 550 mm or longer and 13.7% were 3.0 kg or larger compared to 42.4% and 24.5% of the red snapper from the standing platforms. Significant differences were also observed in the TW-TL equations for red snapper from the two habitats. The TW-TL equation for red snapper from the banks had a significantly larger growth coefficient (*b*) and a significantly

smaller intercept (*a*) than the equation for red snapper from the standing platforms (p=0.0005 and p=0.0001, respectively). The TW-TL equations are:

Shelf Edge Banks $TW = 1.25x10^{-8}(TL^{3.01})$

Standing Platforms $TW = 3.45x10^{-8}(TL^{2.86})$

Overall, red snapper exhibited a truncated age structure with less than 0.5% of the sampled fish older than 10 years. However, the red snapper from the shelf edge banks were, on average, older than the red snapper from the standing platforms (Tukey's test: p=0.0142; Figure 3C). While there was no significant difference among the age frequency distributions between the two habitats (P>KSa: p=0.4976), the shelf edge banks had a slightly higher proportion of red snapper older than 5 yr; 17.3% of the red snapper from the banks were older than 5 yr, compared to 9.9% from the standing platforms (Figure 3C). However, the oldest red snapper (21 yr) was collected at a standing platform.

Red snapper growth was modeled from observed TL at age and TW at age using the von Bertalanffy growth equation for all ages (Figure 4A and B). Significant differences in the TL and TW von Bertalanffy growth models were noted among the habitats (TL models likelihood ratio test; $\chi^2=158.27$; df=2; p<0.0001; TW models likelihood ratio test; $\chi^2=133.12$; p<0.001). However, no significant differences were noted between the von Bertalanffy models for the sexes (TL models likelihood ratio test; $\chi_2=0.4886$; df=2; p=0.7832; TW models likelihood ratio test; $\chi_2=1.8438$; df=2; p=0.3978). Resultant von Bertalanffy growth equations are:

Shelf Edge Banks $TL_t=726.0(1-e^{-0.2451(t)})$

Standing Platforms $TL_t=812.9(1-e^{-0.2440(t)})$

Shelf Edge Banks $TW_t=7.06(1-e^{-0.2060(t)})^{3.01}$

Standing Platforms $TW_t=7.81(1-e^{-0.2362(t)})^{2.86}$

The TL growth model of red snapper from the standing platforms displayed a significantly larger estimate of L_∞ than the estimate from the shelf edge banks model (χ^2=11.54; df=1; p=0.0007). However, no significant differences in growth coefficients (k) were observed between the two models (χ^2=0.00; df=1; p=0.9363). Significant differences were noted between the growth coefficient estimates in the TW models (χ^2=13.01; df=1; p=0.0003), but not between the estimates of W_∞ (χ^2=2.67; df=1; p=0.1021). There were also significant differences in the mean size-at-age of red snapper from the two habitats (Figure 5). Red snapper from the shelf edge banks were consistently smaller at age than red snapper from the standing platforms (p<0.0001). These growth models suggest that red snapper grow rapidly until 6 – 7 yrs of age, after which somatic growth slows considerably.

Reproductive biology

A total of 391 female red snapper were sampled from natural shelf-edge banks (n=174), standing platforms (n=145), and toppled platforms (n=72) in June and July 2009 and July 2010 (Table 1). Unfortunately, sampling efforts in the summer of 2010 were reduced due to the advent of the Deepwater Horizon oil spill. Thus in July 2010, only thirty-one females were sampled from natural habitat, 11 females were collected from standing platforms and 10 females were collected from toppled platforms. However, an additional 57 females were collected in April (n=28) and October (n=29) 2010 at standing and toppled structures to assess spawning activity outside of the known peak

months of reproduction.

Differences in GSI, maturity and spawning frequency were detectable among habitat types. Unfortunately, habitat-specific comparisons of batch fecundity and annual fecundity estimates were not possible due to the limited sample size of hydrated females (n=8).

Oocyte stages were classified for a total of 337 female red snapper collected during the spawning seasons of 2009 and 2010 (June-July 2009 and July 2010) (Table 2). Overall, 55.2% of specimen collected possessed vitellogenic oocytes, indicating capability of spawning, and 12.4% of sexually mature individuals showed signs of eminent or recent spawning activity, indicated by the presence of hydrated oocytes (H) and/or post-ovulatory follicles (POF). The minimum observed age-at-maturity was 2 years old, while the minimum observed size-at-maturity was 320 mm total length (TL). Among hydrated and recently-spawned females (evident by the presence of fresh POF), minimum age was 3 years old, and the smallest sizes were 366 mm and 359 mm TL, respectively.

Fish sampled from toppled platforms produced the highest ratio of mature individuals (66.1%), followed by those collected from natural banks (55.0%) and standing platform structures (49.5%). Highest incidence of POF and hydrated oocytes also occurred at toppled platform sites (POF=12.5%; H=8.9%), while the lowest incidence of each was observed in fish sampled from natural shelf-edge banks (POF=7.6%; H<1.0%).

Mean GSI values greater than 1 occurred at all sites in June and July of 2009 and July of 2010, with the exception of toppled platforms in July 2010 (Table 3). Mean GSI

values less than 1 occurred during the months of April and October 2010 (GSI=0.80 and 0.69, respectively), indicating that overall the mature portion of the female population was not capable of producing optimal batch sizes during these months. However, GSI greater than 0.5 in April indicated the onset of the spawning season (Fitzhugh et al. 2004).

During the spawning season, the highest observed GSI estimates consistently occurred at the natural banks (Table 3). Individuals from toppled platform sites produced the lowest GSI values during the spawning season. At standing platforms, females also yielded relatively low GSI estimates.

In April and October, histological observations indicated mass regeneration and regression spawning phases, respectively. These observations, in conjunction with GSI values less than 1, confirmed suboptimal spawning activity during these months. The majority of mature females sampled in April 2010 (78%) was experiencing the regenerating phase of the annual reproductive cycle and were reproductively inactive; this was indicated by the presence of only primary growth (PG) oocytes in conjunction with atresia and thick ovarian walls (Table 4). Another 25% of April samples were classified as "developing," indicated by the presence of PG oocytes and cortical alveoli (CA), and early pre-vitellogenic oocytes. Only one individual sampled in April 2010 was reproductively active.

Mature fish collected in October were largely undergoing the regressing phase (64%; n=9) of the reproductive cycle; this was indicated by mass atresia in combination with the presence of PG, CA and vitellogenic oocytes. Some of these regressing fish (36%; n=5) also displayed late vitellogenic oocytes indicating the capability of spawning

to some degree. All remaining mature fish in this sample population were in the regenerating phase; that is, incapable of reproduction.

Females from the natural banks reached maturity at a slower pace compared to fish standing and toppled platforms. At the natural banks, females reached 50% maturity by age 5 and 450 mm TL, while fish from standing platforms reached the same maturity benchmark at a similar size, but at a younger age (4 years) (Tables 5 and 6). At toppled platforms, 50% maturity was attained the fastest, by age-3 and 400 mm TL. Natural bank and standing platform habitats each produced the same size-at-100%-maturity estimate of 700 mm TL, however an estimate for 100% maturity at toppled platform habitat could not be determined due to a small sample size.

Batch fecundity was estimated for all fully hydrated females (n=8). Fully hydrated individuals ranged from 366 to 666 mm TL (mean=532 mm TL) and from 3.05 to 7.05 years (mean=4.80 years) in age. All hydrated fish were found during the 2009 spawning season, none of which displayed simultaneous signs of post-ovulatory follicles (POF). Overall, mean batch fecundity was 219,258 ± 113,749 ova per spawning event (Table 7). Red snapper sampled from standing platforms and the natural banks spawned the highest estimated number of ova, while batch fecundity estimates (BFE) for specimens collected at toppled sites were ~2/3 lower. Only one female sampled from the natural banks was found in hydrated condition.

Positive trends in batch fecundity estimates (BFE) emerged when the natural logarithm (ln_e) of BFE was plotted against the natural logs of total length (TL) (Figure 7), eviscerated body weight (EBW) and age. Best-fit regression relationships are shown (Table 8). Due to small sample sizes of hydrated fish, mean batch fecundity estimates

and batch fecundity regression relationships with TL, EBW and age could not be statistically compared between habitat types.

Spawning frequency was estimated for 180 mature female red snapper. On average, spawning occurred once every 6.0 to 7.1 days and 21 to 25 times per reproductive season (Table 9) and spawning frequency estimates (SFE) did not differ between habitats ($p>0.05$) (Table 10). Specimens collected from toppled platforms spawned marginally more often, which was similar to individuals from standing platforms. Fish sampled at natural bank sites ovulated slightly less often.

Overall, age-3 females yielded the lowest SFE values on the Louisiana continental shelf (SFE=11.7-17.5 days; 9 to13 spawning events per season) (Table 11). Fish in the 4- and 5-year age groups spawned at nearly double this rate (age-4: SFE=5.3-8.2; age-5: SFE=6.2-7.4). Fish ages 6-, 7- and 8-years old spawned the most frequently (age-6: SFE=3.0-6.0; age-7: SFE=3.3-3.8; age-8: SFE=3.5-7.0). Age-2 females were excluded from spawning frequency analyses due to small sample size (n=7) and because no fish in this age group were found with POF or hydrated oocytes.

Spawning occurred most frequently for fish that were 725 mm total length or greater (SFE=2.5 days, 60 spawning events per season) (Table 12). In contrast, females in the 325-374 mm total length group spawned at roughly half that rate (SFE=4.0-5.3 days, 28 to 38 times per season). Spawning frequency estimates were highly variable for fish less than 574 mm total length (SFE=4.0-21.0 days). Variability in spawning frequency estimates diminished by the time females reached 575 mm total length (SFE=2.5-6.0); these larger individuals spawned between 25 and 60 times per season.

No statistical difference was found for spawning frequency between habitats in

this study. However, slight dissimilarities were apparent. Females from the natural banks spawned less frequently (SFE=6.7-8.5 days) than did fish from the other habitat types, followed by fish from standing platforms (SFE=5.4-7.0 days). The most frequent spawners were those from toppled platforms (SFE=4.9-5.3 days).

Annual fecundity estimates (AFE) were determined for all hydrated females (n=8). On average, hydrated fish were 4.8 years old and spawned once every 7.1 days (Table 13). The lone hydrated female sampled at the natural shelf-edge banks was capable of producing an estimated 5,765,893 ova per year (age: 7.04 years).

Dietary analysis

In total, 309 red snapper were used in the dietary analysis. The remaining fish collected suffered from barotrauma, and had distended stomachs when brought on deck. Of the usable stomachs sampled, seventy-one were collected from standing platforms, fifty-three were collected from toppled platforms, and 185 were collected from the banks (Table 14).

Diets of red snapper were significantly different among sites, combined over years, based on results of the PERMANOVA ($p < 0.05$). *A posteriori* tests of the results revealed that diets of red snapper collected at toppled platforms were significantly different that those from standing platforms and the banks. Diets were not significantly different between the standing platforms and the banks ($p > 0.05$, PERMANOVA).

Overall, red snapper consumed primarily fish and crustaceans over all sites and years, however the proportions were different (Figure 8). At the standing platform sites, diets were composed of 40% fish, 28% antenna codlet (*Bregmaceros atlanticus*), 19%

squid, and 18% unidentified crustaceans (hereafter crustacean) by dry weight. Fish also contributed significantly at the toppled platform sites, making up 56% of diets, however no antenna codlet were found at these sites. Instead, diets contained more crustaceans, including 4% crustacean, 3% unidentified crabs (hereafter crab), and 25% bathyal swimming crab (*Bathynectes longispina*). Diets at the bank sites were most varied, and consisted of 60% fish, 13% antenna codlet, and 6% crab (including bathyal swimming crab). There was also a significant contribution by gorgonian soft corals, making up approximately 9% of diets by weight, however this was most likely ingested incidentally while the fish were foraging along the bottom. Very little gorgonian was found at the other two habitats, making up less than 1% at the standing platforms, and none found at the toppled platforms. Results of the gut content analysis appear in Table 15.

The IRI generally agreed with the diet analysis by dry weight at the standing platforms, indicating fish, squid and antenna codlet were the more significant contributors to diet. At the toppled platforms, the results of the IRI differ from those of the analysis by dry weight. At these sites, stomachs contained a large number of benthic zooplankton, including amphipods, hyperid amphipods, cavalina, and larval crabs. By weight, these prey items make up less than 3% of diets, but combine to contribute approximately 15% to diets of red snapper. Another 9% contribution was equated to mantis shrimp (stomatopods), which were small but numerous and therefore not as important by dry weight. At the bank sites, the IRI generally agreed with the analysis by dry weight, and indicated that fish were the most important contributor to red snapper diets. The IRI differed with respect to gorgonian soft corals, indicating less influence by this prey item than the analysis by dry weight. This difference is likely due to the fact

that when found, gorgonians were in a large, thick mat, and were therefore massive, though this prey item was found relatively infrequently.

Diets of red snapper were also significantly different between years at all habitats. Several of the predominant prey items found in 2009 were absent in 2010, including antenna codlet and bathyal swimming crab. At the standing platforms, diets were similar between years, aside from the absence of antenna codlet. In 2009, antenna codlet, fish, squid, and crustaceans dominated diets (Table 16). In 2010, there were no antenna codlet and very few crustaceans found, with 76% of diets made up of fish (Table 16). Squid made up 18% of diets in both years (Table 16). This pattern indicates a shift to a majority pelagic prey at the standing platforms. At the toppled platforms and the banks, the shift was towards more benthic prey sources. In 2009, red snapper at the toppled platforms consumed mostly bathyal swimming crabs, fish, squid, and crustaceans. In 2010, diets shifted to primarily fish and mantis shrimp (Stomatopods), with a large percentage of unidentified material (Table 16). At the bank sites, diets shifted from fish, antenna codlet, and lizardfish in 2009, to fish and gorgonian soft corals in 2010 (Table 16).

The IRI more clearly illustrates the differences between years. At the standing platform sites in 2009, the IRI indicates that fish, antenna codlet, crustaceans, and squid all contribute significantly to the diets, whereas in 2010 the diets are dominated by fish and squid (Table 17). At the toppled platforms sites, the diets shifted from hyperid amphipods, fish, crustaceans, and crabs in 2009 to mantis shrimp, fish, amphipods, and cavalina (pteropods) in 2010 (Table 17). While there was some contribution from fish, the majority of diets consisted of small, benthic invertebrates. At the bank sites, the IRI

indicates a shift from fish and codlet in 2009 to lesser contributions of fish, and more contribution from gorgonian soft corals, and the associated invertebrate community (Table 17). Found within the gorgonians were high numbers of amphipods, mantis shrimp, cavalina, and polychaete worms, all of whom contributed more significantly to diets in 2010 based on the results of the IRI (Table 17).

Cumulative prey curves for red snapper reached an asymptote at each habitat at approximately 50 samples. This indicates that there were a sufficient number of stomachs collected at each habitat to accurately assess the dietary habits at each site.

Mean caloric densities (in cal/g) for red snapper were, 5365.22±157.6, 5281.8±224.0, and 5356.11±163.8 for the standing, toppled, and bank sites, respectively in 2009 (Table 18). In 2010, mean caloric densities for red snapper were 5522.52±61.4, 5504.26±69.9, and 5472.67±49.3 for the standing, toppled, and bank sites, respectively (Table 18). Caloric density for individual red snapper ranged from 5054.95 to 5634.14 at the standing platforms, 4811.75 to 5631.52 at the toppled platforms, and 4878.69 to 5548.83 at banks. The greatest range was seen at the toppled platforms, while the smallest range was at the standing platforms, even though the values at the standing platforms were generally higher. The results of the ANCOVA indicate that length was not a significant covariate for the analysis ($p > 0.05$, ANCOVA, SAS Institute), and therefore caloric density was compared among sites using a 2-way ANOVA, comparing year and habitat, and year and site, separately. Results of the ANOVA indicate that caloric density of red snapper was significantly different between years ($p < 0.0001$, ANOVA, SAS Institute). Though mean caloric density values were generally higher at the standing platforms, there were no significant differences between habitat, or sites ($p >$

0.05). Tukey HSD post-hoc confirmed the annual variation (Figure 9).

Stable Isotope Analysis

Mean values for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ for red snapper varied by both habitat and year at the study sites. In 2009, mean values of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ were 12.30, -18.14, and 18.05, respectively, from fish collected at standing platforms, 12.26, -18.18, and 16.99, respectively, from fish collected at the toppled platforms, and 12.45, -17.78, and 18.15, respectively, from fish collected at the banks (Table 19; Figure 10). In 2010, mean values of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ were 13.59, -17.47, and 17.60, respectively, from fish collected at standing platforms, 12.78, -18.05, and 17.65, respectively, from fish collected at the toppled platforms, and 12.45, -17.78, and 18.15, respectively, from fish collected at the banks (Table 19; Figure 10).

Results of the ANCOVA indicated there were significant differences in $\delta^{15}N$ values between habitats ($p = 0.0011$, ANCOVA) and years ($p < 0.0001$). The 2-way ANCOVA indicated a highly significant ($p<0.0001$) interaction between habitat and year. Tukey post-hoc testing, however, found no differences between habitats. The differences observed are therefore likely due to a large difference in the mean value of $\delta^{15}N$ at the standing platforms between 2009 and 2010, which influenced the results of the ANCOVA. There were no significant differences in either $\delta^{13}C$ or $\delta^{34}S$ between either years or habitats ($p > 0.05$, ANCOVA). The observed differences in $\delta^{15}N$ were further analyzed by individual site to determine what was driving the observed difference. Results of the ANCOVA indicate that the standing platform EI-346 was significantly different from all other sites except Alderdice Bank. There was a significant difference between 2009 and 2010, but again this was likely driven by the observed differences at

the standing platform site EI 346. No differences were observed in either $\delta^{\square\square}C$ or $\delta^{34}S$ between either years or habitats ($p > 0.05$, ANCOVA).

Results of the trophic niche breadth analysis show no significant difference in centroid distance (CD) among habitat types ($p > 0.05$, ANOVA) (Figure 11). There was a significant difference between years ($p < 0.001$, ANOVA), with 2010 having a significantly larger CD than 2009, among all habitats combined (Table 20). Results of the 2-way ANOVA show a significant interaction between habitat and year ($p = 0.0028$, ANOVA), with post-hoc testing revealing higher CD in 2010 at both the standing and toppled platforms, with no differences in the shelf edge bank sites between years. Total area (TA) was higher at the banks in 2009, and higher at the standing and toppled platforms in 2010 (Table 20). The TA was similar at standing and toppled platforms in both 2009 and 2010 (Table 20). Overall TA (combined over years) was slightly larger at the bank sites, with standing and toppled platforms having similar overall TA (Figure 11).

Hydroacoustic sampling

Acoustic backscatter was highly variable over both space and time. At the artificial reef sites, biomass was concentrated near the reef structure (Figure 12, rows 1 and 2), and decreased with distance from the structure. At the shelf edge bank sites, biomass was spread more evenly throughout the study area, with peaks concentrated around the numerous bathymetric features of the reefs (Figure 12, rows 3 and 4). No discernable pattern was seen with distance from the center point of the reef study areas. However, work is continuing on the portion of the project.

Tables and Figures

Table 1: Female red snapper, *Lutjanus campechanus*, sampled in 2009 and 2010 at natural bank (Bank), standing platform (Standing), toppled platform (Toppled) sites on the Louisiana continental shelf.

Site	Jun 2009	Jul 2009	Apr 2010	Jul 2010	Oct 2010	Total
Bank	63	80	-	31	-	174
Standing	49	43	15	11	27	145
Toppled	-	47	13	10	2	72
Total	112	170	28	52	29	391

Table 2: Characterization of oocyte maturation for female red snapper, *Lutjanus campechanus*, collected from natural shelf edge bank, standing platform and toppled platform sites during the reproductive season on Louisiana's continental shelf.

Site	n	Unknown Sex	Immature	Mature	LV	POF	H
Bank	175	4	45.0%	55.0%	4.1%	7.6%	<1.0%
Standing	105	2	50.5%	49.5%	1.9%	11.7%	2.9%
Toppled	57	1	33.9%	66.1%	8.9%	12.5%	8.9%
All	337	7	44.8%	55.2%	4.2%	9.7%	2.7%

n=sample size; LV=late vitellogenic; POF=post-ovulatory follicles; H=hydrated

Table 3: Mean monthly gonadosomatic index (GSI) values for female red snapper, *Lutjanus campechanus*, sampled from natural shelf edge banks (Bank), standing platforms (Standing) and toppled platforms (Toppled) on the Louisiana continental shelf.

Bank

Month	n	Mean GSI	SE	StDev	95% CI
Jun-09	26	1.48	0.200	1.018	(1.09, 1.87)
Jul-09	37	1.45	0.196	1.194	(1.07, 1.84)
Apr-10	-	-	-	-	-
Jul-10	25	2.06	0.300	1.498	(1.47, 2.65)
Oct-10	-	-	-	-	-

Standing

Month	n	Mean GSI	SE	StDev	95% CI
Jun-09	20	1.33	0.209	0.935	(0.92, 1.74)
Jul-09	20	1.24	0.210	0.937	(0.83, 1.65)
Apr-10	3	0.80	0.149	0.257	(0.51, 1.09)
Jul-10	9	1.35	0.331	0.993	(0.70, 2.00)
Oct-10	6	0.69	0.268	0.657	(0.16, 1.22)

Toppled

Month	N	Mean GSI	SE	StDev	95% CI
Jun-09	-	-	-	-	-
Jul-09	27	1.07	0.171	0.889	(0.74, 1.41)
Apr-10	-	-	-	-	-
Jul-10	8	0.94	0.166	0.470	(0.61, 1.27)
Oct-10	-	-	-	-	-

n=sample size; StDev=standard deviation; SE=standard error; CI=confidence interval

Table 4: Spawning phases of female red snapper, *Lutjanus campechanus*, sampled from three habitats on the Louisiana continental shelf in April and October 2010 (n=38). Capable=spawning capable. Asterisk indicates n<5.

Apr-10

Site	n	Immature	Mature	Developing	Capable	Regressing	Regenerating
Bank	-	-	-	-	-	-	-
Standing	14	14.3%	85.7%	25.0%	8.3%	-	66.7%
Toppled	8	25.0%	75.0%	-	-	-	100%
All	22	18.2%	81.8%	13.6%	4.5%	-	77.8%

Oct-10

Site	n	Immature	Mature	Developing	Capable	Regressing	Regenerating
Bank	-	-	-	-	-	-	-
Standing	15	13.3%	86.7%	-	38.5%	61.5%	38.5%
Toppled	1	-	*100%	-	-	*100%	-
All	16	12.5%	87.5%	-	35.7%	64.3%	35.7%

*All spawning capable fish sampled in October 2010 exhibited late vitellogenic oocytes in conjunction with significant atresia.

Table 5: Percent maturity at total length for female red snapper, *Lutjanus campechanus,* sampled during spawning season from three habitat types on the Louisiana continental shelf. Bold font marks the size at which 50% maturity was reached for a given size class. Asterisk indicates n<5.

Total Length (mm)	Bank (n=169)	Standing (n=101)	Toppled (n=56)
<274	-	-	-
275-324	50*	-	-
325-374	19	38	50*
375-424	31	10	50
425-474	62	75	71
475-524	59	45	77
525-574	69	40	75
575-624	86	50	40
625-674	78	90	-
675-724	100	100*	100*
725-774	100*	100*	100*
>775	100*	-	-

n=sample size

Table 6: Percent maturity at age for female red snapper, *Lutjanus campechanus,* collected during spawning season from three habitat types on the Louisiana continental shelf. Bold font marks the size at which 50% maturity was reached for a given size-class. Asterisk indicates n<5.

Age (year)	Bank (n=152)	Standing (n=101)	Toppled (n=56)
2	50*	25*	-
3	26	35	58
4	39	50	68
5	66	53	80
6	81	100*	-
7	91	100	-
8	100	100*	100*
9	100*	100*	-
10	100*	-	-
11	100*	-	-

n=sample size

Table 7: Batch fecundity estimates (BFE) for female red snapper, *Lutjanus campechanus,* sampled from three habitat types on Louisiana's continental shelf edge.

Site	n	Mean ± SE	Min	Max
Bank	1	326734	-	-
Standing	3	327955 ± 308584	16363	945114
Toppled	4	110867 ± 70259	4631	316514
All	8	219258 ± 113749	4631	945114

n=sample size; SE=standard error; Min=minimum; Max=maximum;

Table 8: Best-fit batch fecundity regression relationships for Louisiana female red snapper, *Lutjanus campechanus,* from natural shelf-edge bank, standing platform, and toppled platform habitats and from all habitats combined.

Logarithmic Function	p-value	R^2
$Ln_eBFE = 8.4559 * Ln_eL - 41.7636$	<0.0001	0.9578
$Ln_eBFE = 2.9382 * Ln_eW - 10.8473$	<0.0001	0.9553
$Ln_eBFE = 4.9398 * Ln_eAge + 3.6934$	0.0015	0.8344

Ln_e=natural logarithm; BFE=batch fecundity estimate; L=total length; W=eviscerated body weight; A=age

Table 9: Spawning frequency estimates (SFE) for female red snapper, *Lutjanus campechanus,* collected from three habitat types on the Louisiana continental shelf. Spawning frequency estimates are based on the Post-Ovulatory Follicle Method (POF method) and the Time-Calibrated method (TC method).

Site	n day-0	n day-1	n Mature	SFE_{POF}	SFE_{TC}	Spawns* season^{-1} (SFE_{POF})	Spawns* season^{-1} (SFE_{TC})
Bank	8	14	94	6.7	8.5	22	18
Standing	5	9	49	5.4	7.0	28	21
Toppled	8	7	37	5.3	4.9	28	30
All	21	30	180	6.0	7.1	25	21

n=sample size; day-0=late vitellogenic or hydrated; day-1=POF present; SFE_{POF}= spawning frequency estimate based on the POF method; SFE_{TC}=spawning frequency estimate based on the time-calibrated method

Table 10: Chi-Square tests comparing spawning frequency estimates of sexually mature female red snapper, *Lutjanus campechanus,* sampled from A) natural banks and standing platforms B) standing platforms and toppled platforms and C) natural banks and toppled platforms on the Louisiana continental shelf.

A	Bank vs Standing		
	df	χ^2	p
SFE_{TC}	1	0.1954	0.6585
SFE_{POF}	1	0.2880	0.5915

B	Standing vs Toppled		
	df	χ^2	p
SFE_{TC}	1	0.5386	0.4630
SFE_{POF}	1	0.0042	0.9481

C	Bank vs Toppled		
	df	χ^2	p
SFE_{TC}	1	1.6071	0.2049
SFE_{POF}	1	0.3196	0.5719

SFE_{TC}=spawning frequency estimate based on the time-calibrated method; SFE_{POF}= spawning frequency estimate based on the post-ovulatory follicle method

Table 11: Mean spawning frequency estimate at age for female red snapper, *Lutjanus campechanus*, sampled on the Louisiana continental shelf. SFE are based on the post-ovulatory follicle method (POF) and the time-calibrated method (TC). Asterisk indicates n < 10.

Age	n Mature	n Day-0	n Day-1	SFE (POF)	SFE (TC)	Spawns* season^{-1} (POF)	Spawns* season^{-1} (TC)
2	2	0	0	-	-	-	-
3	35	4	2	17.5	11.7	9	13
4	53	3	10	5.3	8.2	28	18
5	37	7	5	7.4	6.2	20	24
6	12	0	4	3.0	6.0	50	25
7	15	5	4	3.8	3.3	40	45
8	7	0	2	3.5	*7.0	43	21
≥9	5	1	0	-	*10.0	-	15

n=sample size; Day-0=hydrated females; Day-1=females with POF

Table 12: Average spawning frequency estimates (SFE) at total length for female red snapper, *Lutjanus campechanus*, sampled on Louisiana's continental shelf. SFE are based on the post-ovulatory follicle method (POF) and the time-calibrated method (TC).

TL (mm)	n Mature	n Day-0	n Day-1	SFE (POF)	SFE (TC)	Spawns* season^{-1} (POF)	Spawns* season^{-1} (TC)
275-324	1	0	0	-	-	-	-
325-374	8	1	2	4.0	5.3	38	28
375-424	18	0	0	-	-	-	-
425-474	32	6	3	10.7	7.1	14	21
475-524	45	5	8	5.6	6.9	27	22
525-574	21	2	1	21.0	14.0	7	11
575-624	19	2	6	3.2	4.8	47	32
625-674	16	2	4	4.0	5.3	38	28
675-724	12	1	3	4.0	6.0	38	25
725-774	5	2	2	2.5	2.5	60	60
>775	1	0	0	-	-	-	-

n=sample size; TL=total length; n=sample size; Day-0=hydrated females; Day-1=females with POF

Table 13: Mean batch and annual fecundity estimates (± standard error) for female red snapper, *Lutjanus campechanus,* collected from three habitat types off the coast collected on the Louisiana continental shelf. Spawning frequency was estimated with the time-calibrated method.

Site	n	SFE	BFE ± SE	AFE ± SE	CI
Bank	1	8.5	326734	5765893	-
Standing	3	7.0	327955 ± 308584	7027605 ± 6612523	12960308
Toppled	4	4.9	110867 ± 140518	3393876 ± 2150780	4215451
All	8	7.1	219258 ± 113749	4632217 ± 2467934	4837062

n=sample size; SFE=spawning frequency estimate; BFE=batch fecundity estimate; AFE=annual fecundity estimate

Table 14: Number of viable red snapper stomachs sampled from each site during each year. Sites represent three distinct habitats, including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank) in the Gulf of Mexico.

Habitat	**Site**	**2009**	**2010**	**Total**
Standing	EI-325	39	19	58
	EI-346	11	5	16
	SS-296	3	0	3
Toppled	EI-322	52	12	64
	EI-324	46	11	57
Bank	Alderdice	41	22	63
	Bouma	103	0	103
	Jakkula	73	23	96

Table 15: Composition of red snapper diets, combined over years, by percentage dry weight (%DW) and Index of Relative Importance (IRI) at three different habitats, including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank).

Prey Item	Standing		Toppled		Bank	
	%DW	IRI	%DW	IRI	%DW	IRI
Unidentified	1.92		4.56		3.86	
Fish	39.83	55.47	55.95	39.19	59.18	68.66
Squid	18.61	8.50	5.34	2.62	0.27	0.14
Crustacean	7.56	6.01	4.36	12.28	2.67	4.91
Shrimp	1.36	0.73	0.00	0.00	2.22	2.33
Mantis Shrimp	0.43	1.02	1.79	9.11	0.13	0.40
Amphipod	0.03	1.45	0.03	5.34	0.05	2.51
Crab	0.39	0.01	2.56	0.39	5.16	8.72
Hyperid Amphiod	0.02	0.35	0.24	12.83	0.04	0.02
Lizardfish	1.37	0.08	0.00	0.00	4.24	0.18
Antenna Codlet	28.01	26.33	0.00	0.00	12.90	9.35
Bathyal Swimming Crab	0.00	0.00	24.49	6.53	0.49	0.01
Crab Larvae	0.01	0.00	0.29	4.11	0.07	0.10
Cavalina	0.00	0.00	0.21	5.67	0.04	0.48
Polychaete	0.00	0.00	0.16	1.93	0.17	1.27
Gorgonian	0.46	0.04	0.00	0.00	8.53	0.91

Table 16: Percentage dry weight (%DW) of red snapper diets, by year at three different habitats, including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank).

Prey Item	Standing		Toppled		Bank	
	2009	2010	2009	2010	2009	2010
Unidentified	1.56	3.11	4.08	30.22	3.11	4.02
Fish	28.84	75.78	44.17	35.94	52.06	67.13
Squid	18.73	18.21	7.30	0.44	0.33	0.14
Crustacean	9.81	0.21	5.99	0.00	3.37	0.76
Shrimp	1.57	0.67	0.00	0.07	3.18	0.39
Mantis Shrimp	0.55	0.05	0.61	25.56	0.03	0.28
Amphipod	0.05	0.00	0.00	0.55	0.02	0.10
Crab	0.51	0.00	3.52	0.00	5.94	3.42
Hyperid Amphiod	0.03	0.00	0.34	0.00	0.06	0.00
Lizardfish	1.79	0.00	0.00	0.00	6.51	0.00
Antenna Codlet	36.57	0.02	0.00	0.00	19.53	0.45
Bathyal Swimming Crab	0.00	0.00	33.62	0.00	0.75	0.00
Crab Megalop	0.01	0.00	0.38	0.28	0.00	0.20
Cavalina	0.00	0.01	0.00	3.90	0.00	0.09
Polychaete	0.00	0.00	0.00	3.03	0.00	0.35
Gorgonian	0.00	1.95	0.00	0.00	0.00	22.66

Table 17: Index of relative importance (IRI) of red snapper diets, by year at three different habitats, including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank).

Prey Item	Standing		Toppled		Bank	
	2009	2010	2009	2010	2009	2010
Fish	45.21	78.36	27.63	15.25	85.59	59.94
Squid	7.56	17.32	3.56	0.10	0.04	0.17
Crustacean	8.15	0.45	22.18	0.00	2.65	0.41
Shrimp	0.77	0.92	0.00	0.01	2.35	0.52
Mantis Shrimp	0.75	1.72	1.16	49.65	0.00	2.13
Amphipod	1.84	0.00	0.00	12.97	0.03	12.46
Crab	0.02	0.00	0.54	0.00	0.10	1.58
Hyperid Amphiod	0.44	0.00	28.01	0.00	0.02	0.00
Lizardfish	0.12	0.00	0.00	0.00	0.12	0.00
Antenna Codlet	35.14	0.43	0.00	0.00	9.10	0.12
Bathyal Swimming Crab	0.00	0.00	8.36	0.00	0.01	0.00
Crab Megalop	0.01	0.00	8.54	0.08	0.00	0.78
Cavalina	0.00	0.43	0.00	15.88	0.00	3.57
Polychaete	0.00	0.00	0.00	6.07	0.00	8.22
Gorgonian	0.00	0.38	0.00	0.00	0.00	10.11

Table 18: Caloric density in cal/g for red snapper collected over three habitats including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank), in the northern Gulf of Mexico. Mean values, standard deviations (SD), and sample number (N) are shown.

Habitat	**N**	**Mean Caloric Density (cal/g)**	**SD**	**N**	**Mean Caloric Density (cal/g)**	**SD**
		2009			**2010**	
Standing	30	5365.22	157.63	15	5472.67	49.36
Toppled	19	5281.81	224.05	16	5522.52	61.39
Bank	41	5356.11	163.80	20	5504.26	69.97

Table 19: Mean values of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ for red snapper (*Lutjanus campechanus*) collected over three habitat types in the Gulf of Mexico, including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank (bank) habitat, per year. Total number of samples run (n) and standard deviations (sd) are also shown.

Habitat	Year	n	Mean $\delta^{15}N$	sd	Mean $\delta^{13}C$	sd	Mean $\delta^{34}S$	sd
Standing	2009	48	12.30	0.90	-18.14	0.46	18.05	0.72
	2010	16	13.59	1.26	-17.47	0.70	17.60	1.01
Toppled	2009	23	12.26	0.67	-18.18	0.53	16.99	0.38

	2010	16	12.78	1.36	-18.05	0.70	17.65	0.75
Bank	2009	178	12.45	0.85	-17.48	2.38	18.19	3.03
	2010	36	12.45	0.69	-17.78	0.36	18.15	0.48

Table 20: Metrics for assessing trophic niche breadth

Habitat	Year	TA	Mean CD	$\square^{15}$N Range		$\square^{13}$C Range	
				Low	High	Low	High
Bank	2009	3.57	0.61 ± 0.45	11.17	16.03	-18.77	-16.60
	2010	1.50	0.66 ± 0.48	11.2	15.5	-18.4	-16.23
Standing	2009	1.98	0.66 ± 0.61	11.01	15.85	-18.62	-16.39
	2010	2.31	1.14 ± 0.52	11.53	15.68	-18.68	-16.12
Toppled	2009	1.51	0.52 ± 0.40	11.54	13.96	-18.78	-16.44
	2010	2.64	1.14 ± 0.79	11.35	15.74	-19.10	-16.57

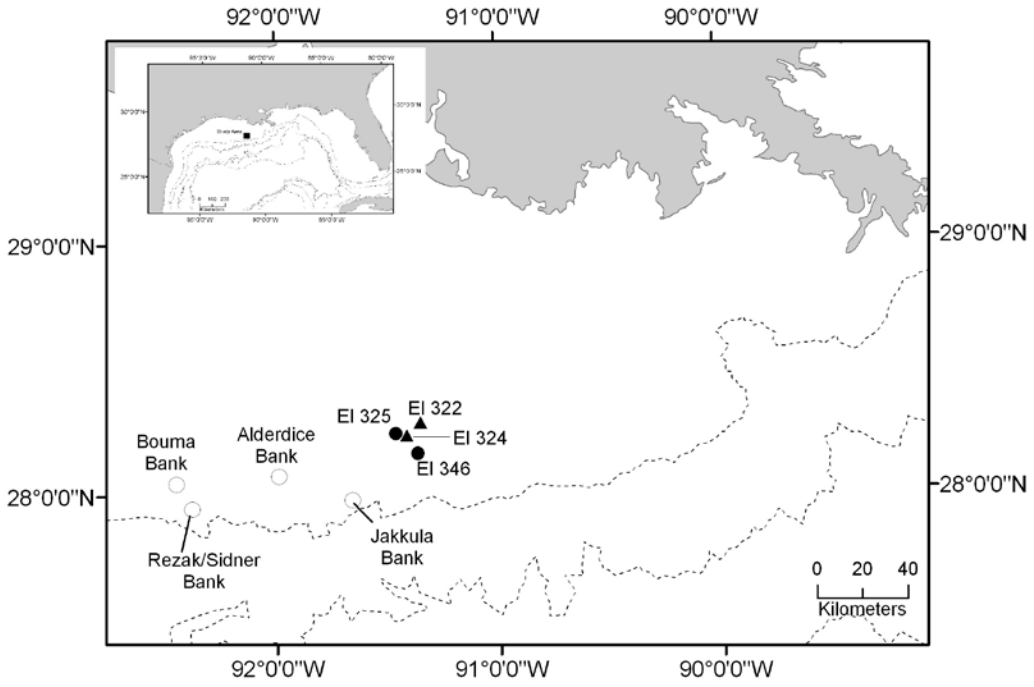


Figure 1: Location of red snapper (*Lutjanus campechanus*) sampling sites in the Gulf of Mexico. Open circles represent shelf edge bank (bank) sites. Closed circles represent standing oil and gas platform (standing) sites. Triangles represent toppled oil and gas platforms (toppled) sites. Dotted lines represent the continental shelf edge break.

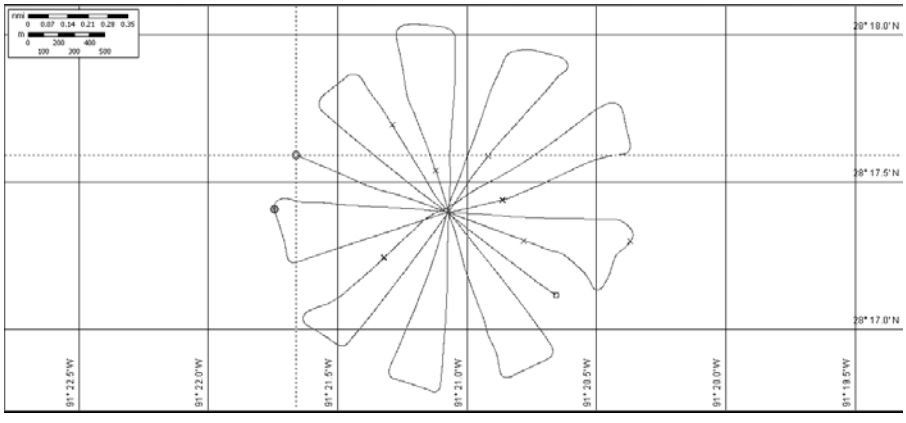


Figure 2: Sample cruise track for hydroacoustic transects around artificial reefs.

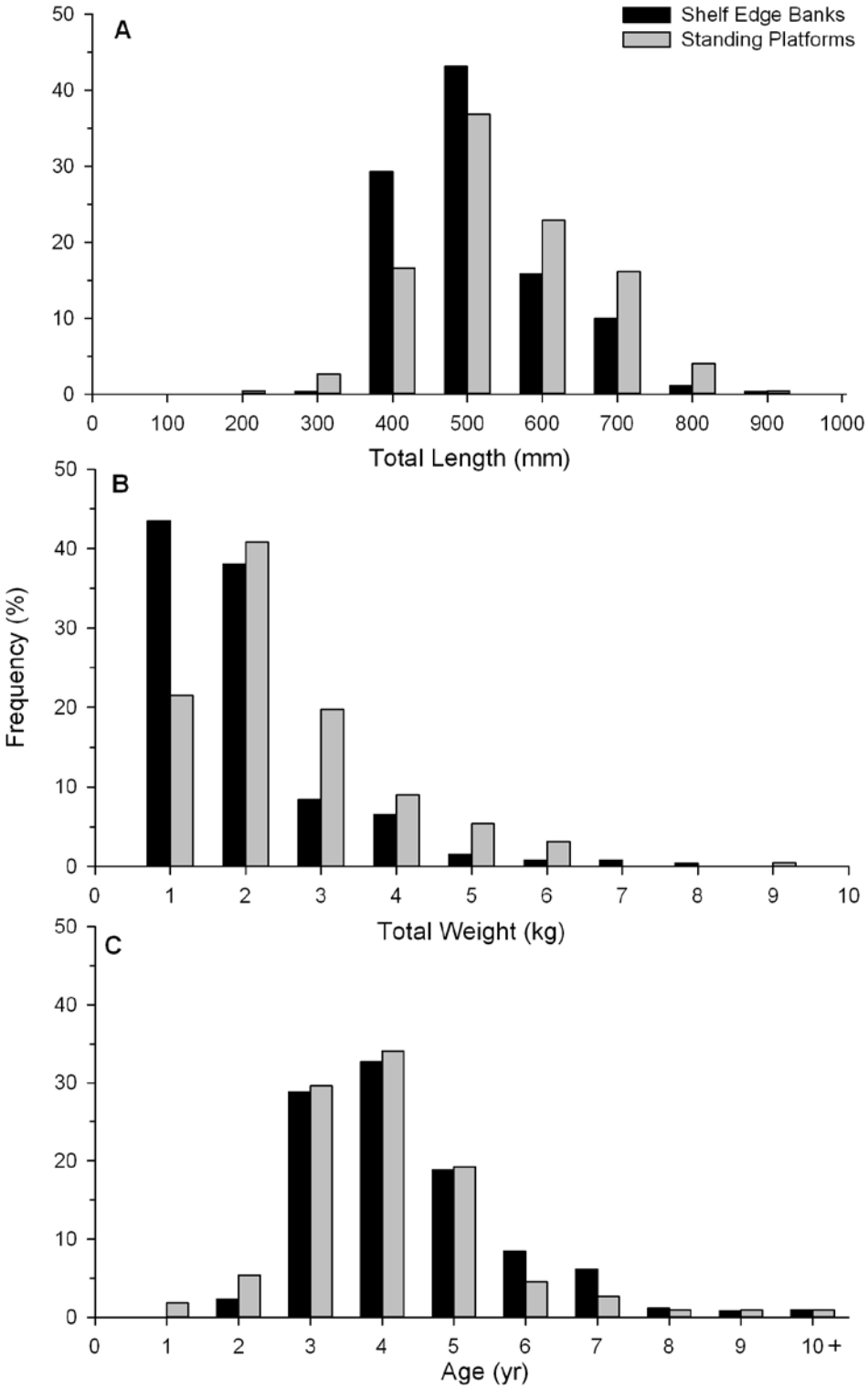


Figure 3: Frequency distribution of (A) total length (mm), (B) total weight (g), and (C) age (yr) for red snapper, *Lutjanus campechanus*, sampled from Louisiana's shelf-edge banks (n=260) and standing platforms (n=223) in 2009, 2010 and 2011.

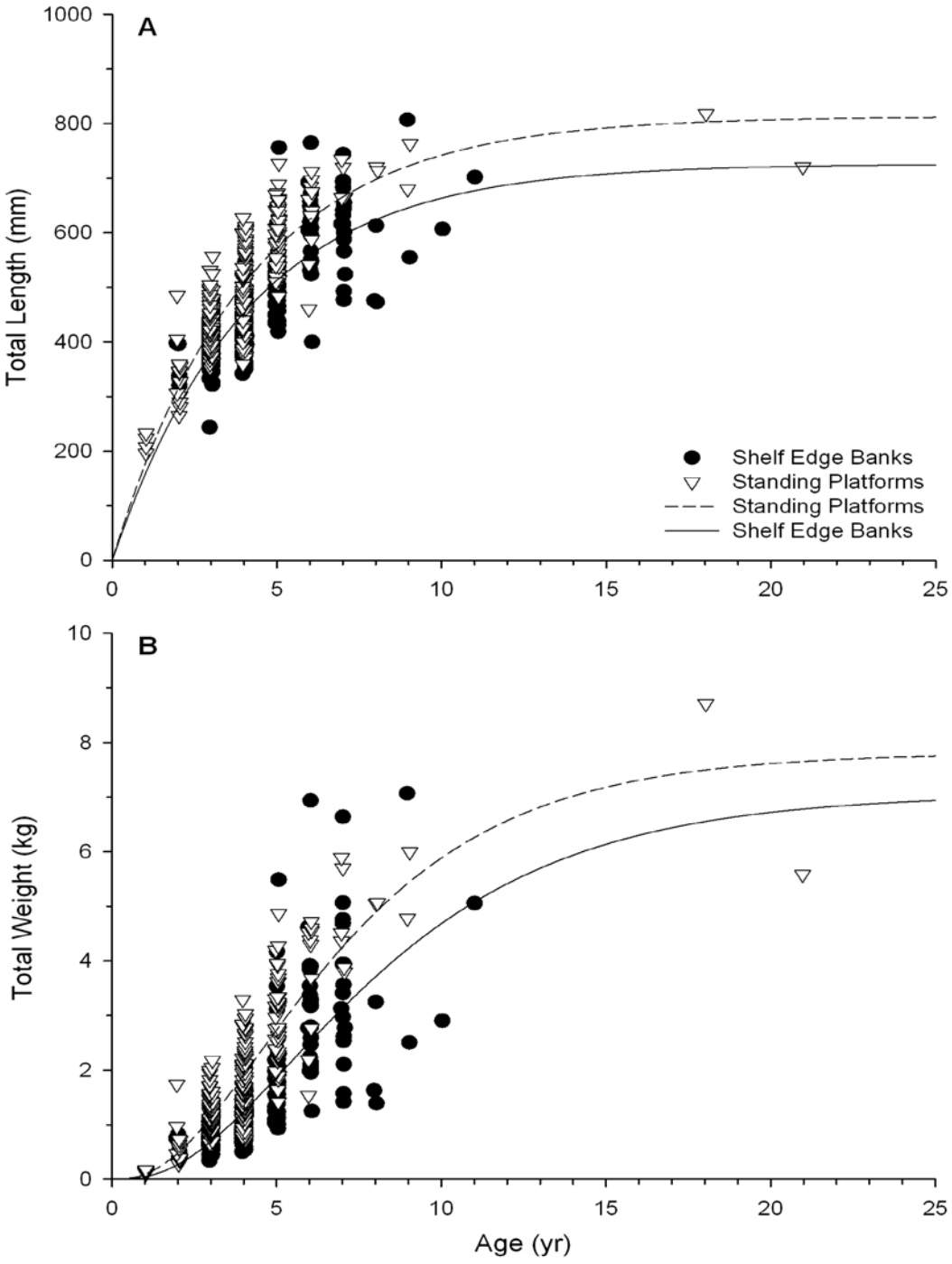


Figure 4: Observed (A) total length (mm) at age (yr) and (B) total weight (kg) at age (yr) for red snapper, *Lutjanus campechanus*, collected from Louisiana's shelf-edge banks (n=317) and standing platforms (n=219) in 2009, 2010 and 2011. Plotted lines indicate von Bertalanffy growth functions fitted to the data from each habitat type.

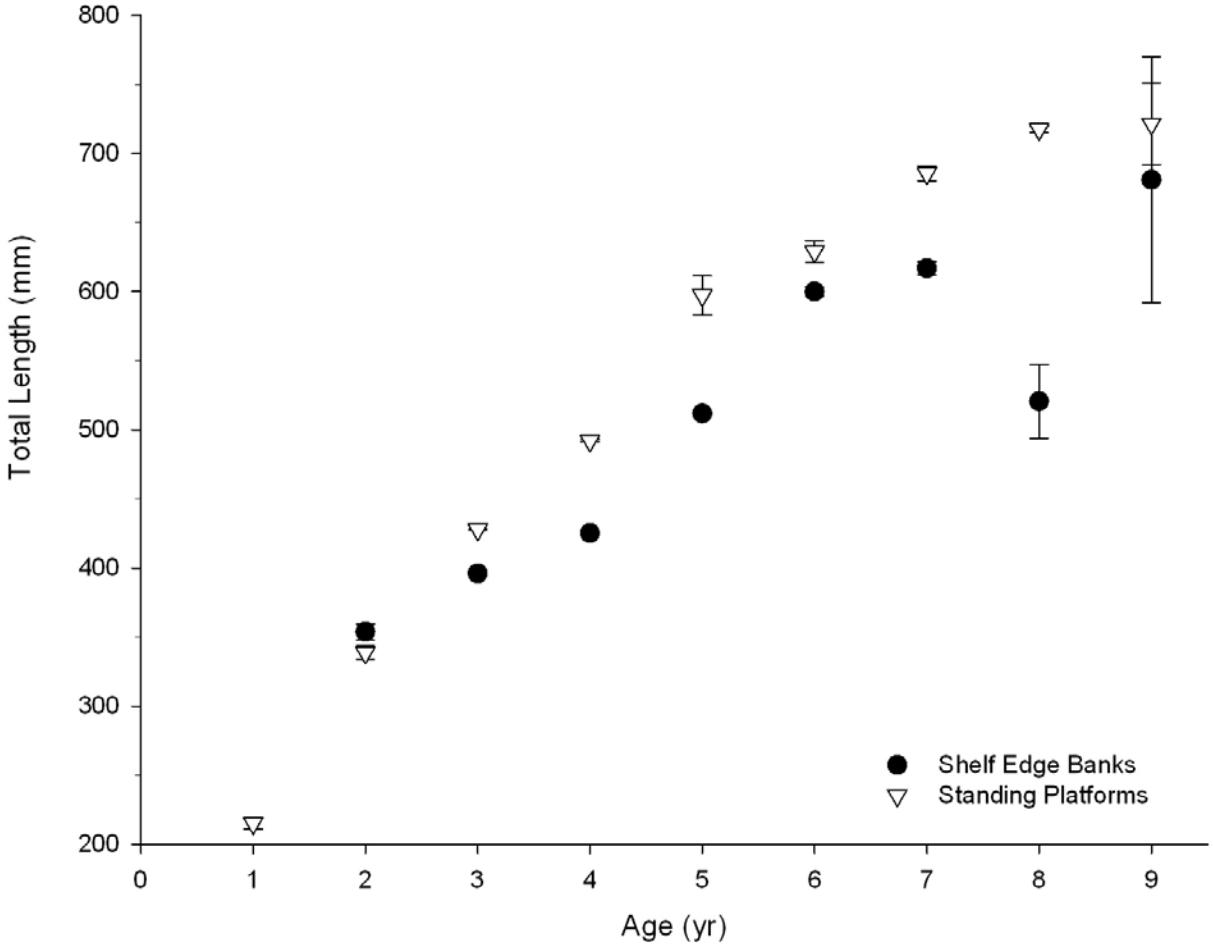


Figure 5: Mean size at age ± standard error of red snapper, *Lutjanus campechanus*, collected from Louisiana's shelf-edge banks (n=317) and standing platforms (n=219) in 2009, 2010 and 2011. Error bars represent standard error of the mean.

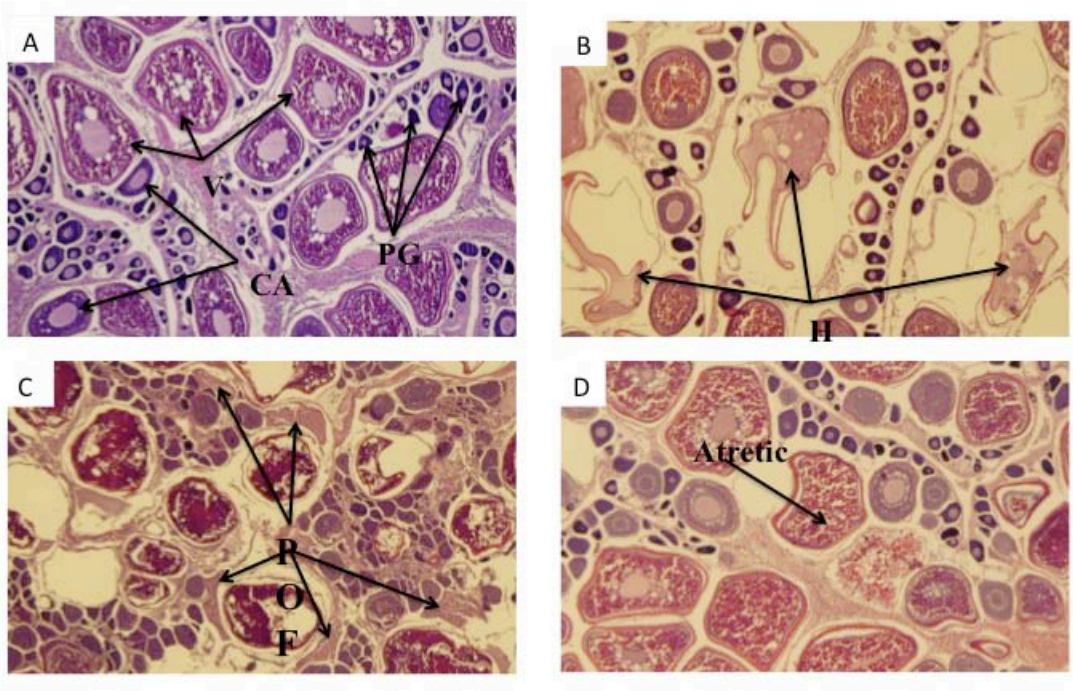


Figure 6: Various stages of red snapper oocyte maturation and post-spawning including: A.) Three stages of red snapper oocyte maturation: PG is primary growth, CA is cortical alveoli, V is vitellogenic. B.) Hydration, final stage of oocyte maturation, occurring < 24 hours before spawning. H indicates hydrated oocytes. C) Post-ovulatory follicles (POF), which remain in the ovary for 24 hours post-spawning before degradation and resorption. D.) Atretic oocyte, indicating the breakdown and resorption of an oocyte prior to ovulation.

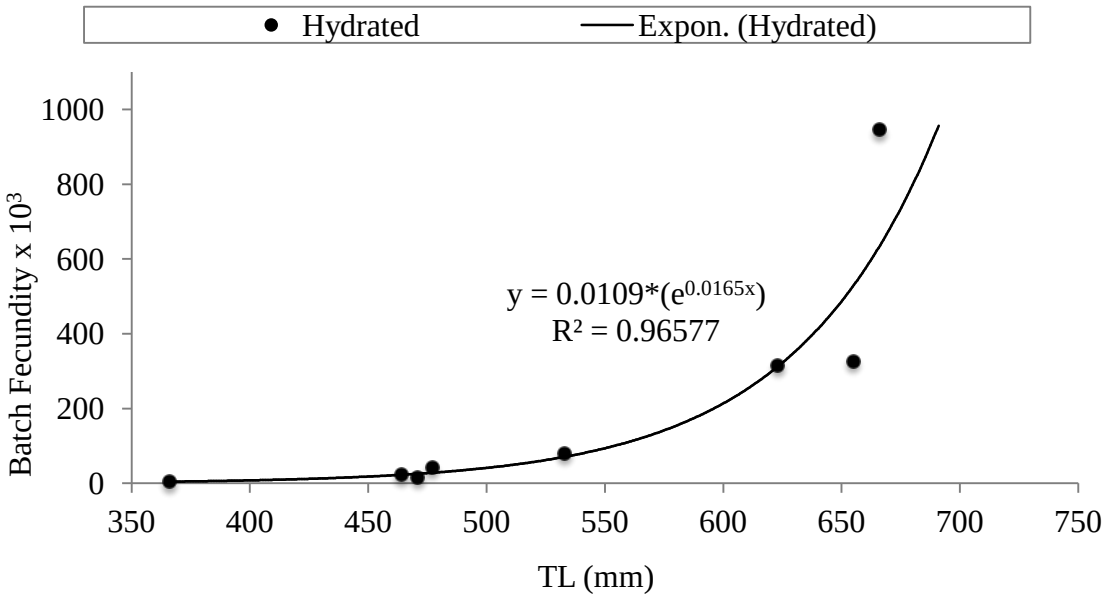


Figure 7: Relationship between batch fecundity and total length (TL) for female red snapper, *Lutjanus campechanus*, collected on the Louisiana continental shelf from three habitat types: natural banks (n=1), standing platforms (n=3) and toppled platforms (n=4).

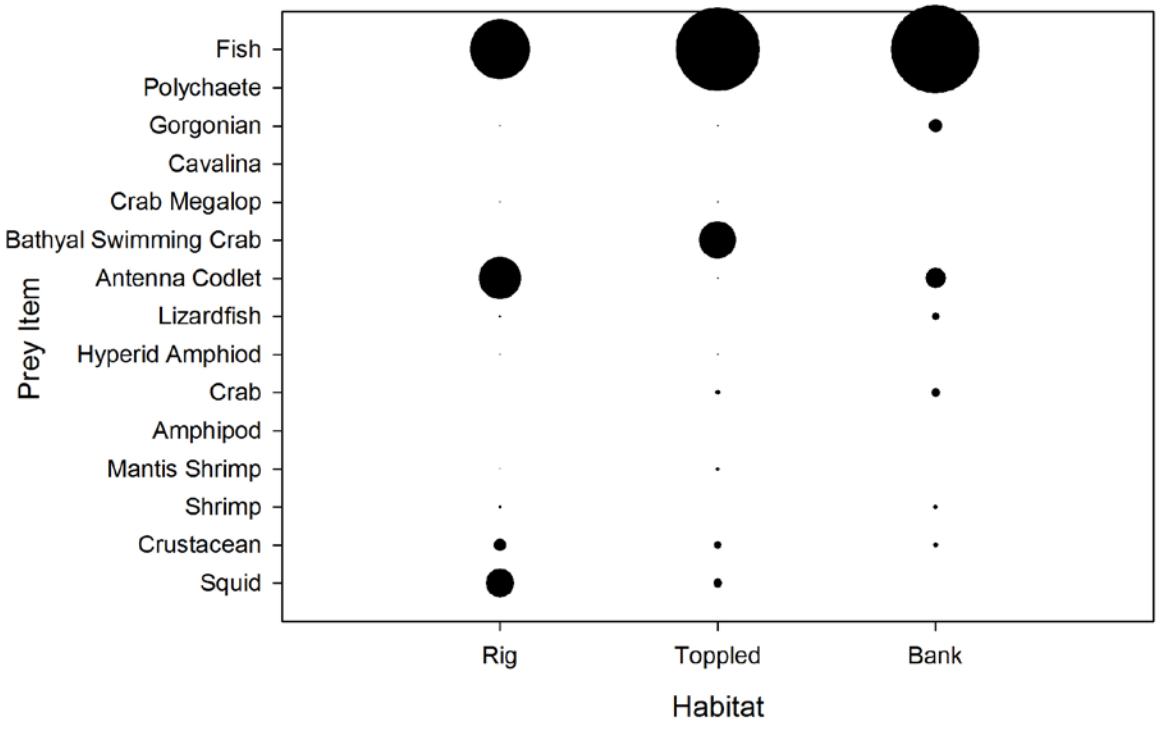


Figure 8: Diets of red snapper (*Lutjanus campechanus*) combined over years by

percentage dry weight over three habitats in the Gulf of Mexico including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank). Samples are from red snapper collected in the summer of 2009 and 2010. Only the 15 most common prey items are displayed.

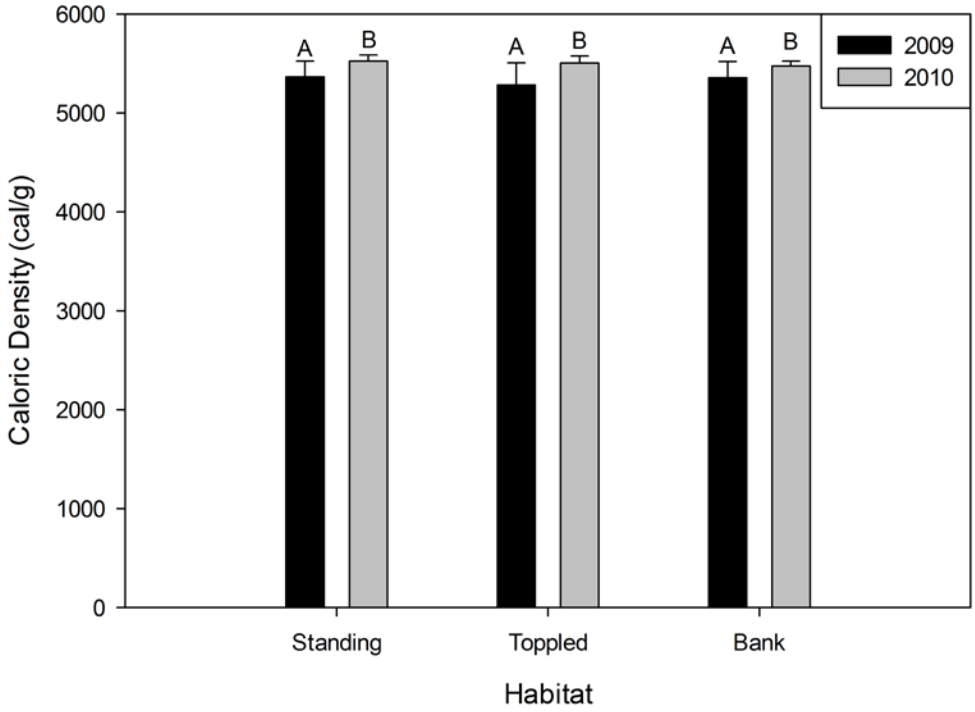


Figure 9: Caloric density in cal/g for red snapper collected over three habitats in the Gulf of Mexico including standing oil and gas platforms (standing), toppled oil and gas platforms (toppled) and natural shelf-edge bank habitat (bank) during two years of the study. Letters represent significant difference. Standard deviation is also shown.

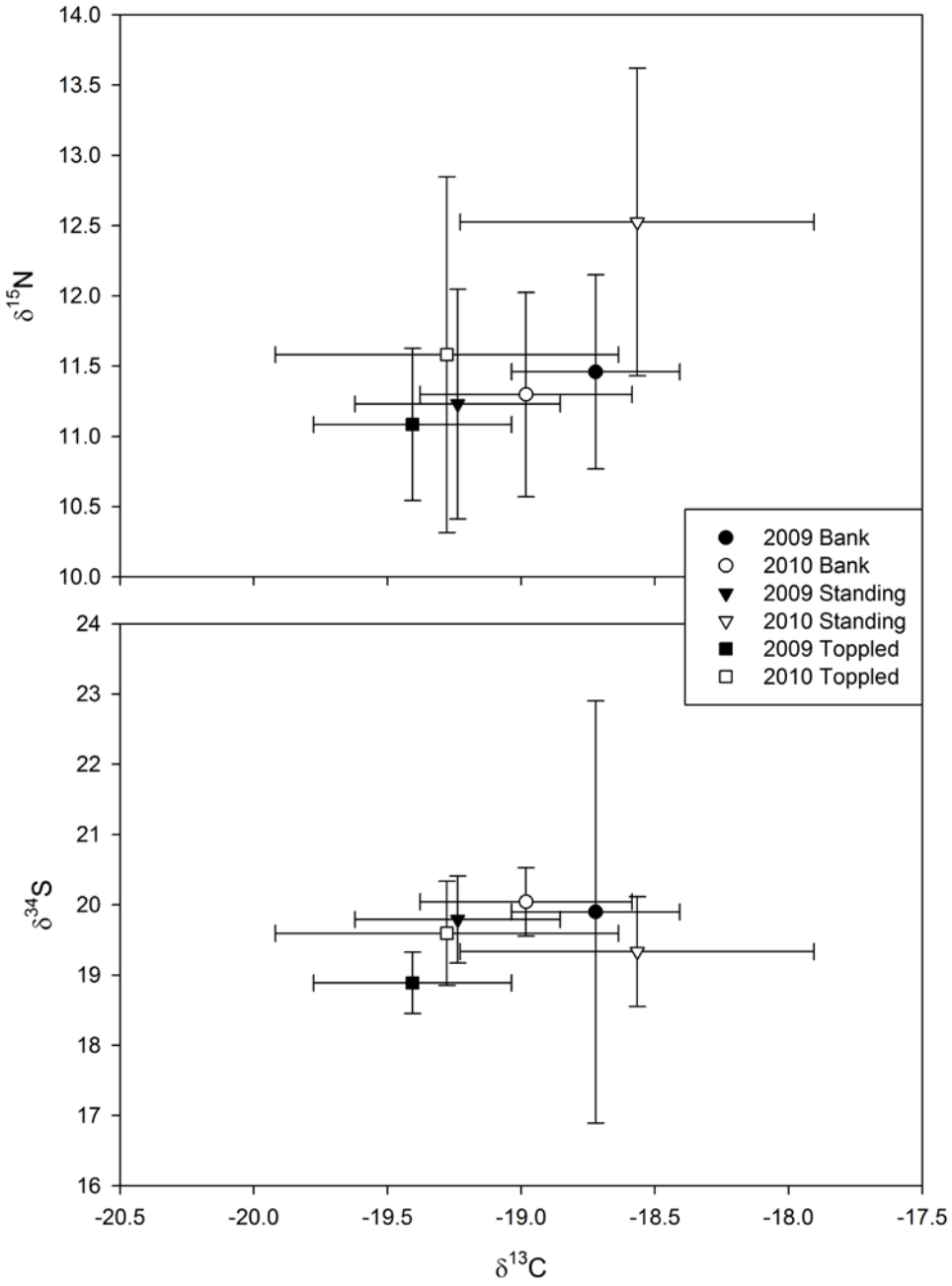


Figure 10: Mean stable isotope results values of red snapper (*Lutjanus campechanus*) as visualized by C-N (top) and C-S (bottom) biplots for three habitats in the Gulf of Mexico, including shelf-edge banks (bank; circle), standing platforms (standing; triangle), and toppled platforms (toppled; square). Standard error bars are shown around the mean. Years 2009 (closed symbol) and 2010 (open symbol) are represented separately due to observed differences between years at the standing platform sites.

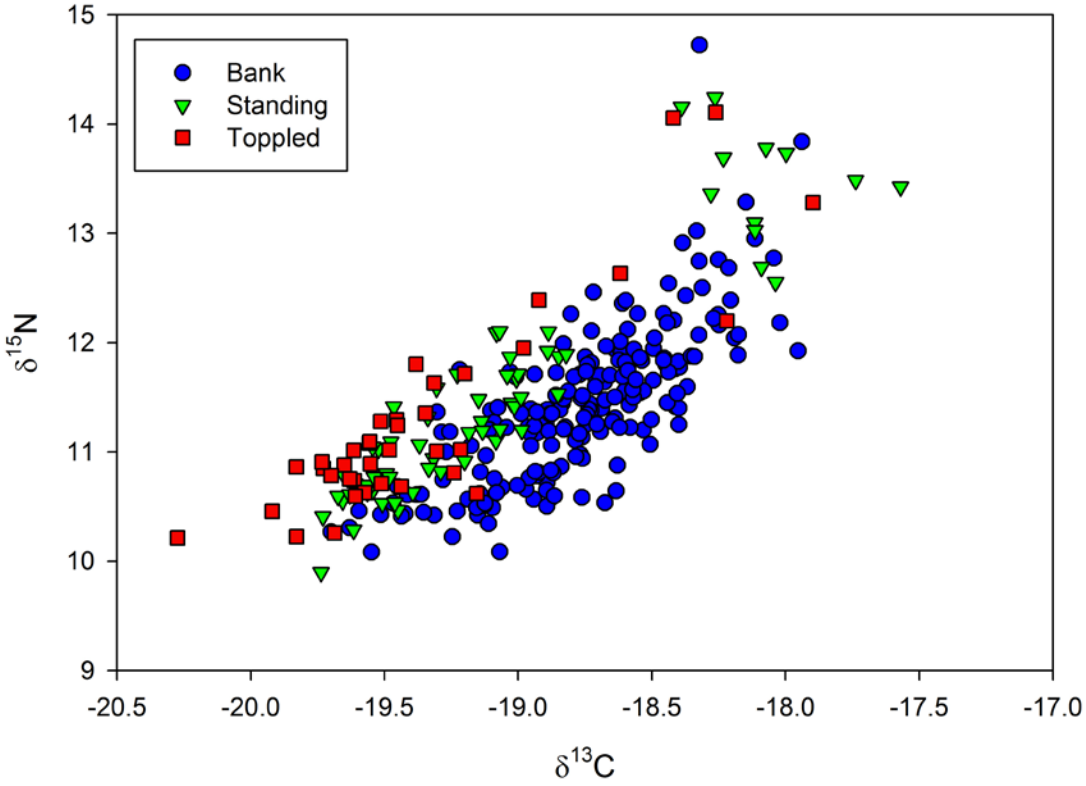


Figure 11: Stable isotope results from tissues samples of red snapper (*Lutjanus campechanus*) as represented by their carbon-nitrogen biplot. Data are length corrected and used to assess trophic niche breadth of three different habitats in the northern Gulf of Mexico including shelf-edge banks (bank; circle), standing oil and gas platforms (standing; triangle), and toppled oil and gas platforms (toppled; square).

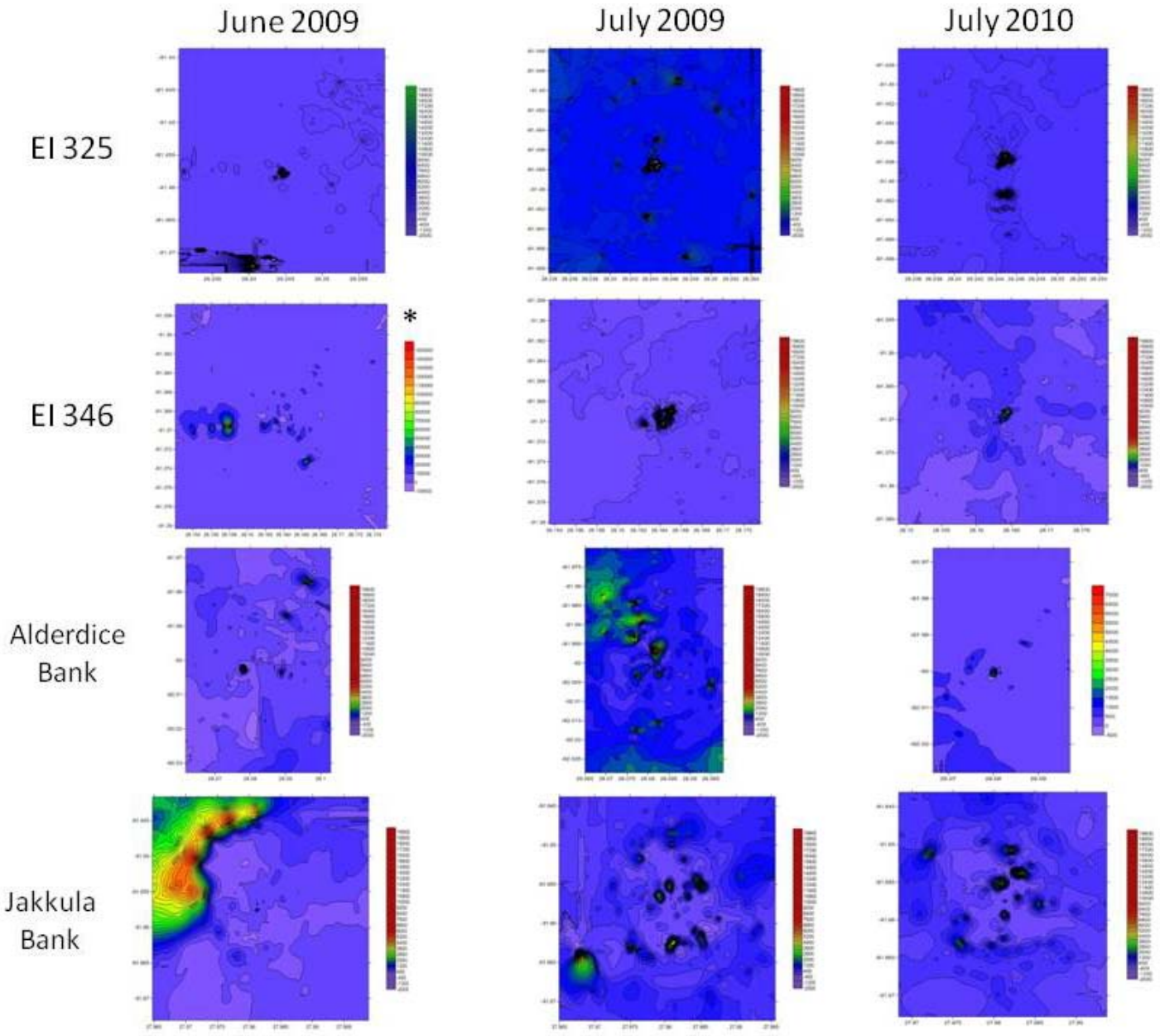


Figure 12: Contour plots of acoustic backscatter data as represented by nautical area scattering coefficient (NASC) at each site over the three sample periods. All plots are constructed in the same scale (minimum contour: -2000, maximum contour: 20000, contour interval: 200), except for EI346 in June 2009 (marked with an asterisk (*)), which was an order of magnitude higher than other samples. X-axis is latitude and Y-axis is longitude. Data is combined over depth to show spatial distribution of biomass.

References

Beamish, R. J. and D. A. Fournier (1981). "A Method for Comparing the Precision of a Set of Age Determinations." Canadian Journal of Fisheries and Aquatic Sciences 38(8): 982-983.

Beckman, D. W., G. R. Fitzhugh and C. A. Wilson (1988). "Growth Rates and Validation of Age Estimates of Red Drum, Sciaenops Ocellatus, in a Louisiana Salt Marsh Impoundment." Contributions in Marine Science 30: 93-98.

Bowen, S.H. 1996. Quantitative description of the diet. In: Murphy BR, Willis DW (eds) Fisheries techniques, 2nd edn. American Fisheries Society, Bethesda, MD, p 513–532.

Chang, W. Y. B. (1982). "A Statistical Method for Evaluating the Reproducibility of Age Determination." Canadian Journal of Fisheries and Aquatic Sciences 39(8): 1208-1210.

Cowan, J. H., Jr, R. L. Shipp, H. K. Bailey, IV and D. W. Haywick (1995). "Procedure for Rapid Processing of Large Otoliths." Transactions of the American Fisheries Society 124: 280-282.

Echoview 2012. Version 5.2; SonarData Pty. Ltd., Hobart, Tasmania, Australia.

Ferry L.A. and G.M. Cailliet. 1996. Sample size and data analysis: are we characterizing and comparing diet properly? In: MacKinlay D, Shearer K (eds) Gutshop '96. Feeding ecology and nutrition in fish symposium proceedings. San Francisco State University, pp 71–80.

Foote, K.G., H.P. Knudsen, G. Vestnes, D.N. MacLennan, and E.J. Simmonds. 1987. Calibration of acoustic instruments for fish density estimation: A practical guide ICES Cooperative Research Report No 144. ICES, Copenhagen, Denmark.

Haddon, H. (2001). Modelling and Quantitative Methods in Fisheries. Boca Raton, Chapman & Hall/CRC Press.

Hunter, J. R. and S. R. Goldberg. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. Fisheries Bulletin. 77:641-652.

Hunter, J. R., N. C. H. Lo and R. J. H. Leong. 1983. Batch fecundity in multiple spawning fishes. Pp. 67-94 *in* R. Lasker, editor. An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*. U.S. Dep. Commer., NOAA Tech. Report. NMFS 36.

Hunter, J. R., and B. J. Macewicz. 1985a. Measurement of spawning frequency in

multiple spawning fishes, p. 79-94 i*n* R.L. Lasker (ed.), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*. NOAA Tech. Rep. NMFS 36, 99 p.

Layman, C.A., D.A. Arrington, C.G. Montana, and D.M. Post. 2007a. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88(1): 42 – 48.

Layman, C.A., J.P. Quattrochi, C.M. Peyer, and J.E. Allgeier. 2007b. Niche width collapse in a resilient top predator following ecosystem fragmentation. Ecology Letters 10: 937 – 944.

MATLAB version 7.0.4. 2007. Computer software, The MathWorks Inc., Natick, Massachusetts

McCawley, J. R., and J.H. Cowan, Jr. 2007. Seasonal and size specific diet and prey demand of red snapper on Alabama artificial reefs. In W.F. Patterson, J.H. Cowan Jr., G.R. Fitzhugh, and D.L. Nieland, editors. Red Snapper Ecology and Fisheries in the U.S. Gulf of Mexico. American Fisheries Symposium 60, Bethesda, Maryland. pp 77 – 104.

McCawley, J. R. 2003. Diet and prey demand of red snapper, *Lutjanus campechanus*, on Alabama artificial reefs. Thesis. University of South Alabama, Mobile, Alabama, USA.

MacLellan, P.G. Fernandes, and J. Dalen. 2002. A consistent approach to definitions and symbols in fisheries acoustics. ICES J. Mar Sci 59: 365 – 369.

Nieland, D. L. and C. A. Wilson. 1993. Reproductive biology and annual variation of reproductive variables of black drum in the northern Gulf of Mexico. Transactions of American Fisheries Society 122:318-327.

PRIMER (Plymouth Routine in Multivariate Ecological Research); Warwick, 1990

SAS Institute. 2010. "Statistics, Version 9.2." SAS Institute, Inc., Box 8000, Cary, NC.

Simmonds, E.J. and D.N. MacLennan. 2005. Fisheries Acoustics: Theory and Practice, 2nd edition. Blackwell Science, Oxford, U.K.

Soldal, A. V., I. Svellingen, T. Jørgensen, and S. Løkkeborg. 2002. Rigs-to-reefs in the North Sea: hydroacoustic quantification of fish in the vicinity of a ‘‘semi-cold’’ platform. ICES Journal of Marine Science, 59: S281–S287.

Wallace, R. A. and K. Selman. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. Amer. Zool. 21:325-343.

Wells, R.J.D. J.H. Cowan, Jr., and B. Fry. 2008a. Feeding ecology of red snapper *Lutjanus campechanus* in the northern Gulf of Mexico. Mar Ecol Prog Ser 361: 213 – 225.

Wilson, C. A., J. H. Render and D. L. Nieland. 1994. Life history gaps in red snapper (*Lutjanus campechanus*), swordfish (*Xiphias gladius*), and red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico: age distribution, growth, and some reproductive biology. Final report to U.S. Department of Commerce, National Marine Fisheries Service, Marine Fisheries Initiative, Cooperative Agreement NA17FF0383-02.

Wilson, C. A. and D. L. Nieland (2001). "Age and Growth of Red Snapper, *Lutjanus Campechanus*, from the Northern Gulf of Mexico Off Louisiana." Fishery Bulletin 99(4): 653-664.