# Identification of Critical Spawning Habitat and Male Courtship Vocalization Characteristics of Red Drum, *Sciaenops ocellatus,* in the Lower Neuse River Estuary of North Carolina.

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**Principal Inverstitor: Anna Barrios Beckwith**
**Cooperative Invesigators: Captain George Beckwith and Peter Rand Ph.D.**

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## Abstract

The Neuse River Estuary presents a unique set of characteristics not previously noted in estuarine systems where red drum spawning has been confirmed. Passive acoustic monitoring techniques offer a viable and non-invasive method to locate spawning aggregations, describe diel and seasonal spawning patterns, and determine affinities to a particular habitat and ambient water quality conditions. Two previous field seasons (2003 and 2004) were supported through the National Marine Fisheries Service MARFIN program, which made available the required research equipment. The 2003 and 2004 field season have shown that while red drum are understood to spawn within the NRE, distribution of spawning efforts and the nature of courtship vocalizations can be highly variable between years. The 2005 field season sought to define critical habitat for red drum spawning by establishing a baseline of behavior and habitat utilization.

We addressed three explicit research hypothesis based on our data, and conducted an exploration of the data to investigate how certain environmental variables may be influencing male courtship vocalizations in the NRE. Our first hypothesis sought to determine if there is a significant relationship with our measured response variable (characteristics of the male courtship vocalizations) to successful spawning activity as determined by the presence of sciaenid type eggs in the water column at the time the recordings were made. Our results indicated that this association was significant. Our second hypothesis sought to determine if there is a high degree of persistence of habitat use at a given site throughout the spawning season. Our results showed that once spawning vocalizations are detected at a site, there was strong evidence of persistent use of that site over the perceived spawning season. Our third hypothesis sought to determine if vocalizations were more prevalent at hard bottom versus soft bottom sites. Our results found that bottom type was not a significant factor in explaining presence of male courtship vocalizations. Finally, the 2005 data was considered collectively with the 2003/2004 data to establish a baseline of spawning distribution, and to develop a predictive model of critical habitat based on habitat features and ambient water quality conditions. During the 2003- 2005 field seasons, we found that red drum vocalizations (and, by association, spawning activity) were found to be related to day of year, year, depth, and salinity.

# Introduction

*Background and Recent Research*

Assessing the distribution and abundance of the spawning stock of red drum has presented a difficult problem to fisheries scientists and managers in this region. The ecosystems which drum typically inhabit are expansive and open, frustrating any attempt at estimated distribution and abundance by means of traditional methods (e.g. mark-recapture, gill net catch per unit effort). The Neuse River Estuary (NRE) presents a unique set of characteristics not previously noted in estuarine systems where red drum spawning has been confirmed. These include a meso-saline environment that experiences periodic water column stratification, often with hypoxic or anoxic bottom waters. Passive acoustic monitoring techniques offer a viable and non-invasive method to locate spawning aggregations, describe diel and seasonal spawning patterns, and determine affinities to particular habitats. Improving upon previous passive acoustic spot sampling techniques as a non-invasive method for delineating spawning aggregations has provided an important first step in understanding distribution and habitat use of the species during the critical spawning period. This effort may help lead to a more direct method for enumerating the number of spawning adults in the populations, a critical need for conservation.

During the summers of 2003 and 2004 courtship vocalizations were monitored to help locate spawning aggregations and characterize important attributes of the spawning grounds. Spawning sites by red drum within the NRE were located by a combination of passive acoustic recordings of courtship vocalizations and presence of eggs and early stage larvae. During these seasons predictable seasonal and diel patterns of red drum vocalizations were identified (Barrios 2004). A link was demonstrated between vocalization and the presence of Sciaenid eggs in the study area, helping to confirm that vocalizations are related to active spawning (Barrios 2004). Barrios (2004) found evidence that spawning aggregations are stationary once vocalizations begin based on a high degree of vocalization persistence and no significant shift in time of highest vocalization activity within the study area that would imply net movement of aggregations over a given night's sampling period.

Given the relatively fixed position of vocalizing aggregations, it was determined that depth and proximity to the river mouth were the dominant factors defining distribution of aggregations in the NRE during the 2003 field season (Barrios 2004). Drum appeared to have a strong affinity for deeper water during spawning. Some likely explanations for the utilization of this habitat for spawning may include: 1) greater habitat volume that better accommodates courtship behavior leading to gamete release, 2) more favorable conditions for egg and larval dispersal as compared to near-shore shallow water sites, and/or 3) greater availability of hard bottom habitat which is raised from the bottom offering higher levels of dissolved oxygen during hypoxic conditions.

Vocalization activity and Sciaenid-type egg presence was more prevalent near the river mouth, suggesting that spawning activity was centered in this area. Higher received levels at the hydrophone (dB re 1 $\mu$Pa 1- 1000 Hz, Bandwidth, P-P, as an indicator of vocalizing red drum presence) were found at sites containing greater than 2.5 mg/l dissolved oxygen in bottom waters. Egg presence was also positively associated with bottom waters containing greater than 3 mg/l dissolved oxygen. This evidence suggests that hypoxia may limit available spawning sites in the NRE (Barrios 2004).

Results from the 2004 field season (A.B. Beckwith, unpublished data) indicate that water quality conditions did not play a role in limiting available spawning sites in the NRE. The water column was atypically mixed beginning in July, which elongated the spawning season by allowing for an earlier than usual first spawn (as indicated by persistent courtship vocalizations). Data indicates that the poor water quality condition during the 2003 field season (stratified water column/low salinities) concentrated drum aggregations near the mouth of the Neuse River, where large groups of fish were recorded and numerous eggs were collected (Barrios 2004). During the 2004 field season (drier/mixed water column/elevated salinities) the spawning area extended upriver to Oriental. This increase in suitable spawning habitat was due to elevated salinities (compared to 2003) and increased dissolved oxygen levels throughout the water column extending upriver to Oriental. No sampling was conducted further upriver than Oriental. Red drum aggregations were detected often but measured intensity of vocalizations were lower than the previous season. Typically during the 2004 season individual males could be identified within the recordings, whereas, this was routinely impossible during the 2003 field season. Having fish spread out over a larger area caused reduced amounts of eggs and larvae to be collected due to lower concentrations as compared to 2003.

*Current Study Objectives*

The funding of this research was of critical importance for the understanding of red drum reproductive life history and identification of critical habitat for the species in North Carolina. The 2005 field season assisted in establishing a baseline from which conservation actions can be more meaningfully discussed during the revision of the current Fisheries Management Plan.

The previous field seasons were supported through the National Marine Fisheries Service MARFIN program, which made available the required research equipment. The 2003 and 2004 field seasons have shown that while red drum are understood to spawn within the NRE, distribution of spawning efforts and the nature of courtship vocalizations can be highly variable between years. The 2005 field season sought to define critical habitat for red drum spawning by establishing a baseline of behavior and habitat utilization. Data has clearly shown that predominant wind patterns, stratification of the water column and salinity and dissolved oxygen levels below the pycnocline dramatically impact site choice and intensity of vocalizations for red drum in the NRE (Barrios, 2004; unpublished 2004 field season data). The third season of data allowed a more accurate predictive model of red drum critical habitat that describes courtship vocalizations and habitat utilization (as it pertains to depth, area of river, habitat features, and water column conditions). This model can be used to concentrate future efforts to measure the abundance and health of the spawning stock in the most likely "hot spots" based on ambient and water column conditions.

The second focus of the study was to understand the seasonal persistence of spawning site selection by red drum aggregations once ambient and water column conditions have defined critical habitat for that spawning season. Previous efforts have clearly shown that aggregations are relatively stationary during a night's spawning activity (Tables 16 - 19), but our study design was not developed to address whether the species might "favor" specific locations and revisit them throughout the spawning season. Field efforts sought to determine if spawning site selection is random within "preferred habitats" (defined particularly by depth and bottom type) and water quality conditions (e.g. elevated salinities and dissolved oxygen levels of bottom waters), or if there are specific conditions/locations within more commonly utilized areas that attract aggregations most consistently.

It seems plausible that red drum might cue toward specific habitats (e.g. hard bottom sites), and this issue is very important when defining critical habitat for the species. Numerous studies have described a positive association of fish species richness and oyster reef habitat (Nesterode at al.1998; Coen et al. 1999; Harding and Mann 1999). Although no work has been done to relate this habitat type to spawning of red drum, local recreational and commercial watermen suggest large concentrations of drum frequent hard bottom habitat throughout the spawning season. Attraction of red drum to hard bottom as an aggregating location may be due to favorable foraging opportunities prior to sunset. Oyster reefs have been shown to have higher food availability and a wider diversity of food types (including fish, shrimp and crabs, which red drum consume) because of increased habitat heterogeneity relative to other habitat types such as mud flats (Harding and Mann 2001; Lenihan et al. 2001). Hard bottom may also offer a beneficial acoustic environment for their courtship calls (reflective bottom), or simply because it is different from the surrounding expansive mud bottom and thus may serve as a prominent bathymetric feature to which individuals can be drawn, as has been shown with other marine finfish (Luckhurst and Luckhurst 1978; Sale et al. 1984; Schroeder 1987; Doherty and Williams 1988; Bohnsack et al. 1991).

In the two previous research seasons it was not possible to directly determine if hard bottom sites were utilized at greater or equal rates to available soft bottom habitat. The ability to establish hard bottom as critical habitat for red drum spawning would make an important contribution to the Fisheries Management Plan review. Establishing the link would make increasing efforts for deep-water oyster reef restoration an appropriate conservation tool.

## Methods

*Acoustic Sampling*

The sampling area extended from the mouth of the Neuse River to the village of Oriental, NC, which is approximately 21 km straight-line distance. Five digital submersible autonomous sonobuoys previously used during the 2003 and 2004 sampling seasons were utilized during the 2005 field season (Figures 1a,b,c,d). The sonobuoys facilitated sampling by: 1) reducing post processing of recordings and archiving better quality recordings by completing the analog to digital conversion in real time, 2) allowing synoptic sampling over multiple sampling stations during a given night, and 3) reducing incidence of noise associated with the presence of boats at the sampling site.

Each sonobuoy contained a PocketPC (HP iPAQ Model 5455, Figure 1c) running an embedded Visual Basic program to control interval recording over the sunset period. The PocketPC recorded directly into an uncompressed *.wav file format (single channel, 44.1 kHz sampling rate, 16 bit resolution) and was connected to a Shure FP-23 amplifier (adjustable gain in 4 dB increments), which was connected to a HTI-96 min hydrophone (omni directional with sensitivity –164 dB re: 1V/μPa) sealed into the bottom of the submersible housing (Figures 1b, 1c).

The PocketPC (Figure 1c) was programmed to record for 5 minutes during each of 10 recording intervals: sunset – 1:00, sunset – 0:30, sunset, sunset + 0:30, sunset + 1:05, sunset + 1:40, sunset + 2:15, sunset + 2:50, sunset + 3:25, and sunset + 4:00. Pre-sunset recording at each location were used as an indication of background noise level during a given night. Gain setting of the amplifiers were maintained at a constant level throughout the season to allow comparison among recordings. Equipment was paired (same hydrophone, amplifier and PocketPC consistently used together) and calibrated to give the most accurate measure of sound as received by the hydrophone at each location. Sonobuoys were placed at a sampling location 1-2 meters below the surface prior to sunset (Figure 1d), and retrieved the following morning. Sonobuoys were used to identify and record vocalizing fish by monitoring underwater sound over a given night at a specific location.

To characterize environmental conditions, salinity, temperature, and dissolved oxygen were measured at sampling locations using a YSI 600XLM Multi Parameter Water Quality Monitor that stored information onto a YSI 650 MDS display and data logger. Depth and spatial position (latitude and longitude) at each location was determined using the research vessel's depth finder and Global Positioning System.

Recordings were downloaded from the PocketPC using a compact flash memory card reader. Using the acoustic program Cool Edit Pro the recordings were then decimated from a 44,100 Hz sampling rate down to an 8,000 Hz sampling rate. This was done to reduce background noise and other sounds not in the frequency range of interest. Presence or absence of vocalizing male drum and additional species were confirmed both by listening to each recording, and by viewing sonograms that were low-pass filtered to remove acoustic energy above 1000 Hz. Field recordings were verified against known sound recording of vocalizing red drum provided by Dr. Joseph Luzcovich of East Carolina University.

*Acoustic Sampling Design*

The sampling period began 7 July 2005 and ended 9 October 2005. A sampling design was chosen that allowed researchers to address both identification of critical habitat and the persistence of habitat use. Two different types of sites were identified, fixed and rotating (Figures 2, 3). Sampling occurred 3 nights a week (depended on weather) for 13 weeks using 5 sonobuoys. Of 15 sampled sites per week, 8 were fixed sites and were sampled with first priority. The remaining 7 were rotating sites. The fixed sites were identified as "hot spots" for spawning activity based on 2003 and 2004 findings. Four of the 8 fixed sites were soft bottom substrate and 4 were hard bottom substrate. The fixed sites were located throughout the sampling area but each soft bottom fixed site was in relative close proximity to a hard bottom fixed site, but far enough away to be acoustically independent (Figure 3), and were considered paired. Rotating sites were sampled less frequently and were selected at random with replacement from 2003/2004 sampled locations.

*Egg Sampling*

Synoptic egg sampling was conducted in association with the fixed sampling sites to confirm that vocalizations by red drum in the "hot spots" were associated with spawning activity. Consistent egg sampling effort was not possible due to wind conditions. A Bongo net (60 cm diameter, 333 μm mesh) was deployed off the research vessel and towed for 3 minutes downwind of the acoustic sampling site near the surface and at mid-water column (hereafter referred to as "at depth"). For this report, we simply document whether Sciaenid type eggs were present at the sampling site. Collections were made at two to three sites per night when wind and wave conditions allowed. Neuston collections were preserved immediately in 70 % ethanol. Sorting and enumeration of eggs and larvae were conducted after the field season. We enumerated eggs that fit the description of red drum eggs (spherical 0.86 – 1 mm in diameter containing one oil globule, Holt et al. 1981). Sampled eggs fitting this description have been sent to Smithsonian Environmental Research Center for DNA confirmation. We are still awaiting results of this laboratory analysis. At stations where both acoustic and neuston sampling was conducted all vocal species were identified in recordings to assess possible spawning contributions from species other than red drum.

*Measured Variable And Statistical Models*

Measured variables included: (1) presence or absence of vocalizing male drum, (2) received level of acoustic energy at the hydrophone (dB re 1 μPa, 1 – 1000 Hz, Bandwidth, P-P) integrated over a 60 second recording period chosen at random and 3) presence or absence of Sciaenid type eggs. It is important to recognize assumptions made about the acoustic variables. The ability to detect a vocalizing drum, and the received level of acoustic energy at the hydrophone as an indicator of red drum aggregations can be affected by background noise, presence of other species, varying distances of aggregations or individuals to the hydrophone, and absorptive and scattering characteristics of the environment due to substrate type. Received level does not allow us to gauge aggregation size because sound level from a larger aggregation further away may be similar to that from a few individuals closer to the hydrophone. Since only males vocalize, contributing to the received level, it is not possible to determine total numbers of fish in an aggregation using this method. It is also impossible to identify vocalizations from individual red drum once there are more than a few fish vocalizing simultaneously. Although received level does not allow us to gauge the size of a given aggregation, it offers information on habitat use by the species.

We addressed three explicit research hypotheses based on our data, and conducted an exploration of the data to investigate how certain environmental variables may be influencing male courtship vocalizations in the NRE.

Our first hypothesis was to determine if there is a significant relationship with our measured response variables (characteristics of the male courtship vocalizations) to successful spawning activity as determined by the presence of Sciaenid type eggs in the water column at the time the recordings were made. To test for the association of vocalizations and egg presence, we used two statistical models. A Pearson's chi-square test was applied using EP (egg presence, binary response variable) and PA (presence of vocalizations, defined below). We used a generalized additive model (GAM) to test for association of EP with VP (vocalization prevalence, defined below).

Our second research hypothesis was to determine if there is a high degree of persistence at a give site through the entire spawning season. To determine the degree of persistence at each of our fixed sites, we fit the seasonal trend of VP (vocalization prevalence, defined below) at each of our fixed sites in 2005 separately with a non-linear loess curve. If vocalizations were persistent, we would expect the trend to be increasing over time. We note any cases where no vocalizations were detected in a given week following a positive detection the week prior. We would expect this situation to be rare if vocalizations at sites are highly persistent over the season.

Our third research hypothesis was to determine if vocalizations were more prevalent at hard bottom sites, which would suggest these fish are actively selecting hard bottom sites in the NRE. To test for the effect of substrate type on presence or intensity of drum vocalizations, we analyzed the "fixed sites" during 2005 (131, 172, 129, 120, 78R, 76R, 12R, 16R) using ANOVA. The model included either PA or VP as response variables, and WEEK and BOTTOM as independent variables.

The remainder of this section all relates to the analysis of the data collected from our rotating sites to explore relationships between vocalizations and environmental variables. The 2005 data collected from the rotating sites was considered collectively with the 2003/2004 data. The intent was to establish a baseline of spawning distribution and to develop a predictive model of critical habitat based on habitat features and ambient water quality conditions. To accomplish this we applied a statistical approach to the acoustic data involving 3 separate models.

The first model considered vocalizing males as either present or absent in each recording (assigning values of 1 or 0, respectively), and a binomial error was assumed (measured variable PA = probability of presence or absence of red drum vocalizations in a recording, hereafter referred to as PA model). For sonobuoys, multiple observations at a given sampling location over a night were reduced to one presence or absence score by picking one recording interval at random and using that observation. Only post sunset recordings were used because pre sunset recordings were considered a control to assess background noise levels at each site each night.

For the second model, I calculated the percentage of recordings that contained male vocalizations over the sampling night (e.g., if vocalizations were present in 4 out of 7 recording intervals, the vocalization prevalence would be scored at 57 %). A Gaussian error distribution was assumed (measured variable VP = vocalization prevalence as a percentage of all recording intervals, hereafter referred to as VP model).

In the third model received level at the hydrophone (dB re 1 μPa, 1 – 1000 Hz Bandwidth, P-P) was calculated for each recording interval. The highest received level from all recording intervals for that site was subtracted from the background noise level (considered to be the pre sunset recording). This received level was used as the measured variable. A Gaussian error distribution was assumed (measured variable RL = Highest received level minus background noise level, hereafter referred to as the RL model). Under the assumption that the form of the relationships between the measured variables and the explanatory variables in this analysis could be non-linear, I fitted each data set to a generalized additive model (GAM) of the form:

$$PA = \mu + s(LON) + s(LAT) + s(DEPTH) + s(DOY) + s(YR) + s(TCOL) + s(SCOL) + s(DOCOL) + s(DOPYCNO) + s(DEPTHPYCNO) + BOTTOM$$

(PA model),

$$VP = \mu + s(LON) + s(LAT) + s(DEPTH) + s(DOY) + s\ (YR) + s(TCOL) + s(SCOL) + s(DOCOL) + s(DOPYCNO) + s(DEPTHPYCNO) + BOTTOM$$
(VP model),

$$RL = \mu + + s(LON) + s(LAT) + s(DEPTH) + s(DOY) + s\ (YR) + s(TCOL) + s(SCOL) + s(DOCOL) + s(DOPYCNO) + s(DEPTHPYCNO) + BOTTOM$$
(RL model),

where $\mu$ = mean response, LON = Longitude, LAT = Latitude, DEPTH = depth in meters at each sampling location, DOY = day of year, YR = Year, TCOL = the average temperature the water column measured at each location each sampling night, SCOL = the average salinity of the water column measured at each location each sampling night, DOCOL = the average dissolved oxygen of the water column measured at each location each sampling night, DOPYCNO = the average of the dissolved oxygen measurements taken below the pycnocline at each location each sampling night, DEPTHPYCNO = depth in meters from the pycnocline to the substrate, BOTTOM = a binomial factor indicating hard substrate or soft substrate (1 or 0 respectively). The 's' term in the model refers to a spline smoother. Variables entered into the model either as a linear term (df = 1) or as a non-linear, non-parametric term (with estimated degrees of freedom, or edf). A reduced model was fit using only those variables that were significant based on the p-values estimated in the full model. Only those variables where the p-value was below 0.05 were included in the reduced model.

I provide estimates of the contribution of each predictor to the overall model fit using a *t*-ratio statistical inference (similar to the approach in generalized linear models) and an estimate of the nonlinear effect for each of the continuous explanatory variables using a nonparametric *Chisq* or *F* ratio test statistic. Further details can be found in Hastie & Tibshirani (1990). All statistical analyses were conducted using R.

## Results

### *Physical Environment*

From July–September 2005 the average depth of the pycnocline in the lower NRE for shallow, medium, deep-soft bottom, and deep-hard bottom sites was 1.57 m, 3.4 m, 4.66 m, 4.01 m, respectively (Table 9). Temperatures averaged between 26 – 29.5 °C in all months throughout the sampling area (Table 6). Well mixed conditions were typical throughout the season at all site types. There were relatively few instances of hypoxic condition either throughout the water column or below the pycnocline (Tables 6, 9). When comparing fixed and rotating sites, the fixed sites were less likely to be hypoxic in all months sampled (Tables 11 - 12). Fixed deep-soft bottom sites also had consistently higher dissolved oxygen conditions below the pycnocline than the deep-soft bottom rotating sites (Tables 12 - 13). The deep-hard bottom sites in 2005 regardless of site category (fixed or rotating) had relatively high levels of dissolved oxygen below the pycnocline (Tables 12 - 13). It appears from the data that red drum were found present at sites that offered reduced probability of hypoxic conditions (Tables 14 - 15).

### *Hydrophone Recordings*

On 34 dates between July and October 2005, we made hydrophone observations at 141 locations throughout the NRE in an attempt to locate and describe spawning sites for red drum. All sites were sampled with sonobuoys (Figures 1a, b, c, d). Vocalizing drum were detected at one site each on 7/19/05, 7/22/05, and 7/25/05, then consistently after 8/1/05. Red drum vocalizations were last detected on 10/03/05. Sampling occurred between 7 July 2005 and 9 October 2005. Red drum vocalizations were detected most often in August and September (Table 1). During observations which covered the spawning season, vocalizing red drum were detected in at least one recording interval a total of 84 (of 141) times (including both fixed and rotating sites). Red drum vocalizations were detected in at least one interval recording at fixed sites 47 (of 79) times, and at rotating sites 37 (of 62) times.

Vocalization activity was typically recorded during four periods beginning at sunset (as was observed in 2003 and 2004, Tables 16 - 19); however, vocalizations were occasionally detected 30 minutes prior to sunset (5 of 136 times, Tables 18 - 19). The probability of first detecting vocalizing drum at a location is typically highest at sunset, and the recording interval following sunset. Following first detection of vocalizations is has been confirmed over three field seasons that vocalizations are very persistent at a given site, with high probabilities of detection in all recordings following the initial detection with the exception of the final recording (typically occurring near midnight), when detection is less likely.

*Eggs Sampling*

Neuston tows were conducted at fixed sites on 19 days between 7 July 2005 and 2 October 2005 in association with acoustic sampling. Eggs matching the description of red drum were collected on 3 nights at a total of 5 sites were red drum presence was confirmed (Tables 2- 3). Three sites did have weakfish, *Cynoscion regalis,* present, although red drum were the vocally dominant species. Eggs fitting the description of red drum eggs (spherical 0.86 – 1 mm in diameter containing one oil globule, Holt et al. 1981) were sent to Smithsonian Environmental Research Center for DNA confirmation but results will not be available until a later date. DNA results will be added to the report upon their arrival.

*Statistical Results*

**First Hypothesis:** Vocalizations are associated with the presence of sciaenid-type eggs (i.e. vocalizations serve as a proxy for detecting active spawning activity)?

We pooled data for the three years of observations (2003-05), and considered whether presence/absence of vocalization (PA) and vocalization prevalence (VP) could serve as proxies for active spawning activity in the study region. For the former, we applied a chi-square test to examine the significance of association between vocalizations and presence of eggs. This association was shown to be significant (X-squared = 12.5344, df =1, p = 0.0004). We used a generalized additive model (GAM) to test for a significant relationship between egg presence and vocalization prevalence. This was also found to be significant (p = 0.002). We did not find an association with received level (RL) and presence of eggs.

**Second Hypothesis:** Once detected, vocalizations are persistent at a given site over the season.

Once detected, all of our fixed sample sites showed strong evidence of persistence over the season. All showed a clear pattern of increasing VP from ~ Day 200 to Day 260, as indicated in the loess fits to the data (Figure 4). This pattern was broken only on three separate occasions (two on hard bottom sites, one on a soft bottom site), where no vocalizations were detecting during a nocturnal interval following a positive detection the week prior (Site 12R, 16R and 129).

**Third Hypothesis:** Vocalizations are more common at sites with hard substrate compared to soft bottom sites.

We found bottom type was not a significant factor in explaining vocalizations (both PA and VP) among the fixed sites in 2005. In an ANOVA with WEEK and BOTTOM as independent variables, we found WEEK to be a significant factor ($p = <0.001$ for both PA and VP), whereas BOTTOM was insignificant ($p = 0.55$ for PA and $p = 0.67$ for VP).

**Data Exploration:** What environmental factors appear to be significantly related to vocalizations (and, by association, spawning activity)?

Environmental conditions in the NRE have varied considerably during 2003-2005. The 2005 field season was marked with the highest water temperatures, intermediate salinities, and the lowest incidence of bottom water hypoxia (Figure 5). The year 2004 was marked with a drought, leading to significantly higher salinities, while 2003 exhibited the highest degree of bottom water hypoxia (Figure 5). This range of conditions allowed us to explore important relationships between patterns of red drum vocalizations and inter-annual habitat changes.

During 2003-2005, the presence of drum vocalizations were found to be related to DEPTH ($p=0.008$), DOY ($p<0.001$), YR ($p<0.001$) and SCOL ($p<0.001$, Figure 6). We did not find any relationship between the variables associated with dissolved oxygen in the water column (DCOL, DOPYCNO, DEPTHPYCNO). While we have documented the importance of depth and day of year in our previous work (Barrios, 2004), here we report for the first time a year effect (with 2004 exhibiting the highest presence of vocalizing drum), and the influence of salinity (a positive, linear relationship, Figure 6). This model explained 48% of the deviance ($N = 313$).

We found similar relationships of these environmental parameters to VP. We found VP was significantly related to DOY ($p<0.001$), YR ($p=0.02$), and SCOL ($p<0.001$, Figure 7). As above, we did not find any significant relationship between VP and the variables involving the level of dissolved oxygen (DOCOL, DOPYCNO, DEPTHPYCNO). For this model, we found DEPTH to be marginally significant ($p = 0.08$). This model explained 57.2% of the deviance ($N = 228$).

Results for our analysis of RL differed somewhat from the other models. As above, we did not find any significant relationship with the variables associated with dissolved oxygen (DOCOL, DOPYCNO, DEPTHPYCNO). As above, we did find a significant effect of YR ($p<0.001$) and DOY ($p<0.001$). With respect to DOY, there appears to be two periods during the season where peaks in low frequency sound intensity occur, and they correspond with the full moon phases during the years of study (~DOY 230, mid to late August, and ~DOY 260, mid to late September). We also observed a trend with high RL in the northern part of the study area (LAT, $p = 0.02$) and during periods of warmer water temperature (TCOL, $p = 0.005$). We did not find a relationship of RL to salinity. This model explained 39% of the deviance (N = 214).

## Discussion

*Persistence of Habitat Use*

Data collected from 2003 – 2005 have all indicated that red drum are stationary once courtship vocalizations have begun (Tables 16 – 19; Barrios, 2004; unpublished data). The fixed locations sampled during the 2005 season showed both a nightly persistence of use once vocalizations began and a seasonal pattern of use with aggregation forming at these locations throughout the sampling season. The averaged ambient conditions between 2005 fixed and rotating sites were not highly variable (Table 10 – 13). When averaged over the season or by site type fixed sites had similar salinities, temperatures and dissolved oxygen values as the rotating sites. Fixed sites, on average, did have a lower incidence of hypoxic conditions below the pycnocline. During the 2003 field season hypoxic conditions were common (Tables 4, 7) and evidence suggested a limiting of available spawning habitat due to these conditions (Barrios, 2004). During the 2004 and 2005 field seasons dissolved oxygen conditions were higher and the incidence of hypoxia were lower than 2003, indicating that dissolved oxygen conditions were not a limiting factor for spawning success in either of these years. This could help explain why dissolved oxygen did not enter in our models as a significant factor explaining variability in courtship vocalizations for the species.

*Substrate Affinity*

During the 2005 season substrate type was not determined to be a significant factor when predicting probability of drum presence. Hard bottom sites are typically raised off the bottom (less then 1 m). In years were hypoxic conditions are typical (as in 2003), we believe that hard bottom sites may offer a more attractive aggregating location than the surrounding expansive soft bottom due to its increased relief providing a normoxic environment in an otherwise hypoxic benthic habitat. In 2003 an earlier "peak" (highest received levels) was noted on hard bottom sites as compared to soft bottom sites (Barrios, 2004). This earlier "peak" time suggest these fish were selecting this normoxic benthic habitat over other areas dominated by hypoxic conditions. These sites may support higher prey biomass, and thus serves to attract foraging red drum.

*Role of Salinity*

Through this research a clear pattern seems to be emerging, with salinity functioning as a driver of "spawning activity". Low salinity and extensive hypoxia, which are coupled, can limit available spawning habitat. The MARFIN research that made available all the research equipment for this study will allow the researchers to further explore this question via otolith microchemistry results. In 2003 eggs in the surface waters of the study appeared to be rare, while similar neuston sampling in 2004 yielding more eggs. This variation in success supports the positive role of higher salinities for spawning success. We intend to explore trend in egg catches over the years of the study to begin to explore questions about the magnitude of spawning/egg recruitment. This salinity issue may be a critical factor for more effective "seeding" of primary nursery habitat that line the Neuse River. It is possible that increased runoff and greater sustained hypoxic conditions in the lower Neuse River may "displace" red drum spawning eastward. This shift would potentially cause a separation of the spawning grounds from the nursery habitat. While eggs and larvae may still advect from spawning grounds to nursery habitat, the trip will be extended, thus exposing eggs and larvae to increased predation, or advection losses. Poorer water quality in those low salinity years could also result in much higher egg mortality. Further discussion of how all this may be tied to inter annual patterns of recruitment for red drum would help extend knowledge of life history patterns and assist in managing the fishery.

*Lunar Periodicity and Spawning*

Results from our analysis of RL found a significant effect of DOY. There appears to be two periods during the season where peaks in low frequency sound intensity occur, and they correspond with the full moon phases during the years of study (~DOY 230, mid to late August, and ~DOY 260, mid to late September). A full moon brings stronger tides resulting in increased egg and larvae dispersal distances. Behavior that synchronizes gamete release during these periods of stronger tidal flux is understood to be an important fitness component of populations inhabiting the coastal shelf environment. There does not appear to be any clear explanation of why this trait might be retained in the NRE spawning population, where egg viability and dispersal appears to be strongly influenced by the direction and intensity if wind events. Perhaps red drum in the NRE are linked to shelf meta populations, and the resulting gene flow is sufficient for the trait to be retained across the larger, regional population of red drum. Even without the benefit of increased egg and larval dispersal by tidal fluxes, synchronous behavior assures a large number of fish are ready to spawn at the same time, and can offer anti-predatory benefits.

## Impact and Benefits

This research confirms that passive acoustic monitoring techniques offer a viable and non-invasive method to locate spawning aggregation, describe diel and seasonal spawning patterns, and determine affinities of spawning fish to particular habitats. This represents a significant contribution in understanding red drum spawning life history and describing habitat characteristics of spawning grounds that will benefit management of red drum in the future. Utilization of this passive acoustic method for delineating spawning aggregations, identifying "hot spots" and predicting habitat utilization based on ambient condition can assist in future attempts at quantifying abundance of the spawning stock and development of an assessment method to meet the needs of future conservation efforts aimed at red drum, and perhaps other related species.

The 2005 field season has made possible the establishment of a solid baseline of behavior and habitat utilization by red drum spawning aggregations in the NRE and allowed for a predictive model to be created that can be utilized to identify critical habitat based on ambient conditions. This study has been able to determine that aggregating sites do have unique properties with respect to the larger sampled area. Understanding the persistence of site selection by spawning red drum has increased the knowledge of spawning life history for red drum, and provided a more detailed description of the spatial extent and habitat characteristics of the spawning grounds of the NRE. The development of this predictive model of behavior and distribution, as well as the detailed information on the persistence of habitat use can be used as the basis for a sustained effort to monitor spawning distribution and health as well as an effort to measure (at least in the NRE) the abundance of the adult stock.

Results of this multi-year effort are particularly timely considering work is just beginning on the revision of the current Fisheries Management Plan for red drum. In the event that seasonal or spatial closures of the fishery in the NRE become a necessary conservation tool in the future, a fuller understanding of the spawning behavior and spatial extent of habitat use will allow for more specific closures reducing local economic impact.

The sampling methods developed for this study are currently being used by Biscayne National Park in south Florida to monitor boat noise on and around sea grass beds. Stated goals of the effort include determining areas and times of highest vessel traffic to better distribute management and enforcement personal to reduce propeller damage to critical habitat. They will also monitor habitat utilization of sea grass beds by vocal species.

## Extension of Results

Anna and Captain Gorge Beckwith are strong supporters of efforts to conserve and rebuild the red drum stock. In the past three years interviews discussing conservation of the adult spawning stock for articles in the popular press include: North Carolina Sportsman, Tide, Coastal Watch, Saltwater Sportsman and Sports Fishing Magazine. We have filmed television shows on red drum for "Outdoor Moments" on the National Outdoor Network and the Carolina Outdoor Journal hosted by Joe Albae. Captain George Beckwith has given educational seminars to dozens of recreational fishing clubs and schools throughout the state. In each of these instances we have discussed red drum life history and the need to use appropriate gear to reduce impacts on the adult spawning fish. In addition to these efforts, George Beckwith and Dr. Peter Rand have developed fact sheets and educational materials produced through Sea Grant on responsible angling, and have contributed to an educational video on North Carolina red drum developed through Hitchcock Productions. George Beckwith and Dr. Peter Rand have published an article focusing on reducing post-release mortality and hooking trauma on adult fish through proper terminal gear (Beckwith and Rand, 2004). Dr. Rand and Anna Beckwith are preparing multiple articles for publication in scientific journals as well as the final report for 2002 - 2004 research supported through the National Marine Fisheries Service MARFIN Program. We will continue to actively seek out opportunities to disseminate our data to the recreational community, and intend to keep in close contact with Louis Daniel and Lee Paramore at the Division of Marine Fisheries. George Beckwith has been appointed to the advisory committee for the revision of the Fisheries Management Plan and Anna Beckwith will participate as a community member.

**Budget Summary and Disposition of Equipment**

No budget revisions occurred during this project. No individual items costing over $500 where purchased through this grant.

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Figure 1. Sonobuoy Equipment.

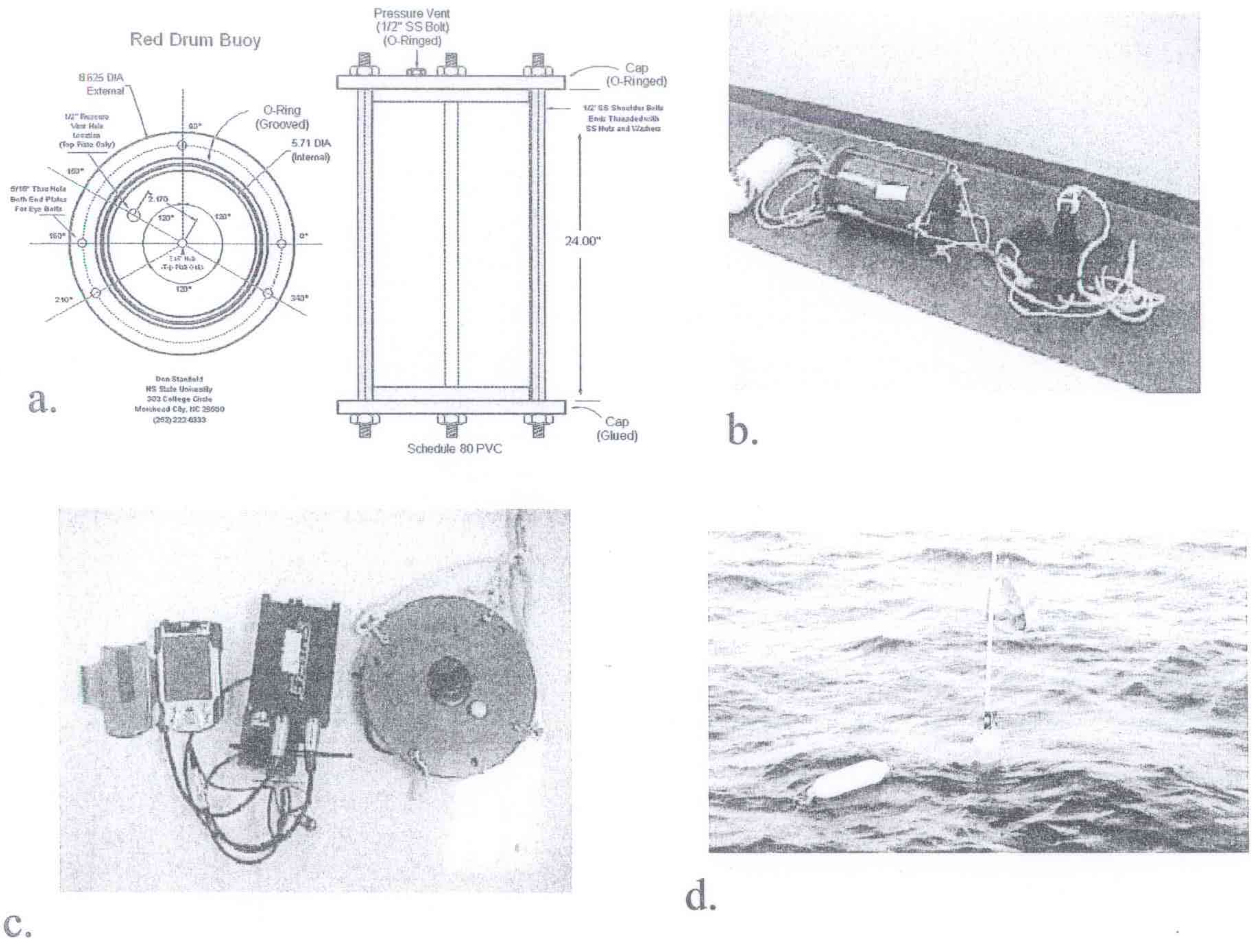


Figure 2. Fixed and Rotating 2005 Sampling Sites.

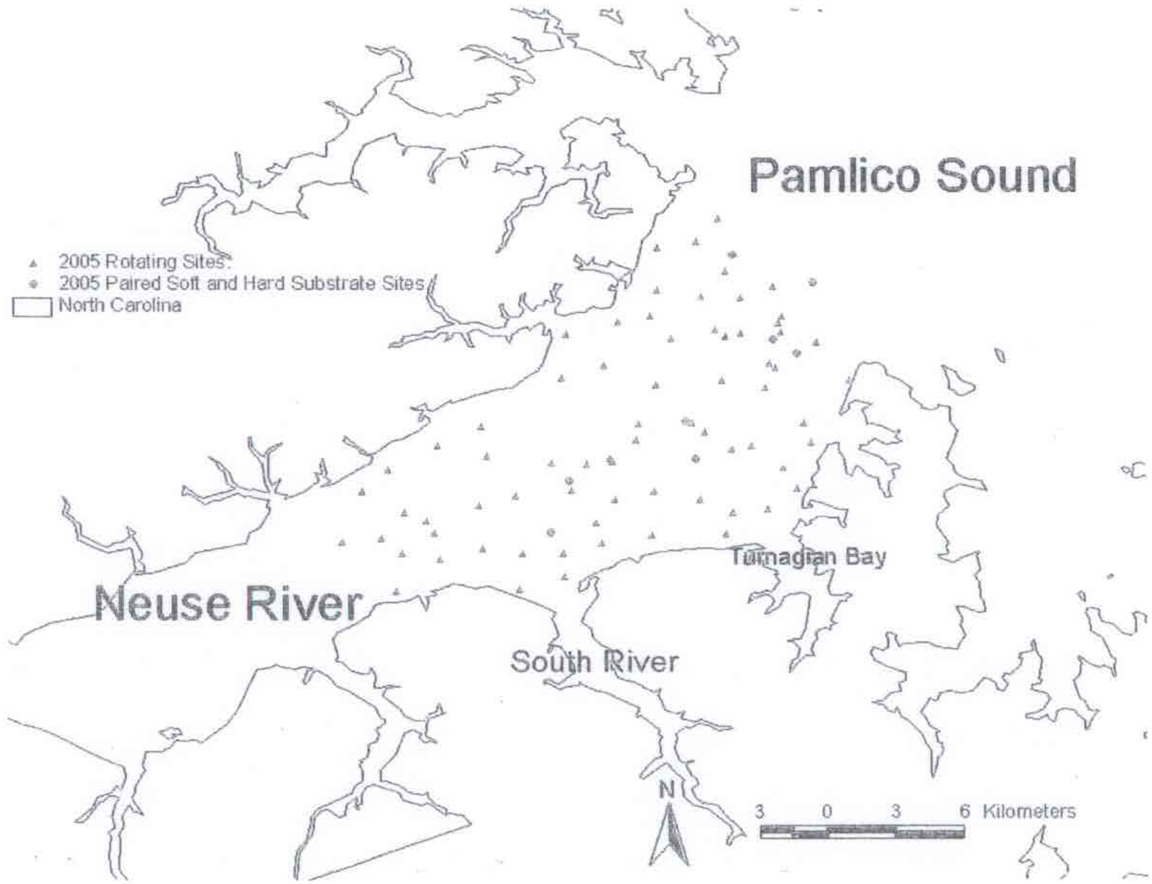


Figure 3. Fixed 2005 Sampling Sites.

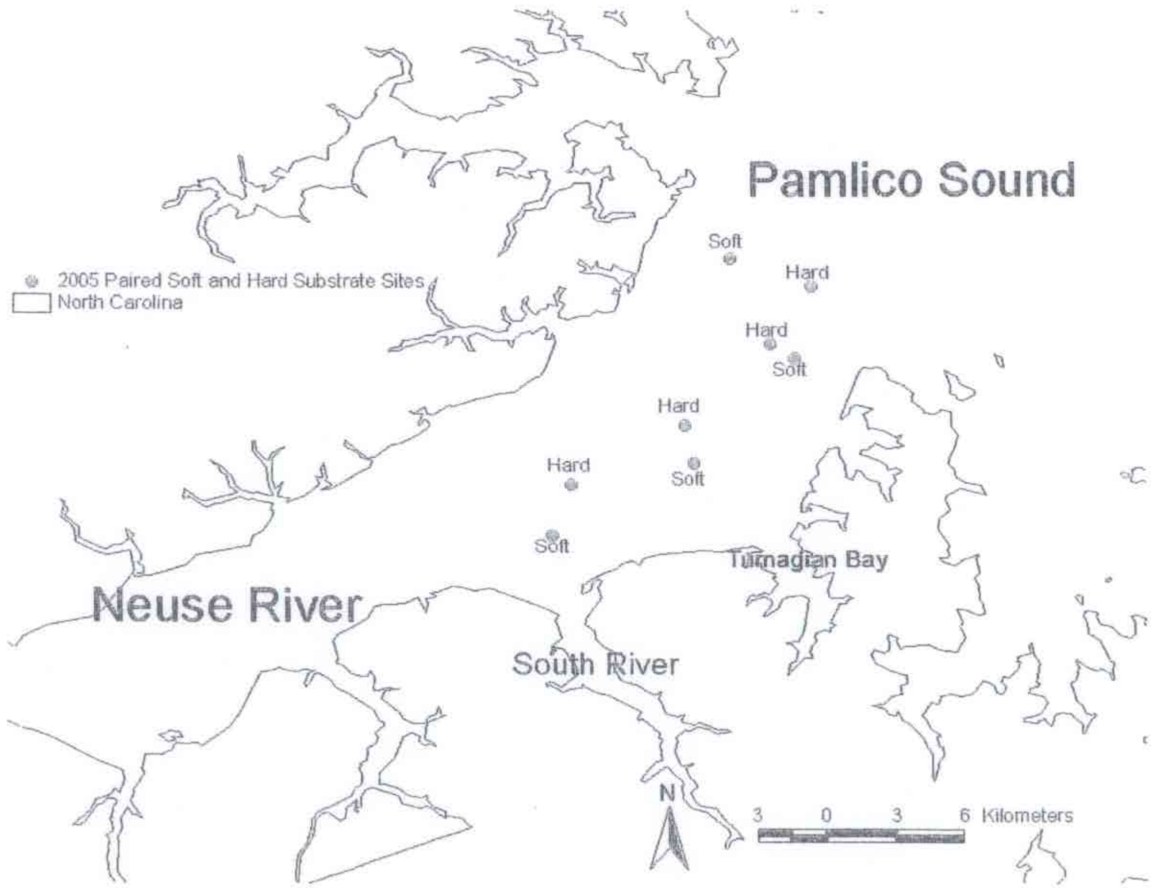
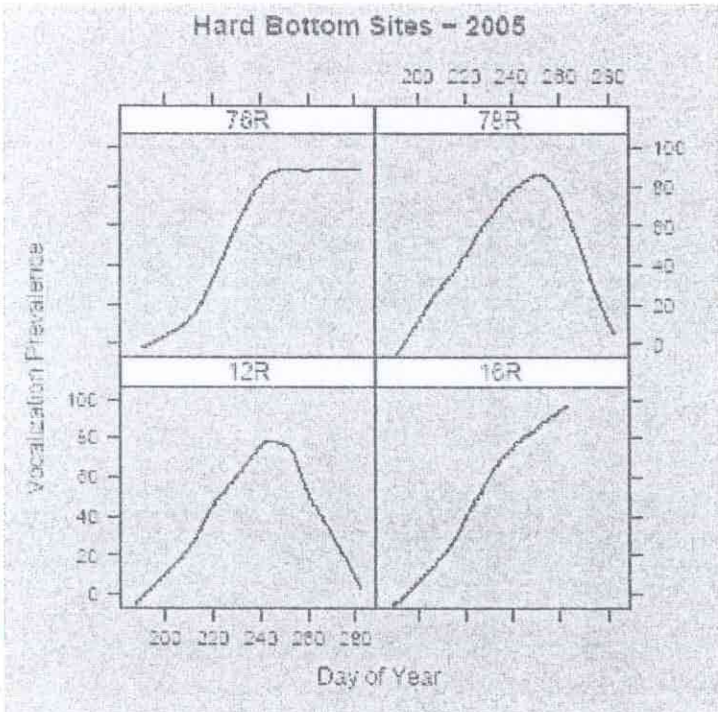
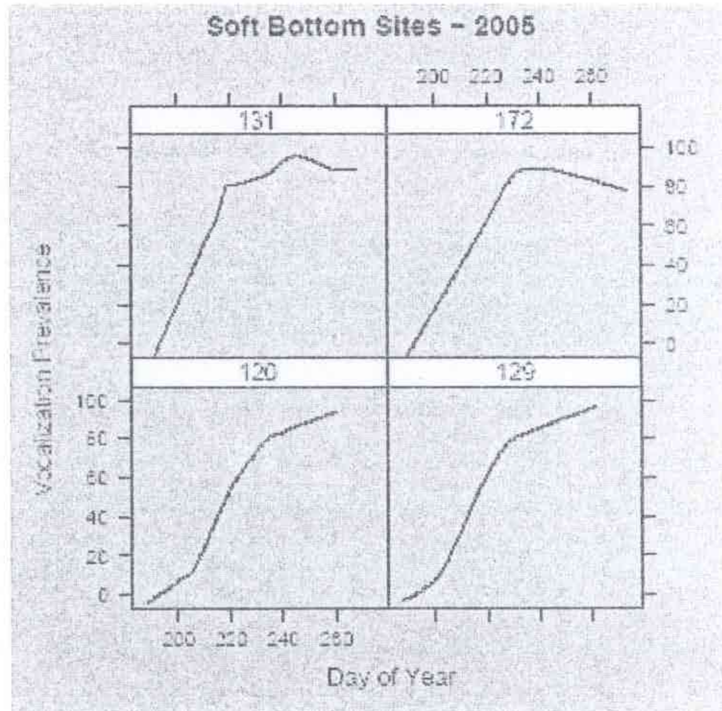


Figure 4. Estimates of red drum courthship vocalizations (VP, or vocalization prevalence, computed as the percentage of nocturnal recording intervals where courtship vocalizations were identified) over the course of the 2005 field season for each of the fixed sampling sites (station identified by number in banner on each graphlet). The data are presented separately for hard bottom sites (left panel) and soft bottom sites (right panel). Estimates of red drum courthship vocalizations (VP, or vocalization prevalence, computed as the percentage of nocturnal recording intervals where courtship vocalizations were identified) over the course of the 2005 field season for each of the fixed sampling sites (station identified by number in banner on each graphlet). The data are presented separately for hard bottom sites (left panel) and soft bottom sites (right panel).



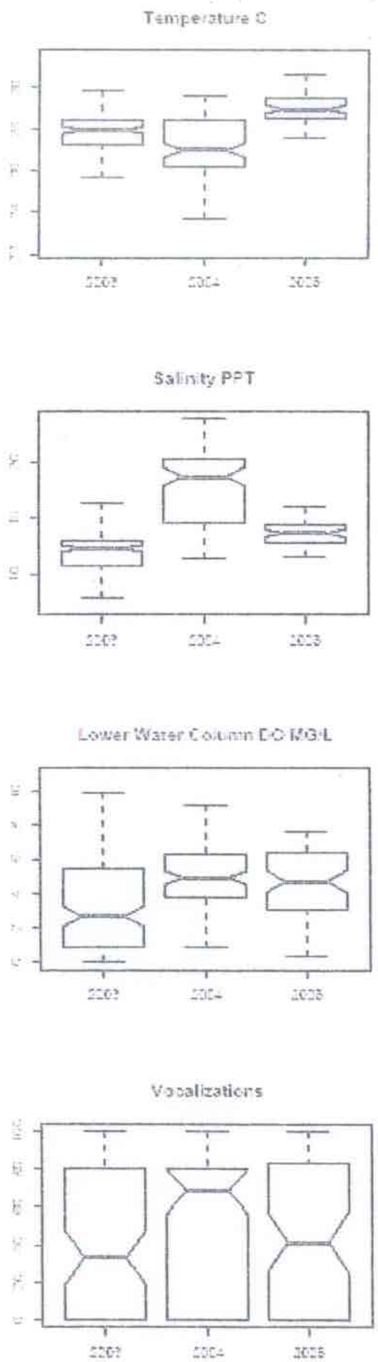
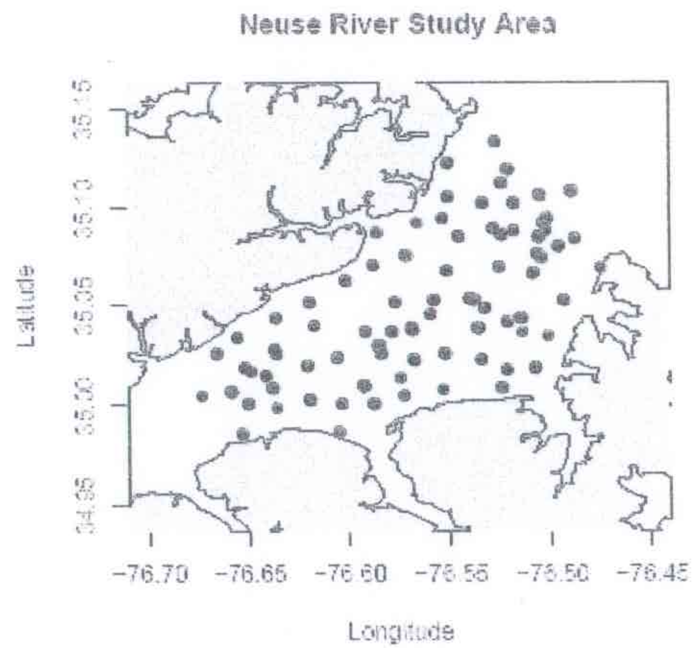



Figure 5. Box and whisker plots of water quality data (water temperature, salinity, lower water dissolved oxygen) and red drum courthship vocalizations (VP, or vocalization prevalence, computed as the percentage of nocturnal recording intervals where courtship vocalizations were identified). The sampling sites in the NRE where data were collected is shown in the right panel of the figure.



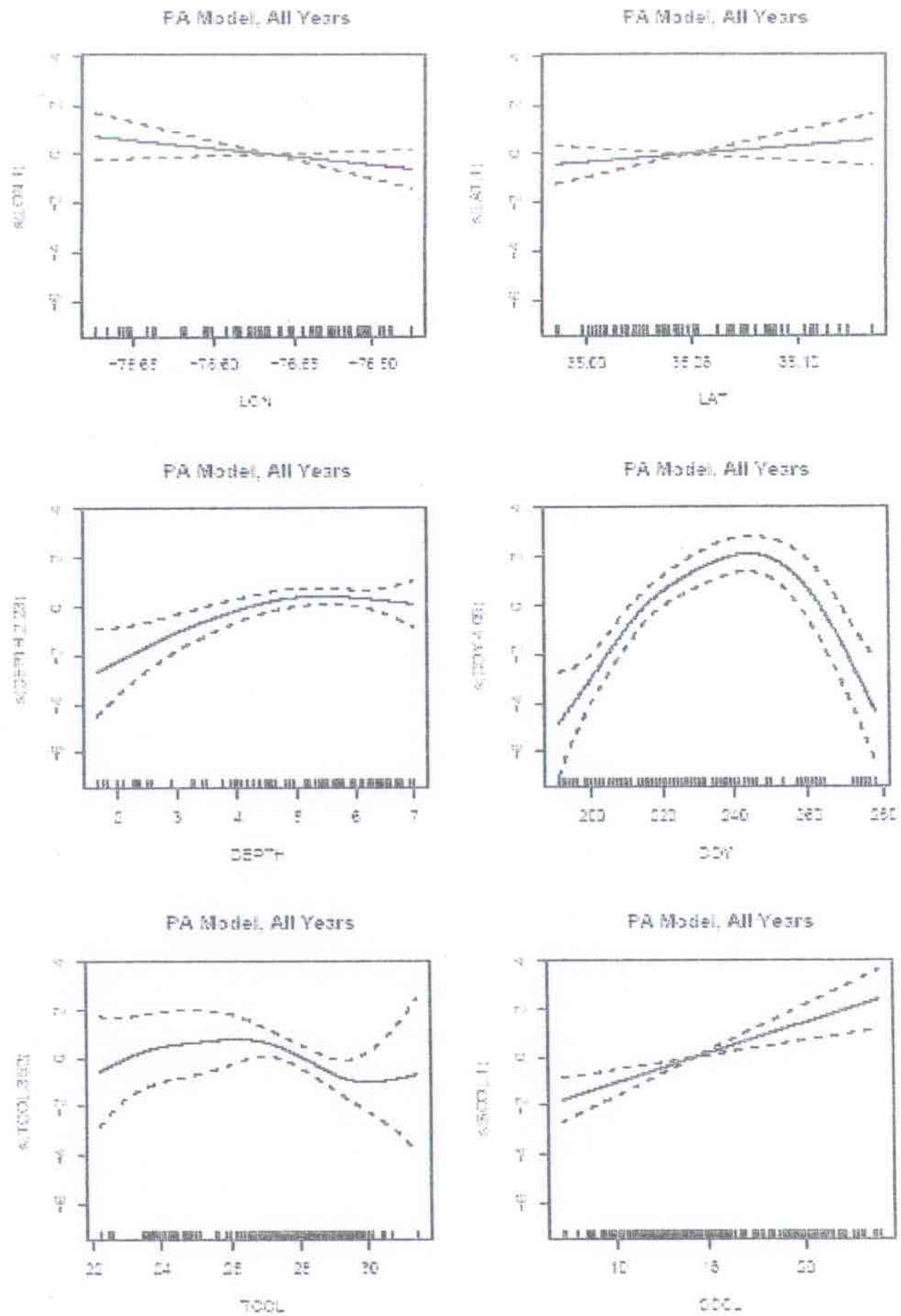
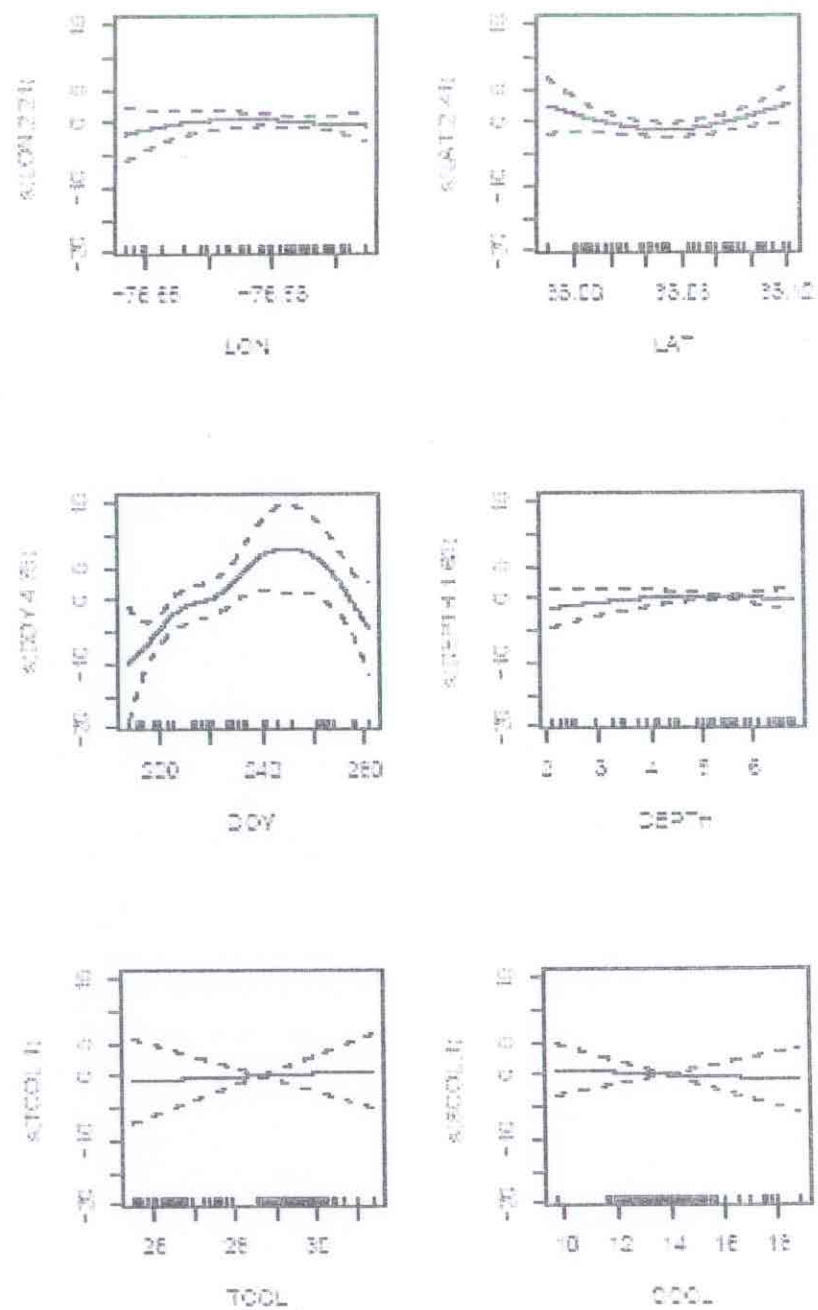



Figure 6. The transformed explanatory variables plotted as smoothed function of the original data values. The smooth fit was accomplished using a Generalized Additive Model explaining variability in the presence of male vocalizations at a given site during 2003-2005 (PA Model) The degrees of freedom applied to each explanatory variable is presented in the Y-axis label. LON = longitude, LAT = latitude, DEPTH = water depth at site, DOY = day of year, TCOL = water temperature, SCOL = salinity.

Figure 7. The transformed explanatory variables plotted as smoothed function of the original data values. The smooth fit was accomplished using a Generalized Additive Model explaining variability in the presence of male vocalizations at a given site during 2005 (PA Model) The degrees of freedom applied to each explanatory variable is presented in the Y-axis label. LON = longitude, LAT = latitude, DEPTH = water depth at site, DOY = day of year, TCOL = water temperature, SCOL = salinity.



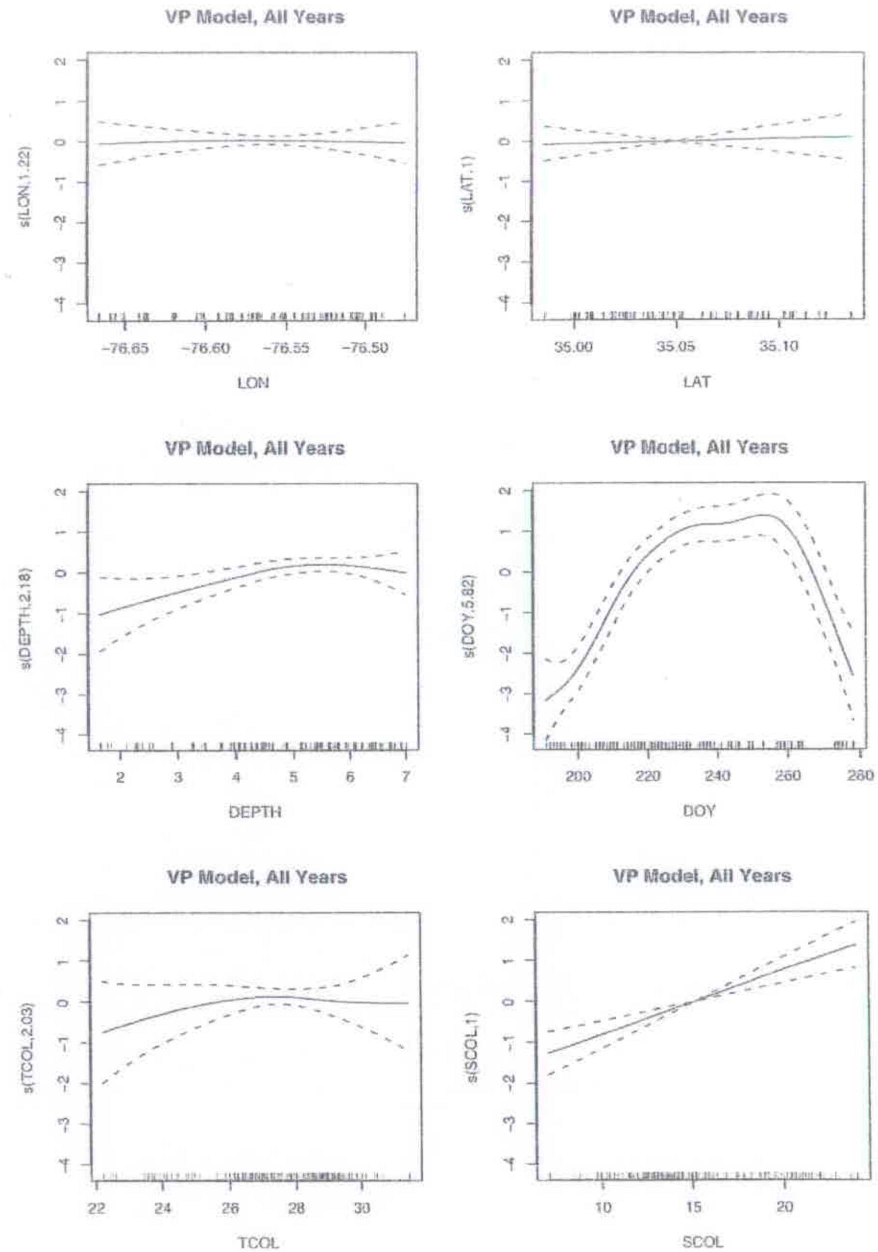



Figure 8. The transformed explanatory variables plotted as smoothed function of the original data values. The smooth fit was accomplished using a Generalized Additive Model explaining variability in the vocalization prevalence at a given site during 2003-2005 (VP Model) The degrees of freedom applied to each explanatory variable is presented in the Y-axis label. LON = longitude, LAT = latitude, DEPTH = water depth at site, DOY = day of year, TCOL = water temperature, SCOL = salinity

Figure 9. The transformed explanatory variables plotted as smoothed function of the original data values. The smooth fit was accomplished using a Generalized Additive Model explaining variability in the vocalization prevalence at a given site during 2005 (VP Model) The degrees of freedom applied to each explanatory variable is presented in the Y-axis label. LON = longitude, LAT = latitude, DEPTH = water depth at site, DOY = day of year, TCOL = water temperature, SCOL = salinity.

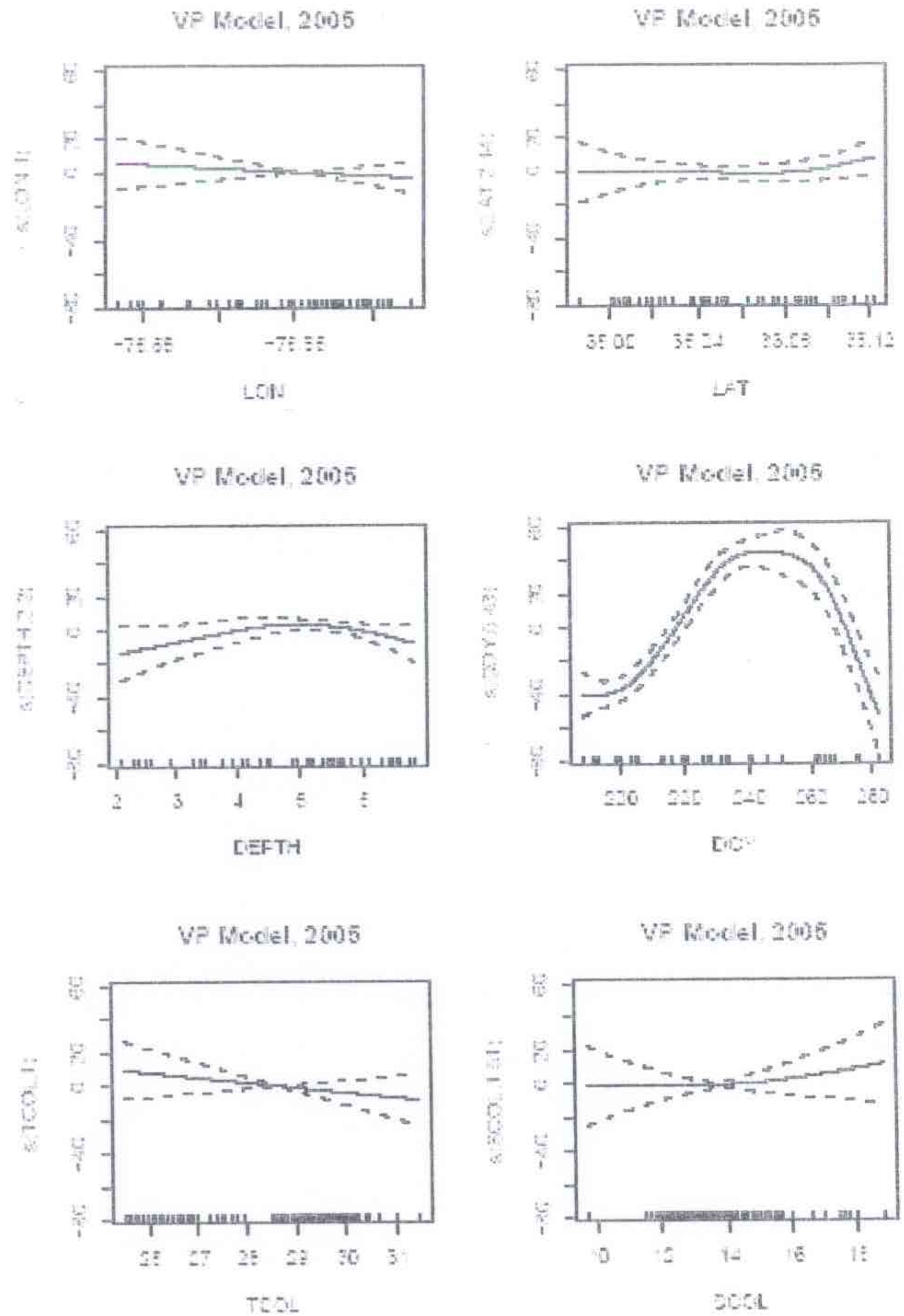


Figure 10. The transformed explanatory variables plotted as smoothed function of the original data values. The smooth fit was accomplished using a Generalized Additive Model explaining variability in the received level (dB) of low frequency sounds in the range of red drum courtship vocalizations at a given site during 2003-2005 (RL Model) The degrees of freedom applied to each explanatory variable is presented in the Y-axis label. LON = longitude, LAT = latitude, DEPTH = water depth at site, DOY = day of year, TCOL = water temperature, SCOL = salinity.

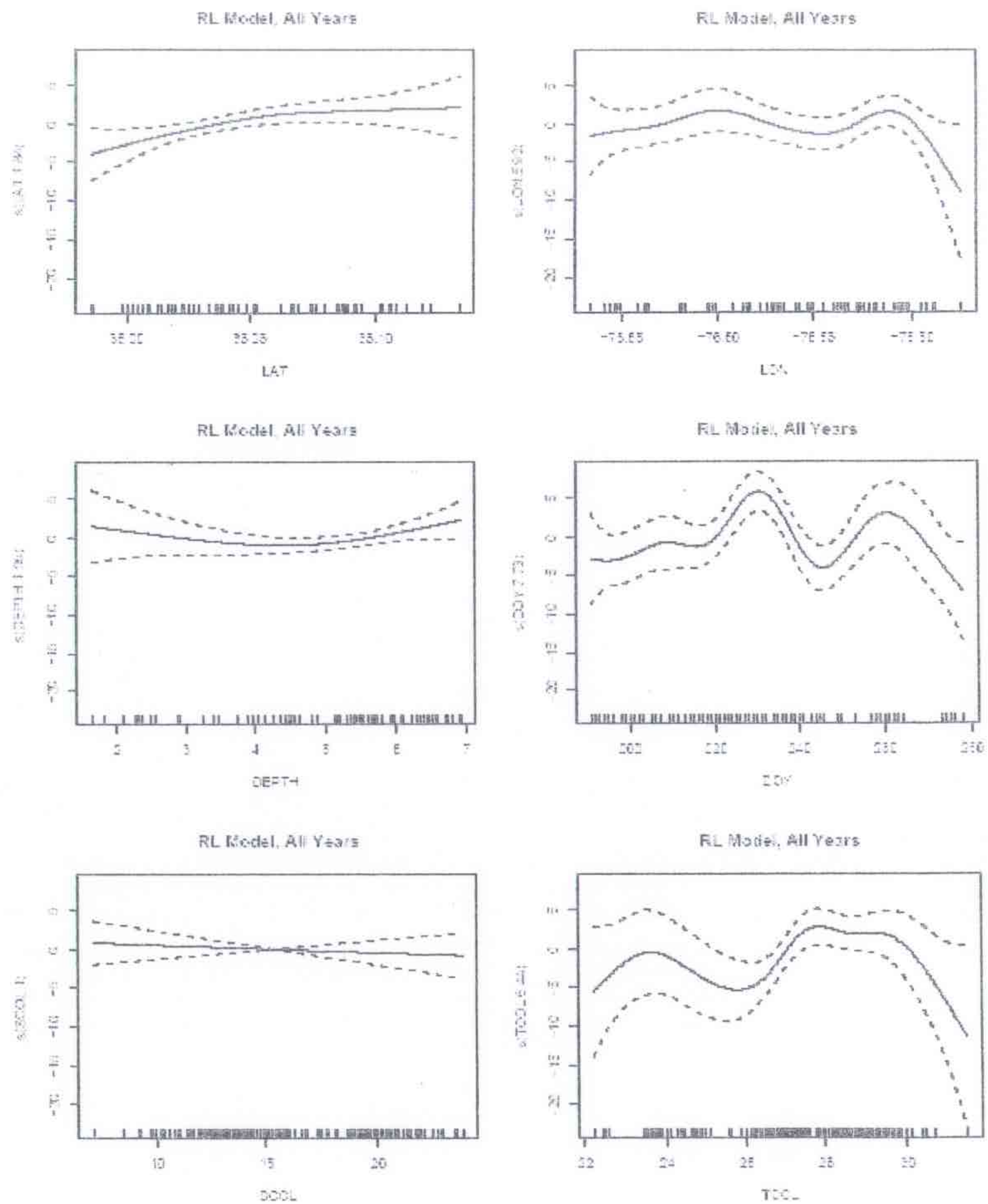


Table 1. Number of times red drum were present in at least of recording per sampling night compared to total number of times the category was sampled organized by month.

Table 1.

| Sampling | Fixed | | | | Rotating | |
|---|---|---|---|---|---|---|
| Month | Deep-Hard Bottom | Deep-Soft Bottom | Shallow | Medium | Deep-Hard Bottom | Deep-Hard Bottom |
| July 2005 | 1 of 13 | 0 of 14 | 0 of 1 | 2 of 6 | 0 of 9 | 0 of 3 |
| August 2005 | 17 of 18 | 16 of 17 | 0 of 2 | 7 of 8 | 10 of 12 | 2 of 3 |
| September 2005 | 6 of 6 | 7 of 7 | 2 of 2 | 4 of 4 | 5 of 5 | 3 of 3 |
| October 2005 | 0 of 4 | 0 of 0 | 0 of 0 | 0 of 1 | 1 of 2 | 1 of 1 |

Tables 2 - 3. Collection dates and number of spherical eggs 0.85 - 1 mm in diameter containing an oil globule (characteristic of Sciaenid family) collected in neuston tows in association with acoustic sampling sites from 7/7/05 - 10/2/05. Neuston tows were only conducted of the fixed sites throughout the season.

Table 2.

| Collection date | Number of Hard Bottom Sites Sampled | Number of Soft Bottom Sites Sampled | Number of "Sciaenid" eggs |
|---|---|---|---|
| 7/7/2005 | 1 | 2 | 0 |
| 7/10/2005 | 2 | 1 | 0 |
| 7/11/2005 | 1 | 2 | 0 |
| 7/17/2005 | 2 | 1 | 0 |
| 7/18/2005 | 1 | 2 | 0 |
| 7/24/2005 | 2 | 1 | 0 |
| 8/8/2005 | 2 | 1 | 0 |
| 8/14/2005 | 1 | 2 | 0 |
| 8/15/2005 | 2 | 1 | 0 |
| 8/21/2005 | 2 | 1 | 428 |
| 8/22/2005 | 1 | 2 | 0 |
| 8/28/2005 | 1 | 2 | 87 |
| 8/29/2005 | 2 | 1 | 166 |
| 9/6/2005 | 1 | 2 | 0 |
| 9/8/2005 | 2 | 1 | 0 |
| 9/20/2005 | 2 | 1 | 0 |
| 9/25/2005 | 1 | 2 | 0 |
| 9/26/2005 | 2 | 1 | 0 |
| 10/2/2005 | 2 | 1 | 0 |
| TOTAL | 30 | 27 | 681 |

Table 3.

| Collection Date | Site | Number of "Sciaenid" eggs | Red Drum Present |
|---|---|---|---|
| 8/21/2005 | Hard Bottom | 247 | confirmed |
| 8/21/2005 | Soft Bottom | 64 | confirmed |
| 8/21/2005 | Hard Bottom | 117 | confirmed |
| 8/28/2005 | Hard Bottom | 87 | confirmed |
| 8/29/2005 | Soft Bottom | 166 | confirmed |

Table 4 - 6. Ambient water quality data collected in association with acoustic sampling for each year averaged by month. Less than 2.5 mg/L DO represents percentage of sites with hypoxic or near hypoxic conditions below the pycnocline. Number of sites with hypoxic or near hypoxic conditions over total number of sites noted in parenthesis.

Table 4.

| | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| **Sampling Month** | **Average Salinity (ppt)** | **Average Temperature (°C)** | **Average DO (mg/L)** | **Average DO (mg/L)** | **Less Than 2.5 mg/L DO** |
| **July 2003** | 9.5 | 27.9 | 6.3 | 4.4 | 22.0% (11/50) |
| **August 2003** | 12.5 | 28.0 | 5.2 | 2.7 | 58.8 % (47/80) |
| **September 2003** | 12.1 | 26.2 | 6.3 | 4.3 | 32.6% (14/43) |

Table 5.

| | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| **Sampling Month** | **Average Salinity (ppt)** | **Average Temperature (°C)** | **Average DO (mg/L)** | **Average DO (mg/L)** | **Less Than 2.5 mg/L DO** |
| **July 2004** | 21.02 | 28.85 | 5.10 | 2.95 | 55.17% (16/29) |
| **August 2004** | 18.35 | 27.17 | 6.12 | 5.13 | 9.86% (7/71) |
| **September 2004** | 14.39 | 24.97 | 6.37 | 5.63 | 2.13% (1/47) |

Table 6.

| | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| **Sampling Month** | **Average Salinity (ppt)** | **Average Temperature (°C)** | **Average DO (mg/L)** | **Average DO (mg/L)** | **Less Than 2.5 mg/L DO** |
| **July 2005** | 12.94 | 29.20 | 6.38 | 5.95 | 4.44% (2/46) |
| **August 2005** | 13.47 | 29.44 | 5.64 | 4.34 | 16.67% (10/60) |
| **September 2005** | 15.36 | 26.69 | 4.86 | 4.28 | 11.11% (3/27) |

Table 7 - 9. Ambient water quality data collected in association with acoustic sampling for each year averaged by depth category. Less than 2.5 mg/L DO represents percentage of sites with hypoxic or near hypoxic conditions below the pycnocline. Number of sites with hypoxic or near hypoxic conditions over total number of sites noted in parenthesis.

Table 7.

| Depth Category | Average Salinity (ppt) | Whole Water Column Average Temperature (°C) | Average DO (mg/L) | Below Pycnocline Average DO (mg/L) | Less Than 2.5 mg/L DO | Average Depth (m) of Pycnocline |
|---|---|---|---|---|---|---|
| Shallow 2003 | 9.3 | 28.1 | 7.1 | 6.4 | 0.0% (0/22) | 1.50 |
| Medium 2003 | 10.5 | 27.6 | 6.0 | 3.9 | 35.0% (14/40) | 3.53 |
| Deep-Soft Bottom 2003 | 12.3 | 27.6 | 5.4 | 2.8 | 54.8% (46/84) | 5.02 |
| Deep-Hard Bottom 2003 | 12.8 | 26.7 | 5.6 | 3.0 | 54.4% (12/22) | 4.50 |

Table 8.

| Depth Category | Average Salinity (ppt) | Whole Water Column Average Temperature (°C) | Average DO (mg/L) | Below Pycnocline Average DO (mg/L) | Less Than 2.5 mg/L DO | Average Depth (m) of Pycnocline |
|---|---|---|---|---|---|---|
| Shallow 2004 | 15.99 | 27.04 | 5.70 | 4.88 | 12.5% (1/8) | 2.75 |
| Medium 2004 | 16.93 | 26.76 | 6.73 | 5.85 | 11.11% (4/36) | 3.50 |
| Deep-Soft Bottom 2004 | 17.67 | 26.64 | 5.89 | 4.59 | 15.94% (11/69) | 4.70 |
| Deep-Hard Bottom 2004 | 18.45 | 26.96 | 5.27 | 4.14 | 22.86% (8/35) | 4.94 |

Table 9.

| Depth Category | Average Salinity (ppt) | Whole Water Column Average Temperature (°C) | Average DO (mg/L) | Below Pycnocline Average DO (mg/L) | Less Than 2.5 mg/L DO | Average Depth (m) of Pycnocline |
|---|---|---|---|---|---|---|
| Shallow 2005 | 13.08 | 28.42 | 5.83 | 5.78 | 0.0% (0/7) | 1.57 |
| Medium 2005 | 13.07 | 28.75 | 6.00 | 5.41 | 15.79% (3/19) | 3.40 |
| Deep-Soft Bottom 2005 | 14.02 | 28.64 | 5.49 | 4.31 | 16.92% (11/65) | 4.66 |
| Deep-Hard Bottom 2005 | 13.89 | 28.71 | 5.80 | 5.22 | 2% (1/50) | 4.01 |

Table 10 - 11. Ambient water quality data collected in association with acoustic sampling for 2005 separated by site type and average by month. Less than 2.5 mg/L DO represents percentage of sites with hypoxic or near hypoxic conditions below the pycnocline. Number of sites with hypoxic or near hypoxic conditions over total number of sites noted in parenthesis.

Table 10.

| Fixed Sites | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| Sampling Month | Average Salinity (ppt) | Average Temperature (°C) | Average DO (mg/L) | Average DO (mg/L) | Less Than 2.5 mg/L DO |
| July 2005 | 12.87 | 29.28 | 6.65 | 6.35 | 0.0% (0/27) |
| August 2005 | 13.53 | 29.41 | 5.56 | 4.38 | 8.57% (3/35) |
| September 2005 | 16.28 | 26.12 | 4.43 | 3.59 | 7.69% (1/13) |

Table 11.

| Rotating Sites | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| Sampling Month | Average Salinity (ppt) | Average Temperature (°C) | Average DO (mg/L) | Average DO (mg/L) | Less Than 2.5 mg/L DO |
| July 2005 | 13.04 | 29.08 | 5.98 | 5.37 | 10.53% (2/19) |
| August 2005 | 13.39 | 29.50 | 5.75 | 4.27 | 28.0% (7/25) |
| September 2005 | 14.65 | 27.35 | 5.19 | 4.93 | 15.38% (2/13) |

Table 12 - 13. Ambient water quality data collected in association with acoustic sampling for 2005 separated by site type and averaged by depth category. Less than 2.5 mg/L DO represents percentage of sites with hypoxic or near hypoxic conditions below the pycnocline. Number of sites with hypoxic or near hypoxic conditions over total number of sites noted in parenthesis.

Table 12.

| Fixed Sites Depth Category | Average Salinity (ppt) | Whole Water Column Average Temperature (°C) | Average DO (mg/L) | Below Pycnocline Average DO (mg/L) | Below Pycnocline Less Than 2.5 mg/L DO | Average Depth (m) of Pycnocline |
|---|---|---|---|---|---|---|
| Deep-Soft Bottom 2005 | 13.88 | 28.62 | 5.59 | 4.51 | 2.44% (1/41) | 4.57 |
| Deep-Hard Bottom 2005 | 13.91 | 28.69 | 5.78 | 5.24 | 7.89% (3/38) | 4.10 |

Table 13.

| Rotating Sites Depth Category | Average Salinity (ppt) | Whole Water Column Average Temperature (°C) | Average DO (mg/L) | Below Pycnocline Average DO (mg/L) | Below Pycnocline Less Than 2.5 mg/L DO | Average Depth (m) of Pycnocline |
|---|---|---|---|---|---|---|
| Shallow 2005 | 13.08 | 28.42 | 5.83 | 5.78 | 0.0% (0/7) | 1.57 |
| Medium 2005 | 13.07 | 28.75 | 6.00 | 5.41 | 15.79% (3/19) | 3.40 |
| Deep-Soft Bottom 2005 | 14.08 | 28.75 | 5.31 | 4.01 | 30.77% (8/26) | 4.77 |
| Deep-Hard Bottom 2005 | 13.82 | 28.83 | 5.87 | 5.12 | 0.0% (0/9) | 3.59 |

Table 14 - 15. Ambient water quality data collected in association with acoustic sampling for 2005 organized by red drum presence or absence and averaged by month. Less than 2.5 mg/L DO represents percentage of sites with hypoxic or near hypoxic conditions below the pycnocline. Number of sites with hypoxic or near hypoxic conditions over total number of sites noted in parenthesis.

Table 14. Ambient data for acoustic recordings where red drum are not present in any recordings over a nights sampling period.

| | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| Sampling Month | Average Salinity (ppt) | Average Temperature (°C) | Average DO (mg/L) | Average DO (mg/L) | Less Than 2.5 mg/L DO |
| July 2005 | 12.92 | 29.19 | 6.37 | 5.96 | 4.65% (2/43) |
| August 2005 | 13.78 | 29.38 | 5.48 | 3.98 | 37.5% (3/8) |
| September 2005 | | | | | |
| October 2005 | 16.29 | 26.47 | 4.79 | 4.19 | 0.0% (0/6) |

Table 15. Ambient data for acoustic recordings where red drum are present in at least one recording over a nights sampling period.

| | Whole Water Column | | | Below Pycnocline | |
|---|---|---|---|---|---|
| Sampling Month | Average Salinity (ppt) | Average Temperature (°C) | Average DO (mg/L) | Average DO (mg/L) | Less Than 2.5 mg/L DO |
| July 2005 | 13.27 | 29.22 | 6.49 | 5.79 | 0.0% (0/3) |
| August 2005 | 13.43 | 29.45 | 5.67 | 4.39 | 13.46% (7/52) |
| September 2005 | 15.36 | 26.69 | 4.86 | 3.91 | 11.11% (3/27) |
| October 2005 | 14.88 | 26.65 | 5.04 | 4.94 | 0.0% (0/2) |

Tables 16 - 17. Matrix of the probability of first and subsequent detections of vocalizing red drum for 2003 and 2004 via sonobuoy sampling method for each recording interval. Number of presence observations over total number of observations noted in parenthesis.

Table 16.

| Sampling Interval | Probability of First Detection | 2003 Probability of Subsequent Detections | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | sunset | sunset + 0:30 | sunset + 1:05 | sunset+ 1:40 | sunset + 2:15 | sunset + 2:50 | sunset + 3:25 | sunset + 4:00 |
| sunset - 0:30 | 7.55% (4/53) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (3/3) | 100% (3/3) | 100% (2/2) | 100% (2/2) |
| sunset | 16.42% (11/67) | | 100% (11/11) | 90.91% (10/11) | 100% (11/11) | 100% (10/10) | 100% (9/9) | 100% (6/6) | 75% (3/4) |
| sunset + 0:30 | 22.86% (16/70) | | | 81.25% (13/16) | 92.31% (12/13) | 100% (13/13) | 90.91% (10/11) | 100% (4/4) | 50% (1/2) |
| sunset + 1:05 | 10% (7/70) | | | | 83.33% (5/6) | 66.67% (4/6) | 80% (4/5) | 50% (1/2) | 100% (1/1) |
| sunset+ 1:40 | 3.17% (2/63) | | | | | 0% (0/1) | | | |
| sunset + 2:15 | 1.92% (1/52) | | | | | | 100% (1/1) | | |
| sunset + 2:50 | 2.38% (1/42) | | | | | | | 100% (1/1) | 100% (1/1) |
| sunset + 3:25 | 0% (0/21) | | | | | | | | |
| sunset + 4:00 | 0% (0/13) | | | | | | | | |

Table 17.

| Sampling Interval | Probability of First Detection | 2004 Probability of Subsequent Detections | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | sunset | sunset + 0:30 | sunset + 1:05 | sunset+ 1:40 | sunset + 2:15 | sunset + 2:50 | sunset + 3:25 | sunset + 4:00 |
| sunset - 1:00 | 3.88% (4/103) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (3/3) | 100% (3/3) |
| sunset - 0:30 | 12.10% (15/124) | 92.86% (13/14) | 100% (15/15) | 100% (15/15) | 100% (14/14) | 100% (13/13) | 92.86% (13/14) | 100% (12/12) | 100% (10/10) |
| sunset | 28.46% (37/130) | | 97.30% (36/37) | 100% (37/37) | 100% (35/35) | 100% (34/34) | 100% (33/33) | 92.59% (25/27) | 92.86% (13/14) |
| sunset + 0:30 | 20.45% (27/132) | | | 100% (27/27) | 100% (27/27) | 92.00% (23/25) | 95.24% (20/21) | 93.75% (15/16) | 85.71% (6/7) |
| sunset + 1:05 | 3.81%(5/131) | | | | 100% (5/5) | 100% (4/4) | 100% (4/4) | 100% (3/3) | 100% (1/1) |
| sunset+ 1:40 | 1.56% (2/128) | | | | | 100% (2/2) | 50% (1/2) | 100% (1/1) | 0.00% (0/1) |
| sunset + 2:15 | 2.44% (3/123) | | | | | | 66.67% (2/3) | 50.0% (1/2) | 50.0% (1/2) |
| sunset + 2:50 | 1.72% (2/116) | | | | | | | 100% (2/2) | 0.00% (0/2) |
| sunset + 3:25 | 1.15% (1/87) | | | | | | | | 0.00% (0/1) |
| sunset + 4:00 | 0.00% (0/61) | | | | | | | | |

Tables 18 - 19. Matrix of the probability of first and subsequent detections of vocalizing red drum for 2005 fixed and rotating sites via sonobuoy sampling method for each recording interval. Number of presence observations over total number of observations noted in parenthesis.

Table 18.

| Sampling Interval | Probability of First Detection | 2005 Fixed Sites Probability of Subsequent Detections | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | sunset | sunset + 0:30 | sunset + 1:05 | sunset+ 1:40 | sunset + 2:15 | sunset + 2:50 | sunset + 3:25 |
| sunset - 1:00 | 0.0% (0/53) | | | | | | | |
| sunset - 0:30 | 4.0% (3/75) | 100% (3/3) | 100% (3/3) | 100% (3/3) | 100% (3/3) | 100% (3/3) | 100% (3/3) | 66.67% (2/3) |
| sunset | 11.84(9/76) | | 100% (8/8) | 100% (8/8) | 100% (8/8) | 100% (9/9) | 100% (9/9) | 85.71% (6/7) |
| sunset + 0:30 | 22.37% (17/76) | | | 100% (17/17) | 94.12% (16/17) | 100% (17/17) | 94.12% (16/17) | 100% (5/5) |
| sunset + 1:05 | 10.53% (8/76) | | | | 87.50% (7/8) | 75.00% (6/8) | 50.00% (4/8) | |
| sunset+ 1:40 | 8.0% (6/75) | | | | | 100% (6/6) | 66.67% (4/6) | |
| sunset + 2:15 | 3.90% (3/77) | | | | | | 33.33% (1/3) | 100% (1/1) |
| sunset + 2:50 | 1.45% (1/69) | | | | | | | |
| sunset + 3:25 | 0.0% (0/19) | | | | | | | |

Table 19.

| Sampling Interval | Probability of First Detection | 2005 Rotating Sites Probability of Subsequent Detections | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | sunset | sunset + 0:30 | sunset + 1:05 | sunset+ 1:40 | sunset + 2:15 | sunset + 2:50 | sunset + 3:25 |
| sunset - 1:00 | 0.0% (0/38) | | | | | | | |
| sunset - 0:30 | 3.28% (2/61) | 100% (2/2) | 100% (2/2) | 100% (2/2) | 100% (2/2) | 100% (2/2) | 100% (2/2) | 50.00% (1/2) |
| sunset | 6.45% (4/62) | | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) | 100% (4/4) |
| sunset + 0:30 | 24.19% (15/62) | | | 93.33% (14/15) | 86.67% (13/15) | 100% (15/15) | 92.86% (13/14) | 62.50% (5/8) |
| sunset + 1:05 | 16.13% (10/62) | | | | 100% (10/10) | 70.00% (7/10) | 77.78% (7/9) | 50.00% (1/2) |
| sunset+ 1:40 | 6.45% (4/62) | | | | | 75.00% (3/4) | 50.00% (2/4) | 100% (1/1) |
| sunset + 2:15 | 1.62% (1/62) | | | | | | 0.00% (0/1) | |
| sunset + 2:50 | 0.0% (0/51) | | | | | | | |
| sunset + 3:25 | 0.0% (0/19) | | | | | | | |

Tables 20 - 21. Probability of first detecting vocalizing red drum via sonobuoy sampling method for 2003 and 2004 during each recording interval organized by month. Number of presence observations over total number of observations noted in parenthesis.

Table 20.

| Sampling | 2003 P(First Detection) | | |
|---|---|---|---|
| **Interval** | **July** | **August** | **September** |
| sunset - 0:30 | 0.00% | 0.00% | 21.05% (4/19) |
| sunset | 0.00% | 10.71% (3/28) | 29.63% (8/27) |
| sunset + 0:30 | 18.18% (2/11) | 30% (9/30) | 17.24% (5/29) |
| sunset + 1:05 | 0.00% | 20% (6/30) | 3.45% (1/19) |
| sunset+ 1:40 | 0.00% | 7.69% (2/26) | 0.00% |
| sunset + 2:15 | 0.00% | 5% (1/20) | 0.00% |
| sunset + 2:50 | 0.00% | 0.00% | 4.17% (1/24) |
| sunset + 3:25 | 0.00% | 0.00% | 0.00% |
| sunset + 4:00 | 0.00% | 0.00% | 0.00% |

Table 21.

| Sampling | 2004 P(First Detection) | | |
|---|---|---|---|
| **Interval** | **July** | **August** | **September** |
| sunset - 1:00 | 0.00% (0/18) | 7.84% (4/51) | 0.00% (0/31) |
| sunset - 0:30 | 4.17% (1/24) | 9.68% (6/62) | 22.68% (8/35) |
| sunset | 4.17% (1/24) | 44.36% (27/61) | 21.43% (9/42) |
| sunset + 0:30 | 16.00% (4/25) | 30.65% (19/62) | 9.52% (4/42) |
| sunset + 1:05 | 4.17% (1/24) | 6.45% (4/62) | 0.00% (0/42) |
| sunset+ 1:40 | 4.35% (1/23) | 0.00% (0/60) | 2.38% (1/42) |
| sunset + 2:15 | 4.55% (1/22) | 1.78% (1/56) | 2.38% (1/42) |
| sunset + 2:50 | 0.00% (0/20) | 1.92% (1/52) | 2.38% (1/42) |
| sunset + 3:25 | | 0.00% (0/42) | 2.38% (1/42) |
| sunset + 4:00 | | 0.00% (0/17) | 0.00% (0/41) |

Table 22 - 24. Probability of first detecting vocalizing red drum via sonobuoy sampling method for each year during each recording interval organized by depth category. Number of presence observations over total number of observations noted in parenthesis.

Table 22.

| | 2003 P(First detection) | | | |
|---|---|---|---|---|
| Sampling Interval | Shallow | Medium | Deep Soft Bottom | Deep Hard Bottom |
| sunset - 0:30 | 0.00% | 0.00% | 22.22% (4/18) | 0.00% |
| sunset | 0.00% | 0.00% | 21.74% (5/23) | 26.09% (6/23) |
| sunset + 0:30 | 0.00% | 23.08% (3/13) | 34.78% (8/23) | 20% (5/25) |
| sunset + 1:05 | 0.00% | 23.08% (3/13) | 4.35% (1/23) | 12% (3/25) |
| sunset+ 1:40 | 0.00% | 0.00% | 0.00% | 8.33% (2/25) |
| sunset + 2:15 | 0.00% | 12.5% (1/8) | 0.00% | 0.00% |
| sunset + 2:50 | 0.00% | 0.00% | 5.88% (1/17) | 0.00% |
| sunset + 3:25 | 0.00% | 0.00% | 0.00% | 0.00% |
| sunset + 4:00 | 0.00% | 0.00% | 0.00% | 0.00% |

Table 23.

| | 2004 P(First detection) | | |
|---|---|---|---|
| Sampling Interval | Medium | Deep Soft Bottom | Deep Hard Bottom |
| sunset - 1:00 | 0.00% (0/25) | 6.38% (3/47) | 3.22% (1/31) |
| sunset - 0:30 | 6.25% (2/32) | 15.52% (9/58) | 11.76% (4/34) |
| sunset | 21.21% (7/33) | 30.16% (19/63) | 32.35% (11/34) |
| sunset + 0:30 | 17.65% (6/34) | 23.81 (15/63) | 17.14% (6/35) |
| sunset + 1:05 | 5.88% (2/34) | 3.17 (2/63) | 2.92% (1/34) |
| sunset+ 1:40 | 0.00% (0/32) | 3.17% (2/63) | 0.00% (0/33) |
| sunset + 2:15 | 9.38% (3/32) | 0.00% (0/58) | 0.00% (0/33) |
| sunset + 2:50 | 6.67% (2/30) | 0.00% (0/55) | 0.00% (0/31) |
| sunset + 3:25 | 0.00% (0/23) | 2.32% (1/43) | 0.00% (0/21) |
| sunset + 4:00 | 0.00% (0/17) | 0.00% (0/29) | 0.00% (0/15) |

Table 24.

| | 2005 Fixed Sites P(First detection) | |
|---|---|---|
| Sampling Interval | Deep Soft Bottom | Deep Hard Bottom |
| sunset - 1:00 | 0.00% (0/26) | 0.00% (0/26) |
| sunset - 0:30 | 5.71% (2/35) | 0.00% (0/38) |
| sunset | 16.67% (6/36) | 5.40% (2/37) |
| sunset + 0:30 | 25.71% (9/35) | 21.05% (8/38) |
| sunset + 1:05 | 8.57% (3/35) | 13.16% (5/38) |
| sunset+ 1:40 | 8.82% (3/34) | 5.26% (2/38) |
| sunset + 2:15 | 0.00% (0/36) | 7.89% (3/38) |
| sunset + 2:50 | 0.00% (0/32) | 2.94% (1/34) |
| sunset + 3:25 | 0.00% (0/8) | 0.00% (0/9) |