10-19-2005

Mapping Tampa Bay Cynoscion nebulosus Spawning Habitat Using Passive Acoustic Surveys

Sarah Lyle Walters
University of South Florida

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Mapping Tampa Bay *Cynoscion nebulosus* Spawning Habitat Using Passive Acoustic Surveys

by

Sarah Lyle Walters

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science College of Marine Science University of South Florida

Major Professor: David A. Mann, Ph.D. Susan Lowerre-Barbieri, Ph.D. Joseph J. Torres, Ph.D.

Date of Approval: October 19, 2005

Keywords: spotted seatrout, mobile hydrophone, courtship sounds, sciaenid reproduction, estuary

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ACKNOWLEDGEMENTS

I would like to thank my committee, Dr. David Mann, Dr. Sue Lowerre-Barbieri, and Dr. Jose Torres, for all of their support and guidance throughout this process. I especially would like to acknowledge Dr. Sue Lowerre-Barbieri for her assistance in helping balance my commitments towards this degree with my obligations for work as well as all her advice in both science and life in general. Data collections as well as mapping were a direct result of Joel Bickford’s dedication (and enthusiasm!) for this work. I’d also like to thank Janet Tunnell for all of her efforts collecting data as well as analyzing sounds in the laboratory. The Fisheries Independent Monitoring Program (FIM) at the Florida Wildlife Research Institute provided the expertise and software knowledge responsible for the SAS sampling selection program. Many thanks to my USF lab mates, Jim Locascio, Brandon Casper, Mandy Hill-Cook, Randy Hill, and Bryan Nichols, as their humor and assistance have facilitated as well as brought joy to this process. And, most importantly, I’d like to thank my family for all their love and support and for raising me to value and respect nature. Evidently, my simple love of the outdoors evolved into a professional direction, one which allows (and even encourages) me to get wet, go fishing, and truly appreciate the intricacies of God’s great world.
NOTE TO READER

Note to Reader: The original of this document contains color that is necessary for understanding the data. The original thesis is on file with the USF library in Tampa, Florida.
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MAPPING TAMPA BAY CYNOSCION NEBULOSUS SPAWNING HABITAT
USING PASSIVE ACOUSTIC SURVEYS

Sarah Lyle Walters

ABSTRACT

Spotted seatrout, *Cynoscion nebulosus*, spawning locations as well as associated environmental variables were determined for Tampa Bay, Florida during the 2004 spawning season using a mobile hydrophone survey. Hydrophones, a type of underwater microphone, can be used to detect and record spawning sounds of soniferous fishes. During their spawning season in Tampa Bay which generally occurs between March and September, mature male spotted seatrout generate sounds associated with courtship in the crepuscular and evening periods by vibrating sonic muscles against the swim bladder. Active spawning sites can be located using hydrophones to find these calling males.

Using a random stratified sampling method, 760 stations within Tampa Bay (46% of the sampling universe) were sampled over the 2004 spawning season. Only 8% of sampled stations had large aggregations of spotted seatrout. Spawning, determined by the sound produced by large aggregations, was detected throughout the bay except for Hillsborough Bay and was most common in the lower bay and eastern region of the middle bay. Presence of submerged aquatic vegetation (SAV), proximity to shoreline, as well as high dissolved oxygen values and shallow depth were positively correlated with spawning areas. Courtship calls of sand seatrout, *Cynoscion arenarius*, and silver perch, *Bairdiella chrysoura* were also detected during the survey as they share an overlapping spawning season with spotted seatrout. Aggregations of all three species rarely occurred simultaneously. Sand seatrout and silver perch used different habitats within Tampa Bay.
to spawn and spawned with a much greater frequency than spotted seatrout. Courtship calls of spotted seatrout were analyzed both by ear and by received sound level to determine if signal processing could be used to assess courtship sound recordings. However, there was no clear relationship between the two methods.
INTRODUCTION

Spotted seatrout are estuarine-dependent batch spawners, but their preferred spawning habitat, as well as environmental parameters corresponding to spawning site selection, has yet to be fully determined (Brown-Peterson et al., 2002). Mature male spotted seatrout generate courtship sounds associated with spawning. These sounds are made in the crepuscular and evening periods by vibrating sonic muscles against the swim bladder (Tavolga, 1969, Fish and Mowbray, 1970). Active spawning sites can be located using underwater microphones, or hydrophones, to find these calling males (Mok and Gilmore, 1983; Saucier and Baltz, 1992; Saucier and Baltz, 1993; Luczkovich et al., 1999). Passive acoustic studies in coastal North Carolina, South Carolina, Louisiana, and the Florida east coast have examined spotted seatrout spawning sites using a non-random approach. Results from these studies, as well as traditional reproductive biology studies, indicated spotted seatrout use a wide range of habitats during courtship and reproduction, including bays, lagoons, channels, deep passes adjacent to open water, deep channels adjacent to vegetated shallow areas, and seagrass in non-tidal areas (Hein and Shepard, 1979; Brown-Peterson et al., 1988; Saucier and Baltz, 1993; Gilmore, 2003).

Previous work on Tampa Bay spotted seatrout reproduction has focused on spawning activity in the lower and middle portions of the bay. Using data on the distribution and average size of larval spotted seatrout, McMichael and Peters (1989) concluded that spawning occurred in middle and lower Tampa Bay and also in nearshore Gulf waters. Actively spawning spotted seatrout targeted and collected by Lowerre-Barbieri (2004) were captured primarily in lower Tampa Bay but location of specific
spawning aggregations was difficult. Capture efforts were focused on lower Tampa Bay with some capture in the southern portion of the middle bay, but the middle and upper Bay were not sampled. McMichael and Peters (1989) also did not include the upper bay for larval collection. Further research investigating spotted seatrout spawning site locations is necessary in order to verify spawning sites in the lower bay, determine the extent of spawning in the middle bay, and establish if the upper bay is being used for spawning. Passive acoustics is an ideal tool for characterization of spawning habitat in a large area such as Tampa Bay. While traditional collection gear typically limits the time and geographic area sampled in a study, passive acoustic methodology permits comprehensive coverage in a fraction of the time. Additionally, as a noninvasive tool, passive acoustics does not interrupt spawning behavior while data are being collected.

The objectives of this study were to locate spotted seatrout spawning sites as identified by aggregation sounds, identify physical and chemical variables associated with these spawning sites, and determine if significant spawning activity differences exist amongst geographic regions within Tampa Bay.
METHODS

A stratified random sampling design using a mobile hydrophone was developed and tested the last half of the 2003 Tampa Bay spotted seatrout spawning season and found to be successful. Data from this survey was used in constructing the sampling window for the 2004 survey (see Sampling Periodicity in Methods) as well as to determine the amount of sampling possible per evening in 2004 (see Sampling Universe in Methods). A few minor alterations were made to the protocol and the design was used for the 2004-spawning season as detailed below.

Sampling Universe

Tampa Bay, Florida was divided into four zones based on geographic and logistical criteria (Hillsborough Bay, Upper Bay, Middle Bay, and Lower Bay). All zones, except Hillsborough Bay were subdivided into east and west regions (Figure 1): upper west (Region A), upper east (Region B), middle west (Region C), middle east (Region D), lower west (Region E), lower east (Region F). Hillsborough Bay was considered both a region (Region G) and a zone. Each region was a stratum, with sampling units composed of 1-nm² grids. Grids with a high percentage of land or very shallow water (95% or more of the area comprised of land or water < 1.5 m in depth) were excluded from sampling. Grids were categorized as either “open water” or “shoreline”. Shoreline grids were those that had more than 5% of their area consisting of either land or water 1.5 m or less adjacent to land. An open water grid had 95% of its area covered by water deeper than 1.5 m and it was not adjacent to land.
Figure 1 Tampa Bay Sampling Universe

Tampa Bay Hydrophone Survey
Sampling Universe (Regions and Grids)
Sampling was based on the lunar calendar, as spotted seatrout spawning frequencies have been attributed to lunar influences (McMichael and Peters, 1989; Gilmore, 2003; Kupschus, 2004). Two nights per week were selected for sampling, both nights falling within two days of the quarter, full, three-quarter, or new moon. One region was sampled per evening. One zone was sampled per week. Zones were rotated monthly so that each zone was sampled over the various possible lunar phases during the course of the spawning season. Grids were randomly selected with replacement in order to account for seasonal variability. During preliminary testing in 2003, it was found that six grids per evening was the maximum number of grids that could be sampled within the sound sampling window (see Sampling Periodicity in Methods).

Regions also varied in the number of “shoreline” and “open water” grids sampled per evening. It was necessary to sample the shoreline/open water grids per region proportionally to what was present in each region. This was necessary because spotted seatrout are reported to spawn in shoreline areas, and certain regions contained more of this habitat than others. Sampling was also proportional for the number of grids per region (Table 1).

Four stations per grid were sampled to ensure representative sampling of the area within each grid. Stations were as evenly distributed as possible over the grid area as well as the available substrata (Figure 2A). Targeted substrata for both shoreline and open water grids included submerged aquatic vegetation (SAV), structure, channel, and non-channel. The SAV category included areas either directly on top of seagrass or adjacent to the flats containing seagrass. Structure encompassed any artificial construction in the water including pilings, jetties, bridges, artificial reefs/fish havens, and range markers.
Table 1 Number of grids in each region characterized by grid type. Total number of grids in a particular region is listed with the number of grids sampled per evening in parenthesis.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of Open Water Grids</th>
<th>Number of Shoreline Grids</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9 (1)</td>
<td>31 (4)</td>
<td>40 (5)</td>
</tr>
<tr>
<td>B</td>
<td>20 (2)</td>
<td>20 (3)</td>
<td>40 (5)</td>
</tr>
<tr>
<td>C</td>
<td>38 (4)</td>
<td>13 (2)</td>
<td>51 (6)</td>
</tr>
<tr>
<td>D</td>
<td>29 (3)</td>
<td>22 (3)</td>
<td>51 (6)</td>
</tr>
<tr>
<td>E</td>
<td>19 (2)</td>
<td>32 (4)</td>
<td>51 (6)</td>
</tr>
<tr>
<td>F</td>
<td>29 (3)</td>
<td>27 (3)</td>
<td>56 (6)</td>
</tr>
<tr>
<td>G</td>
<td>10 (1)</td>
<td>29 (4)</td>
<td>39 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>174</td>
<td>328</td>
</tr>
</tbody>
</table>

The channel substratum was defined as a clearly navigable deeper passage of water surrounded by shallower water. The non-channel substratum was a depth independent describer of areas that were not channels and did not have structure or SAV. If the four different substrata were not present within a grid, then the four sampling stations were selected based on differences in depth. If depth was constant throughout the grid, then the four sample stations were distributed equally based on area of the grid (Figure 2B).
Figure 2 Sampling station distribution when stations determined by available substrata (A) and by area (B).
**Sampling Periodicity**

Hydrophone sampling began on April 5, 2004 and continued through the first week of October 2004. April was selected as the start month because Tampa Bay spotted seatrout males and females typically begin spawning by mid to late March (Lowerre-Barbieri, 2004) or April (McMichael and Peters, 1989). Similarly, October was chosen as the end month as spawning has been reported to cease in mid-September (Lowerre-Barbieri, 2004) or October (McMichael and Peters, 1989).

Because of the diel periodicity associated with spotted seatrout courtship sounds, sampling was conducted during the window of maximum sound production. This window was based on both the 2003 preliminary hydrophone survey data, indicating aggregations were detected between sunset and 02:30 and previous research reporting spotted seatrout spawning aggregation sounds from 17:00-01:00 (Saucier and Baltz, 1993), and sunset to 24:00 (Gilmore, 1994). Based on these sources of information, the sampling window for the 2004 hydrophone survey was set to begin at sunset (roughly 20:00 EDT) and continue for five hours (until roughly 01:00 EDT).

Seasonal start and stop dates for the 2004-spawning season were confirmed based on data from a known spawning site. Lowerre-Barbieri (2004) reported a very high percentage of the females collected at Bunces Pass (Figure 3) were actively spawning (100% in 2001 and 96.5 % in 2002). A long-term acoustic recording system (LARS) was deployed in Bunces Pass for the 2004-spawning season. Anchored 0.5 m off the bottom at the mouth of the pass, the LARS was programmed to sample ten continuous seconds of sound every ten minutes at a 2634 Hz sampling rate and record to onboard Compact Flash memory. Data from the LARS were analyzed both by ear and spectrographically in
Figure 3 Bunces Pass, Florida. Known spotted seatrout aggregation spawning site and location of the long-term acoustic recording system (LARS).

Cool Edit. Temperature data recorded 40 km north from the LARS during the 2004 spotted seatrout spawning season was obtained from NOAA. Although another agency (consulting firm Delta Seven) collected temperature data 1.0 km from the LARS during the same time period, this data set was incomplete. However, as the daily temperature averages did not significantly differ between these two sites during the first and last
months of the spawning season (Mann-Whitney Rank Sum Test, n=98, p=0.201), the NOAA temperature data set was applied to the LARS data.

**Data Collection**

Hydrophone recordings and environmental data were taken at all stations. Once at a sampling station within a grid the engine was turned off, GPS and depth measurements were recorded, and a mobile hydrophone (HTI, model 96-min, sensitivity –164 dBV/µPa) was lowered one meter in the water. Recordings were made after a two-minute period in the event the spotted seatrout calling ceased because of engine noise disturbance. During the two-minute waiting period, a YSI Model 600 QS was lowered into the water mid-depth on the opposite side of the boat from the hydrophone to measure salinity, temperature, and dissolved oxygen. Mid-depth measurements were taken, as Tampa Bay is a well-mixed estuary with little difference in bottom and surface temperature and salinity (Goodwin, 1989). Substrata type (described in the previous section to include submerged aquatic vegetation (SAV), structure, channel, and non-channel) and times were also recorded.

If sciaenid courtship calls were heard, then a recording was made. All recordings were made for a thirty second period in “A-time” on the Sony digital audio tape (dat) recorder model TCD-D8. Recordings on the dat recorder used the “line-in” jack with all recordings on level 10 and a sample rate of 44.1 kHz. Record mode was set to “manual” and microphone sensitivity was set on “low”. A miniature Marshall Amplifier with the tone set in the middle and the volume on 10 was used to listen to all sounds prior to recording. Headphones were worn if a sound was difficult to detect through the amplifier
or if there was too much background noise. Tape label name, program number, tape start and end time, and comments accompanied each recording.

Spotted seatrout males produce distinct courtship calls. These calls have been classified into four major sound types: dual-pulse calls, a long grunt call, multiple-pulse calls, and a stacatto call (Mok and Gilmore, 1983, Gilmore, 2003). Their calls can be distinguished from other soniferous fishes based on pulse duration and intensity of sound by frequency range (Figure 4). Estimated number of spotted seatrout producing sound were categorized as (1) 1-2 individuals, (2) 3-5 individuals, (3) small aggregation with individuals still distinguishable, or (4) large aggregation. Distance to the fish was categorized as: “directly on-top of”, “close-by”, or “in the distance”. The directly on-top of category was defined as fish sounds audible through the bottom of the boat without the aid of the hydrophone. Sounds categorized as “in the distance” were quiet, and difficult to hear through the amplifier. “Close-by” included a wide range of sounds falling between “directly on-top of” and “in the distance” categories.

Courtship calls of two other sciaenid species, sand seatrout, *Cynoscion arenarius*, and silver perch, *Bairdiella chrysoura*, were regularly heard in Tampa Bay. Males in both of these species also make courtship calls associated with spawning. However, their calls are easily distinguished from spotted seatrout (Figures 5 and 6). Sand seatrout calls resemble a “purring” and silver perch have a distinctive high-pitched “knock” (Mok and Gilmore, 1983, Locascio and Mann, 2005, Joel Bickford, pers. comm.). Although sand seatrout and silver perch share overlapping spawning seasons with spotted seatrout and apparently similar windows of maximum sound production, there may be species-specific variability that was not accounted for in this sampling design. Calls made by sand
Figure 4  Spectrograph of (A) an individual spotted seatrout call composed of three sets of multiple-pulses followed by a long grunt and (B) a large aggregation. Darker shading corresponds to higher decibel levels.
Figure 5 Spectrograph of an individual sand seatrout. Darker shading corresponds to higher decibel levels.
Figure 6 Spectrograph of an individual silver perch. Darker shading corresponds to higher decibel levels.

seatrout and silver perch were noted on the datasheet and the number of fish was estimated as (1) 1-2 individuals, (2) 3-5 individuals, or (3) aggregation. Distance from the hydrophone for these two species was categorized identically for spotted seatrout as either “directly on-top of”, “close-by”, or “in the distance”. All recordings made in the field were reviewed in the lab by ear and verified with known recorded fish sounds.

Statistical Analysis

A program written in Statistical Analysis System (SAS) was used to randomly select sampled grids. Arc View GIS 3.3 was used to map the location of courtship sounds. Differences in water temperature between April and May, differences in temperature, salinity, and dissolved oxygen between stations with and without large
spotted seatrout aggregations, and differences in depth between shoreline and offshore-categorized grids were examined using the Mann-Whitney Rank Sum test. The $\chi^2$ test with Yates correction for continuity was applied to test differences between expected and actual aggregation presence of all three sciaenids amongst all regions, by grid type, and by substrata. While examination of sciaenid aggregations by region and grid type included all aggregations regardless of distance, associations with bottom type and environmental parameters were examined at stations categorized as “directly on-top of” or “close-by”. Aggregation stations classified as “in the distance” were not used, as those aggregations may not have been in proximity to the in situ measurement locations. The $\chi^2$ analyses were based on the assumption that the ratio of the number of large spotted seatrout aggregations (and aggregations for sand seatrout and silver perch) heard throughout Tampa Bay divided by the number of stations sampled was the expectation if no significant regional, grid type, or substrata effects existed. This ratio was then used to determine the number of expected large aggregations in each of the categories (by region, grid type, and substrata) and those numbers were then compared to the number of large aggregation stations that actually occurred. When spotted seatrout aggregations were compared to sand seatrout and silver perch, the large and small spotted seatrout aggregation categories were combined in order to have comparable categories for all species.

**Acoustic Analysis**

To determine if signal processing to determine sound level could be used to assess courtship sound recordings, comparisons were made between spotted seatrout number as categorized by the human ear and by received sound level. Each recording was read into
MATLAB and analyzed by performing a 44100-point Fast Fourier Transform (FFT). Average sound spectrum levels were then calculated over 100 Hz wide bins. Signals were calibrated using the hydrophone calibration and a calibration of the DAT recorder. Sound energy for spotted seatrout calls is concentrated in the 200-300 Hz frequency range (Figure 4). Sound pressure levels within the 200-300 Hz frequency range were compared to all spotted seatrout numerical categories assigned by trained technicians.
RESULTS

Seasonality

Although hydrophone survey sampling occurred from April through October, only data from May 3rd through September 19th were included in the analyses. Spotted seatrout typically begin spawning in mid-March/April in Tampa Bay, however in 2004 the start of the spawning season was delayed. Data from the hydrophone survey indicated an absence of sounds of large aggregations until May. All seven regions were sampled in April and only six stations out of the 144 sampled stations had spotted seatrout calling, none of which were large aggregations. Conversely, thirty stations (n=148) in May had spotted seatrout calling, three of which were large aggregations and three of which were small aggregations. Water temperatures were also quite low in April, ranging from 19.2 °C to 26.2 °C, and averaging 22.2 °C (Figure 7). Monthly water temperature significantly increased to 26.8 °C in May (Mann-Whitney Rank Sum test, n=292, p<0.001) and sounds of large aggregations were detected. Due to equipment failure, it was not possible to confirm the start of the spawning season based on the LARS at Bunces Pass. However, large aggregation sounds were only detected on three days in March (19th-21st) but by May, only four days did not have sounds attributable to large aggregations (Figure 8). Although data were not available from April 1st to May 5th due to equipment failure, additional sampling with a mobile hydrophone at Bunces Pass detected sounds of a large aggregation on April 30th. Combining data from Bunces Pass with information from the hydrophone survey, the start of the spawning season as defined by the sounds produced by large aggregations was estimated to begin in early May.
Figure 7 Temperature (°C) at all stations sampled in the 2004 Tampa Bay hydrophone survey during April and May. The threshold water temperature of 23 °C has been cited as the water temperature necessary to initiate spawning (Brown-Peterson et. al, 1988).
Figure 8 2004 Bunces Pass spotted seatrout spawning season start with daily duration, sunset time, and daily average water temperature. The threshold water temperature of 23 °C has been cited as the water temperature necessary to initiate spawning (Brown-Peterson et. al, 1988).

Bunces Pass 2004 Large Aggregation Level Sound, May 6-June 6

Although data were collected through the first week of October, only data collected through September 19th were used in further analyses. After September 12th, only three large aggregations were detected by the hydrophone survey and spawning aggregation sound began to decrease at Bunces Pass by September 13th. Sounds of large aggregations occurred on only one night between September 20th and September 26th (Figure 9). Although large aggregations were heard again from September 27th through October 5th, start times were variable and duration steadily decreased. Sounds produced
by large aggregations terminated by October 6th and were replaced by individuals calling until complete cessation of spotted seatrout calls occurred on October 8th.

**Figure 9** 2004 Bunces Pass spotted seatrout end of season daily duration with associated sunset time and temperature data.

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**Data Collected**

Over the course of the sampling season, 760 stations were sampled from 190 grids (Table 2). Thirty-four of the grids were sampled at least twice, with five of the grids sampled three times. Eight of the 34 repeat grids displayed differences in the amount of
spotted seatrout detected between dates. Region E had the highest percentage (60%) of repeat grids with differences between multiple sampling dates (Table 3). There were no clear trends in average temperature, salinity, dissolved oxygen, sampling time, or sampling date that might explain the changes in sound production (Table 4). Because these differences in spotted seatrout courtship sound occurred in the same grid over different dates, repeated grid stations were considered independent data points.

**Table 2** Number of total grids and stations sampled with the percent of grids sampled in each region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of Grids</th>
<th>Number of Stations</th>
<th>Percent Grids Sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>25</td>
<td>100</td>
<td>50%</td>
</tr>
<tr>
<td>B</td>
<td>25</td>
<td>100</td>
<td>53%</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>120</td>
<td>45%</td>
</tr>
<tr>
<td>D</td>
<td>30</td>
<td>120</td>
<td>41%</td>
</tr>
<tr>
<td>E</td>
<td>30</td>
<td>120</td>
<td>47%</td>
</tr>
<tr>
<td>F</td>
<td>30</td>
<td>120</td>
<td>43%</td>
</tr>
<tr>
<td>G</td>
<td>20</td>
<td>80</td>
<td>46%</td>
</tr>
<tr>
<td>Total</td>
<td>190</td>
<td>760</td>
<td>46%</td>
</tr>
</tbody>
</table>
Table 3  Number of grids repeated and percentage of repeated grids displaying differences in spotted seatrout detections by region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of Repeated Grids</th>
<th>Percent Repeated Grids with Differences in Spotted Seatrout Detections between Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>33%</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>17%</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>13%</td>
</tr>
<tr>
<td>E</td>
<td>5</td>
<td>60%</td>
</tr>
<tr>
<td>F</td>
<td>5</td>
<td>20%</td>
</tr>
<tr>
<td>G</td>
<td>2</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>24%</td>
</tr>
</tbody>
</table>

Forty six percent of all grids were sampled during the May through September sampling season (Figure 10). All sampled grids were examined for proximity of the four sampling stations to one another to account for potential overlapping in detected calls. Each grid was first scored for sampleable area (that with water depth > 1.5 m). Grids where the area was less than 15% were checked for distance between sampled stations. If this distance was less than 150 m, then those two stations were treated as one station. This decision was based on the assumption that those stations were close enough that the same group of fish could be detected in both locations. Two grids qualified as each having less than 15% of the area available for sampling with two stations within 150 m of one another. In each of these grids, the two proximal stations were considered one site, and two stations were thus removed from the universe reducing the number of total stations sampled to 758.
Table 4 Repeated grids displaying differences in spotted seatrout detections between sampling dates. Temperature (°C), salinity (ppt), dissolved oxygen (mg/L), and time measurements for each grid are the average of the four sampling stations in each grid. Spotted seatrout detections are the number of spotted seatrout heard in each station in the grid, with each station separated by a comma. Estimated number of spotted seatrout were categorized as either: 1=1-2 individuals, 2=3-5 individuals, 3=Small aggregation, 4=Large aggregation

<table>
<thead>
<tr>
<th>Region</th>
<th>Grid #</th>
<th>Date</th>
<th>Grid Average Temp</th>
<th>Grid Average Salinity</th>
<th>Grid Average DO</th>
<th>Grid Average Time</th>
<th>Spotted Seatrout Detections</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>65</td>
<td>8/5/04</td>
<td>30.1</td>
<td>19.4</td>
<td>8.7</td>
<td>20:33</td>
<td>2,2,4,4</td>
</tr>
<tr>
<td>A</td>
<td>65</td>
<td>8/27/04</td>
<td>30.6</td>
<td>17.3</td>
<td>9.7</td>
<td>22:02</td>
<td>0,0,0,1</td>
</tr>
<tr>
<td>B</td>
<td>51</td>
<td>5/5/04</td>
<td>24.9</td>
<td>23.9</td>
<td>7.6</td>
<td>21:13</td>
<td>0,0,0,0</td>
</tr>
<tr>
<td>B</td>
<td>51</td>
<td>8/6/04</td>
<td>30.0</td>
<td>20.1</td>
<td>6.7</td>
<td>21:01</td>
<td>0,2,1,0</td>
</tr>
<tr>
<td>B</td>
<td>51</td>
<td>8/30/04</td>
<td>30.8</td>
<td>18.8</td>
<td>5.1</td>
<td>20:50</td>
<td>3,3,3,3</td>
</tr>
<tr>
<td>C</td>
<td>297</td>
<td>6/1/04</td>
<td>30.0</td>
<td>29.9</td>
<td>7.1</td>
<td>20:39</td>
<td>2,0,3,0</td>
</tr>
<tr>
<td>C</td>
<td>297</td>
<td>9/7/04</td>
<td>28.3</td>
<td>26.2</td>
<td>8.1</td>
<td>20:23</td>
<td>1,3,4,3</td>
</tr>
<tr>
<td>D</td>
<td>269</td>
<td>6/24/04</td>
<td>32.4</td>
<td>28.5</td>
<td>7.4</td>
<td>20:50</td>
<td>0,0,1,4</td>
</tr>
<tr>
<td>D</td>
<td>269</td>
<td>7/19/04</td>
<td>27.9</td>
<td>23.6</td>
<td>5.1</td>
<td>23:19</td>
<td>0,0,0,0</td>
</tr>
<tr>
<td>E</td>
<td>274</td>
<td>8/15/04</td>
<td>29.1</td>
<td>29.4</td>
<td>8.5</td>
<td>20:26</td>
<td>3,4,3,2</td>
</tr>
<tr>
<td>E</td>
<td>274</td>
<td>9/12/04</td>
<td>29.2</td>
<td>29.1</td>
<td>5.4</td>
<td>23:43</td>
<td>0,0,1,0</td>
</tr>
<tr>
<td>E</td>
<td>295</td>
<td>7/22/04</td>
<td>29.9</td>
<td>30.9</td>
<td>8.4</td>
<td>21:42</td>
<td>3,2,2,2</td>
</tr>
<tr>
<td>E</td>
<td>295</td>
<td>9/12/04</td>
<td>29.0</td>
<td>23.3</td>
<td>6.52</td>
<td>23:09</td>
<td>0,1,0,1</td>
</tr>
<tr>
<td>E</td>
<td>353</td>
<td>6/30/04</td>
<td>31.5</td>
<td>33.0</td>
<td>7.8</td>
<td>21:44</td>
<td>0,0,4,0</td>
</tr>
<tr>
<td>E</td>
<td>353</td>
<td>7/22/04</td>
<td>30.2</td>
<td>32.1</td>
<td>9.4</td>
<td>23:04</td>
<td>0,0,2,0</td>
</tr>
<tr>
<td>F</td>
<td>391</td>
<td>7/23/04</td>
<td>29.9</td>
<td>31.8</td>
<td>6.9</td>
<td>21:04</td>
<td>3,3,2,2</td>
</tr>
<tr>
<td>F</td>
<td>391</td>
<td>9/13/04</td>
<td>28.6</td>
<td>27.8</td>
<td>5.0</td>
<td>22:45</td>
<td>1,0,0,0</td>
</tr>
</tbody>
</table>
Figure 10 2004 Tampa Bay hydrophone survey sampled grids. Sampled grids are indicated by the pink/coral color.

2004 Tampa Bay Hydrophone Survey
Grids Sampled
**Geographic distribution of spotted seatrout courtship sounds**

Spotted seatrout sounds (all numerical categories) were detected at approximately one-third of all stations (n=758) sampled (Figure 11) throughout Tampa Bay. The majority of the sounds (13% of all sampled stations) were made by 1-2 individual spotted seatrout (Table 5). The sound categories of 3-5 individuals, small aggregations, and large aggregations (regardless of distance from the hydrophone) were each present at roughly 8% of all stations sampled.

Although large aggregations were detected throughout most of Tampa Bay, they were not equally distributed amongst the seven regions. They occurred most commonly in the lower bay and the eastern region of the middle bay (Figure 12). No large aggregations were detected in Hillsborough Bay. The regional differences were significant ($\chi^2, n = 758, p<0.001$) (Figure 13). Compared to the overall expected frequency ($H_o=7.7\%$) of large aggregations throughout Tampa Bay, regions A, C and G had fewer aggregations than expected while regions B, D, E, and F had more (Figure 14). However, only the differences in regions C ($\chi^2, n = 758, p<0.01$), E ($\chi^2, n = 758, p<0.05$), and G were significant ($\chi^2, n = 758, p<0.01$).

Most aggregations (95%) occurred in shoreline grids rather than offshore. These differences were statistically significant ($\chi^2, n = 58, p<0.001$). Although three stations with large aggregations occurred in open water grids, these stations had characteristics similar to shoreline grids. Depth did not exceed 3.3 m at any of these stations and two of these three stations had SAV substrata while the other station was a non-channel substratum.
Figure 11 2004 Tampa Bay hydrophone survey spotted seatrout detections.

2004 Tampa Bay Hydrophone Survey
Cynoscion nebulosus
Table 5 Spotted seatrout detections by category and distance.

<table>
<thead>
<tr>
<th>Spotted Seatrout Category</th>
<th>Number of Stations “Directly on–top of”</th>
<th>Number of Stations “Close by”</th>
<th>Number of Stations “In the distance”</th>
<th>Total Number of Stations</th>
<th>Percent Stations Sampled (total = 758)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 individuals</td>
<td>3</td>
<td>33</td>
<td>62</td>
<td>98</td>
<td>12.9%</td>
</tr>
<tr>
<td>3-5 individuals</td>
<td>0</td>
<td>28</td>
<td>35</td>
<td>63</td>
<td>8.3%</td>
</tr>
<tr>
<td>Small Aggregation</td>
<td>0</td>
<td>35</td>
<td>24</td>
<td>59</td>
<td>7.8%</td>
</tr>
<tr>
<td>Large Aggregation</td>
<td>0</td>
<td>39</td>
<td>19</td>
<td>58</td>
<td>7.6%</td>
</tr>
</tbody>
</table>
Figure 12 2004 Tampa Bay hydrophone survey spotted seatrout large aggregation stations.

Tampa Bay Hydrophone Survey
*Cynoscion nebulosus* Aggregations

Legend:
- Large Aggregation
- All Other Sites
- Close-by
- In The Distance

Regions:
- Region A
- Region B
- Region C
- Region D
- Region G
- Region F
Figure 13 The number of stations within a region where a spotted seatrout large aggregation was detected (black) and the total number of stations sampled within that region (gray). The percent of stations within a region that had large aggregation detections is listed above the bar.
Figure 14 Large aggregation percent by region compared to the expected percent (7.7%) of large aggregation detections.

Large aggregations occurred most frequently over SAV substratum. The frequency of large aggregations differed significantly by substrate type ($X^2$, $n=758$, $p<0.001$). Although non-channel was the most frequently sampled substratum ($n=482$), it had the lowest association (1.7% of stations sampled) with large aggregations (Table 6). Roughly one-quarter of the stations sampled over SAV had large aggregations nearby, the largest percentage of any substrata type (Table 6). SAV was significantly higher and non-channel areas were significantly lower from the expected substrata frequency (SAV: $X^2$, $n=758$, $p<0.001$).
n = 39, p < 0.001, non-channel: $X^2$, n = 39, p < 0.001). SAV was present in all regions with the exception of Hillsborough Bay, but aggregation detections over SAV only occurred in regions D, E, and F. Large aggregations rarely occurred (4.0% of stations sampled) in channels (Table 6). Structure was the least sampled substratum but associated with large aggregation sound more frequently than channel or non-channel (8.2% of stations sampled) with two stations at old range markers and two stations at bridges.

Table 6 Substrata at all sampled stations and stations with large spotted seatrout aggregations categorized as “close-by”. Percent of each substratum used by “close-by” large aggregations is indicated.

<table>
<thead>
<tr>
<th>Substrata</th>
<th>Channel</th>
<th>Non-Channel</th>
<th>Structure</th>
<th>SAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Sampled Stations</td>
<td>150</td>
<td>482</td>
<td>49</td>
<td>77</td>
</tr>
<tr>
<td>Large Aggregation Stations</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>% Used by Large Aggregations</td>
<td>4.0%</td>
<td>1.7%</td>
<td>8.2%</td>
<td>27.3%</td>
</tr>
</tbody>
</table>

31
Environmental variables associated with spotted seatrout courtship sounds

Depth of large aggregation stations was significantly shallower than that of stations without large aggregations (Mann-Whitney Rank Sum test, n=758, p<0.001). Mean depth of stations containing large aggregations categorized as “close-by” was 2.8 m, ranging between 1.6-8.2 m (Figure 15).

Figure 15 Mean depth (m) of stations without large aggregations versus large aggregation stations, +/- one standard deviation. Numbers above and below error bars are the number sampled.
The largest depth value associated with a large aggregation (8.2 m) was taken from under a bridge. All stations without large aggregations had an average depth of 4.2 m, ranging between 1.5-21.4 m. Mean depth of stations containing large aggregations by region ranged from 2.3 m (n=14) in region D to 3.4 m in regions B & C (n=5). In comparison, mean depth of stations without aggregations varied from 3.0 m (n=97) in region A to 5.3 m (n=109) in region E. As spotted seatrout presence was also analyzed according to grid type (shoreline versus open water) and grid type was most likely related to depth, differences in mean grid type depth were examined. Significant differences between depths of shoreline and open water-categorized grids verified these categories were likely a function of depth (Mann-Whitney Rank Sum test, n=758, p<0.001).

As stations containing large aggregations (“close-by”) were found in shallower, shoreline areas of the bay, the water temperature was significantly warmer at these stations than at stations without aggregations in the deeper, open water locations (Mann-Whitney Rank Sum test, n=758, p<0.001). Stations containing large aggregations had an average temperature of 30.3 °C while all other stations averaged 29.3 °C (Figure 16). Also temperature range was smaller at stations containing large aggregations (28.0-31.8 °C) than at stations without aggregations (24.4-33.8 °C). Regionally, mean temperature was relatively consistent for stations without aggregations, ranging from 28.9 °C (n= 96) in region B to 29.9 °C (n=113) in region F.
**Figure 16** Mean temperature (°C) of stations without large aggregations versus large aggregation stations, +/- one standard deviation. Numbers above and below error bars are the number sampled.

Salinity differences between “close-by” stations with and without large aggregations were marginally different (Mann-Whitney Rank Sum test, n=758, p=0.05). Mean salinity across all regions of stations containing large aggregations was 27.6 ppt and stations without aggregations was 26.2 ppt (Figure 17). The salinity range of stations containing large aggregations was 18.3-34.5 ppt while stations without aggregations had a larger range between 13.1-35.4 ppt. Salinity averages at stations of both large aggregations and non-aggregations varied by regions with the two averages within one or two parts-per-thousands of one another at each region.
Figure 17 Mean salinity (ppt) of stations without large aggregations versus large aggregation stations, +/- one standard deviation. Numbers above and below error bars are the number sampled.

Dissolved oxygen (DO) was significantly greater at “close-by” stations containing large aggregations than at stations without aggregations (Mann-Whitney Rank Sum test, n=758, p<0.001). DO values ranged between 5.3-9.9 mg/L for stations with large aggregations and averaged 7.6 mg/L (Figure 18). Stations without aggregations experienced a much broader DO range of 0.2-12.61 mg/L, averaging 6.5 mg/L. The majority (82%) of stations with large aggregations were found at DO values greater than this non-aggregation mean (6.5 mg/L). Regionally, DO of stations with large
aggregations ranged from 6.9 mg/L (n=14) in region D to 8.3 mg/L in region A (n=3) and region E (n=11). Mean regional DO values of stations without aggregations ranged from 5.6 mg/L in region G (n=90) to 7.2 mg/L in region E (n=109).

**Figure 18** Mean dissolved oxygen (mg/L) of stations without large aggregations versus large aggregation stations, +/- one standard deviation. Numbers above and below error bars are the number sampled.
Comparison of spotted seatrout spawning locations with other sciaenids

Although congeners, sand seatrout used different areas to spawn and were more commonly detected within Tampa Bay than spotted seatrout. Sand seatrout sounds were detected more frequently (53% of stations, n=758) than spotted seatrout sounds (Table 7). Aggregation-level sound was also more common in sand seatrout (40% of all stations, n=758) than spotted seatrout (15% of all stations). Sand seatrout aggregations were detected in all regions (Figure 19) whereas spotted seatrout aggregations were nearly absent in region G (n=1) and region C (n=5) (Figure 20). Although sand seatrout aggregations were more equally distributed geographically than spotted seatrout (Figure 21), regional differences in sand seatrout aggregations were significant ($\chi^2$, n =758, p<0.001).

Table 7 Sand seatrout detections by category and distance.

<table>
<thead>
<tr>
<th>Sand Seatrout Category</th>
<th>Number of Stations “Directly on–top of”</th>
<th>Number of Stations “Close-by”</th>
<th>Number of Stations “In the distance”</th>
<th>Total Number of Stations</th>
<th>Percent Stations Sampled (total = 758)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 individuals</td>
<td>0</td>
<td>27</td>
<td>36</td>
<td>63</td>
<td>8.3%</td>
</tr>
<tr>
<td>3-5 individuals</td>
<td>1</td>
<td>30</td>
<td>5</td>
<td>36</td>
<td>4.7%</td>
</tr>
<tr>
<td>Aggregation</td>
<td>26</td>
<td>180</td>
<td>100</td>
<td>306</td>
<td>40.4%</td>
</tr>
</tbody>
</table>
Figure 19 2004 Tampa Bay hydrophone survey sand seatrout detections.
Figure 20 2004 Tampa Bay hydrophone survey spotted seatrout and sand seatrout aggregations.

2004 Tampa Bay Hydrophone Survey
*C. nebulosus* and *C. arenarius*
**Figure 21** The number of stations within a region a sand seatrout aggregation was detected (black) and the total number of stations sampled within that region (gray). The percent of stations within a region that had large aggregation detections is listed above the bar.

Most sand seatrout aggregations (70.3%, n=306) occurred in open water grids ($X^2$, n = 758, p<0.001) while most spotted seatrout aggregations occurred in shoreline grids. The frequency of sand seatrout aggregations differed significantly by substrate type ($X^2$, n=758, p<0.001). Non-channel was the most frequently used substratum (34.6%, n=206) by sand seatrout aggregations while SAV was used the least (Table 8), the opposite of what was seen with spotted seatrout.
Table 8 Substrata at all sampled stations and stations with sand seatrout aggregations and percent of each substratum used by aggregation stations.

<table>
<thead>
<tr>
<th>Substrata</th>
<th>Channel</th>
<th>Non-Channel</th>
<th>Structure</th>
<th>SAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Sampled Stations</td>
<td>150</td>
<td>482</td>
<td>49</td>
<td>77</td>
</tr>
<tr>
<td>Aggregation Stations</td>
<td>23</td>
<td>167</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>% Used by Aggregations</td>
<td>15.3%</td>
<td>34.6%</td>
<td>26.5%</td>
<td>3.9%</td>
</tr>
</tbody>
</table>

Mean depth of sand seatrout aggregations (5.5 m, n=206) was nearly double that of spotted seatrout aggregations across all regions (Figure 22). Depths associated with sand seatrout aggregations (1.8-16.1 m) also exhibited a wider range than values associated with spotted seatrout aggregations. Mean temperature (29.2 °C), salinity (26.5 ppt), and dissolved oxygen (6.2 mg/L) for sand seatrout aggregations were lower than those associated with spotted seatrout aggregation stations (Table 9).
Figure 22 Mean depth (m) of spotted seatrout and sand seatrout aggregation stations, +/- one standard deviation. Numbers above and below error bars are the number sampled.

Table 9 Environmental variables at stations where all three sciaenid species (spotted seatrout, sand seatrout, silver perch) were detected at aggregation levels simultaneously.

<table>
<thead>
<tr>
<th>Species</th>
<th>Depth (m)</th>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>Dissolved Oxygen (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spotted seatrout</td>
<td>2.8</td>
<td>30.1</td>
<td>28.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Sand seatrout</td>
<td>5.5</td>
<td>29.2</td>
<td>26.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Silver perch</td>
<td>3.9</td>
<td>28.5</td>
<td>27.9</td>
<td>6.6</td>
</tr>
<tr>
<td>All 3 species</td>
<td>4.2</td>
<td>30.1</td>
<td>29.3</td>
<td>6.8</td>
</tr>
</tbody>
</table>
Silver perch were also heard more frequently than spotted seatrout but less frequently than sand seatrout (Table 10). Silver perch courtship sounds were heard at almost half of the stations (43.2 %, n= 758) and aggregation level sound (24%) was the most frequently detected silver perch sound category (Figure 23). In contrast to spotted seatrout and sand seatrout, silver perch aggregations were much more evenly distributed geographically (Figure 24) without significant regional differences ($X^2$, n =181, p=0.069). All of the regions had between 15-33% of their sampled stations categorized as silver perch aggregation locations (Figure 25).

Table 10 Silver perch detections by category and distance.

<table>
<thead>
<tr>
<th>Silver Perch Category</th>
<th>Number of Stations “Directly on-top of”</th>
<th>Number of Stations “Close-by”</th>
<th>Number of Stations “In the distance”</th>
<th>Total Number of Stations</th>
<th>Percent Stations Sampled (total = 758)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2 individuals</td>
<td>2</td>
<td>39</td>
<td>31</td>
<td>72</td>
<td>9.5%</td>
</tr>
<tr>
<td>3-5 individuals</td>
<td>3</td>
<td>49</td>
<td>22</td>
<td>74</td>
<td>9.8%</td>
</tr>
<tr>
<td>Aggregation</td>
<td>25</td>
<td>104</td>
<td>52</td>
<td>181</td>
<td>23.9%</td>
</tr>
</tbody>
</table>
Figure 23 2004 Tampa Bay hydrophone survey silver perch detections.

**2004 Tampa Bay Hydrophone Survey**

*Bairdiella chrysoura*

![Map of Tampa Bay hydrophone survey results showing detections in various regions.](image)

**LEGEND**
- Aggregation
- 3-5 Individuals
- 1-2 Individuals
- "Directly On-Top Of"
- "Close-By"
- "In The Distance"
- No *B. chrysoura* Heard
Figure 24 2004 Tampa Bay hydrophone survey silver perch, spotted seatrout, and sand seatrout aggregation detections.

2004 Tampa Bay Hydrophone Survey
*C. nebulosus, C. arenarius, and B. chrysoura*
**Figure 25** The number of stations within a region a silver perch aggregation was detected (black) and the total number of stations sampled within that region (gray). The percent of stations within a region that had large aggregation detections is listed above the bar.

Silver perch aggregation presence was similar at stations from both shoreline and open water grids ($X^2$, $n = 758$, $p=0.198$). Silver perch aggregations also did not differ significantly by substrate type ($X^2$, $n = 758$, $p=0.162$), although they were most frequently detected over SAV (Table 11). Mean depth of silver perch aggregations (3.9 m, $n=129$) was between the mean depths of spotted seatrout and sand seatrout, as was mean DO (Table 9). Mean values of temperature (28.5 °C) at station with aggregations were less for silver perch than the other sciaenids, whereas mean salinities at stations with aggregations were similar for all three species.
Table 11 Substrata at all sampled stations and stations with silver perch aggregations and percent of each substratum used by aggregation stations.

<table>
<thead>
<tr>
<th>Substrata</th>
<th>Channel</th>
<th>Non-Channel</th>
<th>Structure</th>
<th>SAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Sampled Stations</td>
<td>150</td>
<td>482</td>
<td>49</td>
<td>77</td>
</tr>
<tr>
<td>Aggregation Stations</td>
<td>19</td>
<td>87</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>% Used by Aggregations</td>
<td>12.7%</td>
<td>18.0%</td>
<td>10.2%</td>
<td>23.4%</td>
</tr>
</tbody>
</table>

Simultaneous detection of a spotted seatrout aggregation with at least one other sciaenid aggregation occurred at nearly one-quarter (n=29) of stations with spotted seatrout aggregations (n=117). Spotted seatrout aggregations were more commonly detected with sand seatrout aggregations (n=26) than with silver perch aggregations (n=13). At only ten stations (1.3% of all sampled stations, n=758) were all three species simultaneously detected at aggregation levels. When aggregations of all three species were heard at a station, they were always heard in some combination of close-by or in the distance (Table 12). The stations where all three species were detected simultaneously occurred in five of the seven regions (regions B, D, E, F, and H) and the majority (n=6) occurred in shoreline-categorized grids and over non-channel substratum (n=4). Mean salinity at these stations was greater than at aggregation stations for any one of the species, whereas average depth, temperature, and DO were intermediate (Table 9).
Table 12 Stations where all three sciaenid species (spotted seatrout, sand seatrout, silver perch) were detected at aggregation levels simultaneously. Aggregation distance categories are:
1: “directly on-top of”, 2: “close-by”, or 3: “in the distance”.

<table>
<thead>
<tr>
<th>Date</th>
<th>Region</th>
<th>Substrata</th>
<th>Depth (m)</th>
<th>Spotted seatrout aggregation distance</th>
<th>Sand seatrout aggregation distance</th>
<th>Silver perch aggregation distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/26/04</td>
<td>B</td>
<td>Structure</td>
<td>5.1</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6/2/04</td>
<td>D</td>
<td>SAV</td>
<td>1.9</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6/2/04</td>
<td>D</td>
<td>SAV</td>
<td>1.9</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6/9/04</td>
<td>E</td>
<td>Structure</td>
<td>2.4</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6/9/04</td>
<td>E</td>
<td>Channel</td>
<td>10.9</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>7/1/04</td>
<td>F</td>
<td>Non-channel</td>
<td>3.1</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7/19/04</td>
<td>D</td>
<td>Non-channel</td>
<td>4.8</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>7/29/04</td>
<td>H</td>
<td>Non-channel</td>
<td>2.0</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8/6/04</td>
<td>B</td>
<td>Structure</td>
<td>5.6</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8/16/04</td>
<td>F</td>
<td>Non-channel</td>
<td>3.8</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Analysis of spotted seatrout courtship sounds

There was not a significant relationship between decibel level (in the 200-300 Hz frequency range) and abundance category assigned by ear. There was a large range in decibel level within each category and much overlap between categories (R²=0.08) (Figure 26). The large aggregation category had the least spread in sound level but still overlapped extensively with the other categorical ranges. After reducing the data set to those recordings with only spotted seatrout calling, there still remained a large amount of overlap (R²=0.20) (Figure 27). Similarly, even after correcting for distance by using only recordings of spotted seatrout close-by, (Figure 28) there was no clear relationship (R²=0.14).
Figure 26 Decibel level within 200-300 Hz frequency range corresponding to number of spotted seatrout categorized by ear. All of the plotted recordings do not exclusively contain spotted seatrout calls and other sciaenid species may be present. Estimated number of spotted seatrout were categorized as either: 1=1-2 individuals, 2=3-5 individuals, 3=Small aggregation, 4=Large aggregation
Figure 27 Decibel level within 200-300 Hz frequency range corresponding to number of spotted seatrout categorized by ear. All of the plotted recordings are exclusively spotted seatrout calls. Estimated number of spotted seatrout were categorized as either: 1=1-2 individuals, 2=3-5 individuals, 3=Small aggregation, 4=Large aggregation

\[ y = 0.0501x - 1.5703 \]

\[ R^2 = 0.1966 \]
Figure 28 Decibel level within 200-300 Hz frequency range corresponding to number of spotted seatrout categorized by ear. All of the plotted recordings are exclusively spotted seatrout calls detected close-by. Estimated number of spotted seatrout were categorized as either: 1=1-2 individuals, 2=3-5 individuals, 3=Small aggregation, 4=Large aggregation.

\[
y = 0.0527x - 1.9116 \\
R^2 = 0.141
\]
DISCUSSION

**Geographic distribution and environmental variables associated with spotted seatrout spawning**

Spotted seatrout spawning occurred in all zones (lower, middle and upper bay) except Hillsborough Bay, and over a wide range of salinities. Most aggregation-level sound occurred in the lower bay zone and eastern middle region; areas previously reported as having spawning activity (McMichael and Peters, 1989; Lowerre-Barbieri, 2004). Although spawning did not occur as frequently in the upper bay zone, presence of large aggregations indicated that spotted seatrout were not restricted to areas in proximity to the Gulf of Mexico. Mature spotted seatrout have been reported to move to higher salinity areas in the summer months to spawn (Helser, et al., 1993). However, Tampa Bay spotted seatrout spawning locations were present across the latitudinal expanse of the bay and there was only a marginal difference in salinity between aggregation and non-aggregation stations. Although salinity affects spotted seatrout egg buoyancy and diameter as well as juvenile survival (Kucera et al., 2002; Holt and Holt, 2003), spotted seatrout are capable of spawning in a wide range of salinity. Optimal spawning salinities ranged from 15 ppt and 21 ppt in Louisiana (Saucier and Baltz, 1993) and spawning studies conducted in captivity generally maintained salinity between 25-30 ppt (Arnold et al., 1976; Brown-Peterson et al., 1988; Gray et al., 1991) although one study kept salinity within approximately 1 ppt of 35.4 ppt (Wisner et al., 1996). The spread of spawning salinity values range from 7.0 ppt to 25.8 ppt in Louisiana (Saucier and Baltz, 1993) while Tampa Bay larvae were collected between 18 ppt and 32 ppt (McMichael and Peters, 1989). Even higher salinity values were associated with larval collections from
other estuaries with values of 36 ppt (Peebles and Tolley, 1988) and 48 ppt (Holt and Holt, 2003). The moderate range of spawning salinities in Tampa Bay (18.3-34.5 ppt) as well as the range in non-spawning areas (13.1-35.4 ppt) were within the reported range of spawning salinities for spotted seatrout, suggesting that in Tampa Bay salinity is not influential in determining spawning location provided extreme values are not involved.

The percentage of locations with spawning aggregation differed regionally and was greater in those regions with more shoreline grids and SAV substrata. When compared to the percentage of aggregations throughout Tampa Bay (7.7%), the western region of the lower bay had significantly more aggregations and the western region of the middle bay had significantly less, while their counterparts did not differ from this expected number. While the number of aggregations in the two upper bay regions did not differ from the expected, Hillsborough Bay, sharing similar latitude with the upper bay, had less than the expected with no large aggregation detections. The east/west discrepancies in the lower bay are likely attributable to both available and sampled substrata. The western portion of the lower bay has more areas categorized by SAV than its eastern counterpart (or any other region) and it also had more stations sampled over SAV. Other variables, such as depth, temperature, salinity, and dissolved oxygen were similar between the two regions. Discrepancies in the middle bay could again be a result of reduced potential spawning habitat in the western region. Shoreline grids held 95% of spawning locations and in all regions but the western region of the middle bay, between 43-78% of grids were categorized as shoreline and sampled according to this percent. Conversely, the middle bay western region only had 25% of grids categorized as shoreline. This region also had the fewest stations sampled over SAV (besides
Hillsborough Bay where no SAV was ever detected) whereas its eastern counterpart had three times the amount of stations sampled over SAV.

Although spawning aggregations were detected in both regions of the upper bay zone, Hillsborough Bay, a zone of similar latitude, was devoid of spotted seatrout spawning. Larvae have been collected at the southern most end of Hillsborough Bay, although this area had a smaller amount collected than the middle and lower bay areas (McMichael and Peters, 1989). Hillsborough Bay traditionally has the poorest water quality and consistently experiences hypoxia (Janicki, 2001; Janicki, 2001). Reduced abundance of fish and crustaceans, poor flushing rates, low dissolved oxygen values, and seagrass loss make Hillsborough Bay a poor nursery habitat (Sykes and Finucane, 1966; Taylor, 1970; Lewis and Estevez, 1988) as well as spawning habitat. Although SAV coverage has increased from complete absence in 1982 to an estimated 192 acres in 1999, Hillsborough Bay still has the least amount of SAV coverage of any Tampa Bay region (Tomasko, 2002). It also had the lowest mean value of dissolved oxygen of all regions, likely a connection with relatively little SAV, the key spotted seatrout spawning substratum.

SAV areas, especially when in proximity to channels or bottom contours, have consistently been reported as spotted seatrout spawning habitat (Mok and Gilmore, 1983; Holt, et al., 1985; Brown-Peterson, et al., 1988; Crabtree and Adams, 1995). Spawning site selection has been attributed to placing early life history stages in or near habitat types that will foster growth and survival (Peebles and Tolley, 1988) and early stage spotted seatrout eggs have been consistently collected over or near seagrass beds (Holt et al., 1985). SAV is also essential habitat for spotted seatrout larvae (Holt and Holt, 2000)
and was the most important habitat variable associated with young-of-the-year spotted seatrout in Tampa Bay (McMichael and Peters, 1989; Nelson and Leffler, 2001). Spotted seatrout have been reported to spawn in a wide variety of habitats, besides those associated with SAV including deep moving water between barrier islands (Saucier and Baltz, 1993), large bridges (Saucier and Baltz, 1992), and barrier island beaches as well as on natural sand and shell reefs (Hein and Shepard, 1979). Other areas within the spotted seatrout range that have little or no SAV support spawning over available substrata such as soft bottom, oyster beds, and tidal marshes (Mahood, 1975; Brown-Peterson and Warren, 2001; Lowerre-Barbieri et al., in review). In Tampa Bay, at approximately 75% of the stations with SAV substratum, spotted seatrout aggregations were not detected. Use of SAV as spawning habitat also varied regionally. Although SAV was present in all regions with the exception of Hillsborough Bay, no aggregation detections over SAV occurred in the upper bay or the western region of the middle bay. Spawning aggregations were located over all surveyed substrata types in Tampa Bay with structure as the most frequently used substratum following SAV. The four spawning aggregations associated with structure were split between bridges and old range markers. However, both the bridges and old range markers were in the vicinity of SAV.

Spawning locations were primarily located in shallow, shoreline areas of Tampa Bay. Although the average depth varied regionally, aggregations consistently used shallow areas regardless of the available depth. As most aggregations were detected over SAV, mean depth of aggregation stations was relatively shallow. The upper range of mean depth (3.4 m) associated with stations with aggregations occurred in two regions (western upper and middle bay) where aggregations were not detected over SAV. The
deepest area used for spawning (8.2 m) was associated with an approximately 450 m-long bridge under the main span.

Deeper areas have been implicated as spawning habitat in other studies. Optimal spawning depth in a Louisiana acoustic study was reported between 4.0-8.0 m, with mean depth of aggregation sound occurring at 5.2 m (Saucier and Baltz, 1993). Spotted seatrout aggregations were acoustically detected in Indian River Lagoon, Florida over a deep channel area and in shallow SAV habitats (Mok and Gilmore, 1983). Similarly, gravid spotted seatrout females were collected in varying depths of water in Barataria Bay, Louisiana (Hein and Shepard, 1979). However, in Tampa Bay, the distinct differences between mean depths at aggregation and non-aggregation stations demonstrate the influence of this variable both independently and as an associate with substrata type. As temperature is also a function of depth, spawning locations were associated with warmer water temperatures than in areas devoid of spawning.

Temperature is reported to affect spotted seatrout reproductive output (Brown-Peterson et al., 1988; Kupschus, 2004). Initiation of spawning has been linked to temperature, with spawning onset paralleling a 5 °C increase to 23 °C (Brown-Peterson et al., 1988). The lowest reported temperature at which spawning occurs (from Tampa Bay larvae back-calculations) is 20.4 °C (McMichael and Peters, 1989). Tampa Bay spotted seatrout began spawning at least one month later than usual in 2004 and this delay is likely due to cooler spring water temperatures. Although the hydrophone survey started at the beginning of April, large aggregation sounds were not detected until May when the water temperature rose significantly. The first aggregation was detected at 29 °C, and average water temperature of spawning stations (30.3 °C) throughout the season was
similar to the predicted optimum reproductive temperature (Kupschus, 2004; 29 °C) and concurrent with other reported ranges of adult courtship calls and young-of-the-year (Saucier and Baltz, 1992; 27.5-28.8 °C; Saucier and Baltz, 1993; 24.5-33.5 °C; Nelson and Leffler, 2001; 29.9-30.4 °C). Spawning stations were consistently located in warmer areas of the bay. This relationship is likely attributable to depth, as aggregations were habitually located in the shallow, shoreline areas of Tampa Bay.

**Comparison of spawning locations between spotted seatrout and other sciaenids**

Aggregation sounds of spotted seatrout rarely overlapped with aggregation sounds of other sciaenids. Silver perch were found across all regions, substrate types, and grid types. Conversely, spotted seatrout and sand seatrout used specific areas of the bay for spawning, indicating they may actively select spawning habitats. Similarly, in the Indian River Lagoon, Florida, silver perch aggregations were broadly distributed along the Intracoastal Waterway (ICW) whereas spotted seatrout aggregations were predominantly located within a specific southern section (Mok and Gilmore, 1983). While the primary silver perch spawning aggregation was located in the ICW, smaller aggregations were detected in shallower areas, some characterized by SAV (Mok and Gilmore, 1983). Spotted seatrout spawning locations shifted temporally, with isolated aggregations occurring over shallow SAV early in the season, with a shift to deeper SAV areas as well as in the ICW later in the season (Mok and Gilmore, 1983).

Differences in substrate and depth were the primary distinctions between spotted seatrout and sand seatrout spawning habitats. Although spotted seatrout and sand seatrout are congeneres and have courtship calls of similar frequency, their use of distinctly different habitats within the estuary appears to segregate the two and minimize the
opportunity for cross-species fertilization. Spotted seatrout and sand seatrout are able to hybridize, and sand seatrout have been shown to hybridize frequently with weakfish (Cynoscion regalis) along the east coast of Florida, but there have not been many spotted seatrout/sand seatrout hybrids detected in Tampa Bay (Mike Tringali, pers. comm.).

Sand seatrout as well as silver perch also had a much higher percentage of aggregation detections than spotted seatrout. A number of factors could be responsible, including: (1) varying abundance by species; (2) species-specific variation in the level of sound associated with spawning; and/or (3) the interaction between species-specific spawning diel periodicities and the sampling window. Acoustic interactions between silver perch and spotted seatrout in the Indian River Lagoon indicated that differing diel periodicities may result from the two species sharing overlapping spawning locations (Mok and Gilmore, 1983). As peak acoustic activity of silver perch occurred later in the evening during the months when spotted seatrout were spawning, it was suggested that spotted seatrout were responsible for delaying the daily start time of the silver perch aggregation sounds. A similar pattern has been observed at Bunces Pass. The silver perch spawning season begins earlier than the spotted seatrout season with the diel periodicity of the silver perch aggregation shifting to later in the evening once aggregation sounds of spotted seatrout have begun (Sarah Walters, pers. obs). If these three species have different diel periodicities associated with aggregation calls, then the survey could potentially miss aggregation calls depending on the time certain habitats were sampled.

Methodology review

The mobile hydrophone survey is an excellent methodology for assessing the geographic distribution of courtship calls associated with spawning. However, a few
weaknesses are associated with this type of method and should be addressed. First, because of the nature of the survey, only one boat and one hydrophone were used to assess presence/absence of species-specific calls. Although distance of these calls were estimated, it is not possible to accurately assess the true distance of these fish without multiple hydrophones and sound propagation studies. As each station has different substrata, depth, and acoustic interferences, additional testing would have to be conducted at each station in order to accurately determine the distance between the sound source and the hydrophone. However, more traditional techniques to assess spawning location can experience similar problems. Planktonic eggs and larvae can be quickly dispersed by tides, current, and wind from their original spawning site. Similarly, adult capture may occur just prior to spawning, but before the fish are on the spawning grounds.

Another methodology issue involves the sampling window used during the study. The five hour window (starting at sunset, roughly 20:00 EDT, and continuing until approximately 01:00 EDT) did not account for variability of aggregation-associated sound duration within the spawning season. Preliminary examination of the Bunces Pass spawning aggregation diel periodicity indicated that although the average daily duration of aggregation-associated sound over the spawning season was 5.9 hours, duration ranged from 3.0-12.3 hours (Walters et al., in review). Start and end times of aggregation sound varied as well, with the earliest start time beginning at 17:00 EDT and the latest start time occurring 1.5 hours after sunset at 22:04 EDT. End times ranged from 20:44 EDT to 05:30 EDT. Roughly a quarter of days did not have aggregation sound beginning until after sunset. As sunset was the designated sampling start time for the hydrophone survey
and there appears to be diel variability associated with the start and duration of
aggregation-associated sound, it is possible that hydrophone sampling occurred outside
the window of peak sound production. Other studies have reported shorter spawning
durations as well. Holt et al. (1985) found eggs only for a three hour period around
sunset, estimating a spawning window between two hours pre-sunset and 2.5 post-sunset.

Although the LARS at Bunces Pass assisted in reporting spawning aggregation
diel trends, it was only one location used to represent the entire bay. Additionally, the
spawning aggregation at Bunces Pass is one of the largest aggregations in Tampa Bay
and one of the only aggregations detected in a Gulf pass. As an anomaly, the spawning
aggregation at Bunces Pass may behave differently than other estuarine spawning
aggregations. Additional LARS at other spawning aggregation locations in Tampa Bay
would help compare the trends at Bunces Pass to these sites as well as those sampled in
the mobile hydrophone survey. These additional permanent monitors would provide
further resolution to the diel and seasonal spawning periodicities within Tampa Bay.

Multiple studies have verified that sound production is associated with spawning
by coupling acoustic sampling with egg collection (Mok and Gilmore, 1983; Saucier and
Baltz, 1992; Saucier and Baltz, 1993; Gilmore, 1994; Luczkovich et al., 1999), and adult
collection (Crabtree and Adams, 1995; Luczkovich et al., 1999; Lowerre-Barbieri, 2004).
However, further research is necessary to determine the level of sound that is consistently
associated with gamete release. Spotted seatrout were only considered to be spawning in
large aggregations in this study in order to be conservative but spawning could be
occurring in the smaller aggregations as well as with 3-5 individuals. Conversely,
spawning may not be occurring throughout the entire duration of aggregation sound.
**Analysis of spotted seatrout courtship sounds**

Signal processing, using decibel level within a given frequency range, did not match spotted seatrout abundance categories assigned by the human ear. Ultimately, it is not possible to pair decibel ranges with fish number categories as each individual category does not use an exclusive decibel range. More complex signal processing strategies are needed to account for differences in sound levels that might be found in different situations. For example, one close-by spotted seatrout call could be louder than an aggregation located in the distance from the hydrophone. Aggregation density may also influence the decibel level. These signal processing issues must be solved before this type of analysis can be used to assess species, number, and distance.


