Characterization of critical spawning habitats of weakfish, spotted seatrout and red drum in Pamlico Sound using hydrophone surveys

Submitted by:

Principal Investigators

Joseph J. Luczkovich[1], Hal J. Daniel, III[2], and Mark W. Sprague[3]

Graduate Research Assistants

Stephen E. Johnson[2], R. Christopher Pullinger[2], Todd Jenkins[3], and Marcy Hutchinson[2]

[1]Institute for Coastal and Marine Resources, [2]Department of Biology, [3]Department of Physics
East Carolina University, Greenville, NC 27858

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EXECUTIVE SUMMARY

The exact locations of spawning areas used by marine fishes are needed to design marine reserves and protect spawning stocks from fishing activities. The location of spawning areas of soniferous fishes such as weakfish, *Cynoscion regalis*, spotted seatrout, *Cynoscion nebulous*, and red drum, *Sciaenops ocellatus*, can be determined by means of passive hydroacoustic surveys. We conducted nocturnal hydrophone surveys and pelagic egg surveys at sites near the Ocracoke and Hatteras Inlets and sites on the western side of Pamlico Sound to locate potential spawning areas during May - October 1997. After locating potential spawning areas, we used a stratified random sampling design to characterize the spatial and temporal variation (May through October 1998) in the drumming behaviors of the three sciaenid species in two regions in Pamlico Sound: one near Ocracoke Inlet and the other near the mouth of the Bay River. From these latter surveys, maps of likely spawning areas have been produced.

Hydrophones were suspended from a small boat anchored at ten or more locations every two weeks from May through October 1997, and digital audio tapes were made of drumming sounds, and the tapes were analyzed spectrographically. At these same locations, experimental gill nets were set out to capture mature fishes for age and gonadal-somatic-index estimation and plankton net tows were made to capture fish eggs and estimate their density. In 1997 and 1998, recordings of captive weakfish, spotted seatrout, and red drum in spawning condition were made in laboratory tanks and from fish caught by hook-and-line. Captive recordings of drumming fish were used to identify field recordings both by ear and spectrographically. Finally, in 1998, ten custom-built autonomous sonobuoys containing hydrophones, timers, and a cassette recorder were placed at 25 or more random locations each month and were programmed to record at night. The sonobuoys were used to locate potential spawning areas of weakfish, spotted seatrout and red drum on a monthly basis within the two 100 km^2 regions in Pamlico Sound. These potential spawning areas are presented as geographic information system (GIS) coverages in a series of maps generated using Arcview GIS base maps and National Oceanic and Atmospheric Administration/National Ocean Survey estuarine bathymetry for Pamlico Sound.

Weakfish "purring" sounds are produced by males and have been associated with spawning activity in the laboratory (Connaughton and Taylor 1996) and in the field (Connaughton and Taylor 1995, Luczkovich et al. in press). Weakfish "purring" sounds were recorded at all stations and most sonobuoy locations near Hatteras and Ocracoke inlets; very few weakfish were recorded away from the inlets. These sounds were detected on sonobuoys from May through September, but the highest drumming indexes were in May and June. Based on 24-hour sampling with sonobuoys, weakfish began "purring" before sunset at 1800 Eastern Daylight Time (EDT). The drumming index for weakfish purring was greatest at 2200 EDT. The sound production has highest in deep water (> 10 feet deep) sonobuoy sets; the prime spawning areas for weakfish thus seem to be in deeper channel areas near inlets and in the deep parts of Pamlico Sound. High salinities (> 20 ppt) were observed in association with high egg and sound production. Purring sounds reached a maximum of 127 dB (re 1 μPa) in sound pressure level for individual fish. Aggregations of weakfish and silver perch (*Bairdiella chrysoura*) were heard drumming in the same location; at these locations, sound pressure levels reached 147 dB (re 1 μPa). The maximum distance that an individual weakfish “purr” can be detected above the background sound, assuming a cylindrical spreading model, is approximately 50 m. Early-stage sciaenid eggs (<1 day old) were captured in plankton nets in great numbers at the inlet stations where large aggregations of fishes were detected acoustically. There was a strong association

(r = 0.78) between the $\log_{10}$-transformed abundance of early-stage sciaenid-type eggs and maximum sound pressure levels, with the greatest numbers occurring at the inlet stations. This association suggests that sound production may be measured as a surrogate for egg production and result in substantial savings in survey costs. Genetic identification of these sciaenid-type eggs using mitochondrial DNA restriction-fragment-length polymorphism (mtDNA-RFLP) method indicated that both weakfish eggs and silver perch eggs were collected in many of our samples where weakfish were heard drumming. However, silver perch eggs were smaller in diameter and could be separated from the weakfish eggs. Strong correlations were detected between weakfish acoustic signals and weakfish egg abundances; similarly silver perch acoustical signals and egg densities were correlated. Weakfish spawning areas were located near Ocracoke and Hatteras Inlet and spawning occurred from May through September, with a peak of egg production in May.

Spotted seatrout spawning occurred during May through September on both sides of Pamlico Sound. In 1997, spotted seatrout were recorded drumming at locations in Rose Bay, Jones Bay, Fisherman's Bay, Bay River, and near Ocracoke and Hatteras inlets. In 1998, spotted seatrout were heard drumming in both the Ocracoke and Bay River sonobuoy survey areas. There was an increase the drumming activity during the summer, with greatest drumming index values occurring in July. Most drumming by male spotted seatrout occurred after sunset and had ended by midnight. Spotted seatrout eggs were identified definitively using mtDNA-RFLP methods at Wallace Channel in association with spotted seatrout sound production and at Fisherman's Bay not in association with sound production. The lack of association between sound and egg production in the second case is most likely explained by the fact that there was a very short duration of the drumming each night (immediately after sunset) for this species. Thus, eggs were collected later in the evening, but not exactly at the same time and place as the drumming males, which we missed because we did not record immediately after sunset on some nights.

Red drum were heard least frequently of all the species examined. Red drum males were heard producing "knocking" sounds in September 1997 during hydrophone surveys and in August, September, and October of 1998 during sonobuoy surveys. Red drum were heard only at Ocracoke Inlet and in the Bay River areas in both 1997 and 1998. Red drum called most frequently from just before sunset to 2100 EDT, although at least in one case a red drum was heard at 0800 EDT. Red drum eggs (identified using mtDNA-RFLP methods) were collected in plankton tows made at the mouth of the Bay River in September 1997 in association with drumming sounds. In 1998, sonobuoys recorded red drum "knocking" in the same general area (Mouth of the Bay River) as these egg collections in 1997. Red drum were also heard near Ocracoke Inlet in August, September, and October of 1998.

The results reported here, especially the strong association of early-stage sciaenid eggs and male sciaenid drumming, suggests that passive listening using hydrophones can greatly improve a fish biologist's ability to delimit spawning areas for conservation of essential fish habitat and other fishery management purposes. Passive acoustics can address the need for fishery independent monitoring of adult spawning stages of soniferous species such as weakfish, spotted seatrout, and red drum.

INTRODUCTION

Knowledge of spawning habitats and spawning stock biomass is essential for the conservation of exploited fish stocks. Marine reserves and closure of fishing areas have been proposed for conservation of exploited fish stocks (Clark 1996, Ogden 1997, Roberts 1997, Allison et al. 1998, Lauck et al. 1998). The establishment of marine reserves will require precise spatial data on the spawning areas for exploited fishes. In addition, spawning stock biomass estimates are required for fishery management plans; such data are currently obtained by indirect estimates using mathematical models (e.g., virtual population analyses). A direct measurement of both the spawning areas and the spawning stock would be desirable.

Most traditional methods of establishing spawning locations for marine fishes are labor-intensive net-harvest methods directed at the larvae or the adults. One approach involves collection of eggs, larvae, or pelagic juvenile fishes. The spawning areas and times are estimated from age-specific growth data and current patterns, which are projected backwards in time using estimates of fish age, growth and estuarine hydrography (Holt et al. 1985, Peters and McMichael 1987, Johnson and Funicelli 1991). This method is not efficient because of the great amount of work involved in conducting net surveys, the uncertainty over the identity of species collected at early life stages (Daniel and Graves 1994), the extensive knowledge of estuarine hydrography required, and the spatial extrapolation involved. Another method that has been used extensively to locate spawning adults of many species, including weakfish and spotted seatrout (*Cynoscion regalis* and *C. nebulosus*), is to capture fishes with nets and determine the gonadal condition in a variety of areas (Merriner 1976, Brown-Peterson et al. 1988, Murphy and Taylor 1990, Lowe-Barbieri et al 1996). Determining the stage of gonadal development is a time-consuming and subjective technique that can only be made by an experienced observer. It requires that the spawning fishes be captured and dissected for histological samples of the gonad. This method depends on the previous knowledge of spawning locations so that nets can be deployed and spawners captured. Furthermore, the spawning location may not be the same as the location where the gonadally ripe adults occur because fish often migrate prior to spawning, thus introducing error in the position of spawning habitat. The adults examined for gonadal condition are often collected by fishers themselves (e.g., data are obtained from the recreational or commercial catch), so that areas are not sampled randomly, the data may be subject to under-reporting, and the data may contain misleading information on area of capture. Although both methods eventually may provide data on spawning locations and seasons, they are very slow and do not lend themselves to easy use by fishery managers, who must often assess population status quickly and make area and season closure decisions rapidly.

Fishery managers must assess stock abundance patterns rapidly as they change. Traditionally, fishery-dependent approaches are the only available option for estimating spawning stock biomass. Fishery-independent data can be obtained using commercial-scale gear (haul-seines, gill nets, trawls, etc.) deployed using a sampling design that is statistically valid. This is a costly and labor-intensive process. In addition, all such gear is selective to some extent and may under-estimate stocks due to net avoidance by the fish.

Hydroacoustic assessment of the spawning grounds and spawning stock is one alternative to the above methods. For fishes that produce sounds (soniferous fishes), passive acoustics, in which a hydrophone is used to listen for characteristic sounds produced during spawning by the fishes themselves, may be very useful in detecting the presence and estimating the relative abundance of the spawning stocks quickly and efficiently. It has been known for some time now that many fishes, including most members of the Sciaenidae (drums and croakers), make sounds and communicate with one another (Myrberg et al. 1965, Fish & Mowbray 1970, Fine et al. 1977, Myrberg 1981). Furthermore, it is apparent that males of the Sciaenidae, especially the weakfish (*Cynoscion regalis*), spotted seatrout *(C. nebulosus*) and the red drum (*Sciaenops ocellatus*) make species-specific calls during courtship of the females at locations where spawning occurs (Fish and Mowbray 1970, Guest and Lasswell 1977, Mok and Gilmore 1983, Connaughton and Taylor 1995, Connaughton and Taylor 1996). Hydroacoustic monitoring of drumming by male sciaenids as a method of delimiting spawning areas has been undertaken recently in the field and laboratory (Mok and Gilmore 1983, Johnson and Funicelli 1991, Saucier et al. 1992, Saucier and Baltz 1993, Connaughton and Taylor 1995). For example, weakfish drumming has been observed immediately prior to spawning in the laboratory (Connaughton and Taylor 1996). Male weakfish make drumming sounds with their swim bladder (Tower 1908), which we described as “purring” sounds (Luczkovich et al. in press). These "purring" sounds have been correlated with egg abundance in field surveys (See Task 2 below; Luczkovich et al in press). In addition, male spotted seatrout make drumming sounds in conjunction with the presence of large numbers of spotted seatrout eggs in the water column (Mok and Gilmore 1983). Finally, Guest and Lasswell (1977) observed courtship and spawning behavior of red drum along with their sound production in a laboratory tank. All of these sciaenid drumming sounds have been analyzed spectrographically and are unique for each species (Fish and Mowbray 1970, Guest and Lasswell 1977, Mok and Gilmore 1983; Luczkovich et al in press). The sounds produced by these fishes in the field can thus be identified using these spectrographs of species-specific sounds. It is now possible to monitor the spatial distribution and relative abundance of drumming male sciaenids using hydrophones and the Global Positioning System (GPS) of navigation satellites and to establish the probable spawning locations and seasons using an unequivocal, rapid, and cost-effective technique. Eventually, once passive acoustic methodology is calibrated to traditional methods of stock assessment, it will be possible to monitor spawning stock biomass as well.

In this project, it was our purpose to ascertain if male weakfish, spotted seatrout, and red drum drumming sites can be identified and accurately mapped. In order to do this, the weakfish, spotted seatrout, and red drum calls heard at a location must be differentiated from other species of soniferous fishes that may also be present. There may be as many as 15 species of sound-producing fishes co-occurring in the estuaries of the Southeastern United States in the families Ariidae, Batrachoididae, Blenniidae, Carangidae, Gobiidae, Haemulidae, Lutjanidae, Sparidae, and Sciaenidae (Fish & Mowbray 1970, Myrberg 1981, Mok and Gilmore 1983). We have been able to separate our species of interest from these other species by ear and on the basis of spectrographs made from calls recorded on captive fishes. We also estimated the acoustical background noise during daylight at the site to establish a threshold for background noise. Using a cylindrical spreading model, we estimated the greatest distance over which the dominant sound frequency produced by a drumming male sciaenid could be heard under those conditions. This allowed us to plot the area of maximum likelihood in which the male sciaenid could be producing sounds. Finally, to determine if variation in fish sound production was associated with variation in spawning behavior, we compared sound pressure levels associated with acoustic recordings of fish sounds for each location with an ichthyoplankton net survey, which is a traditional method of assessing spawning.

Sciaenid Fisheries In North Carolina

Sciaenid fishes are targeted by both recreational and commercial fishers in North Carolina. In 1997, commercial weakfish harvests were worth $ 1,869,212, spotted seatrout were valued at $ 284,128 and red drum were worth $ 57,007. These values are lower than they have been in past years due to declining catches and the harvest limits imposed by fishery managers. The economic activity associated with recreational harvest of fishes in North Carolina is not known for certain, but is large (estimated to be $59.5 billion for fishing and hunting in the entire US). In Virginia, the recreational saltwater catch was recently estimated to be valued at $353.5 million (Kirkley et al. 1999). In 1997, North Carolina recreational fishers harvested 158,454 pounds of weakfish, 299,587 pounds of spotted seatrout, and 38,327 pounds of red drum, and an unknown number were caught and released. Kirkley et al (1999) estimated the value of spotted seatrout recreational catch in Virginia to be $ 47/pound; if we use that estimate for sciaenid fishes, North Carolina's catch for these three species alone are valued at about $ 23 million. Thus, the recreational harvests of these three species are valuable to North Carolina's coastal economy as recreational fishers bring economic activity to the coastal tourism industry.

Although they had been in decline in the late 1980’s and early 1990’s (Vaughan et al.1991), weakfish stocks are now recovering (Personal Communication, Louis Daniel, Atlantic States Marine Fisheries Commission Technical Committee on Weakfish Assessment, 1998). Recent stock assessment of red drum (Vaughan 1996) suggests that the stock is declining along the Atlantic coast of the US. Management options include protection of critical spawning areas for these species. This report identifies and characterizes some of these spawning areas in Pamlico Sound, which are thought to represent the major spawning areas along the Atlantic coast for weakfish and red drum. Fishery management plans that describe and identify essential fish habitat, minimize the adverse effects on such habitat, and identify other actions to encourage the conservation of such habitat are now required by the federal law [Magnuson-Stevens Sustainable Fisheries Act, Section 108(a)(3)]. The development of fishery management plans for red drum and other sciaenids is a high priority for North Carolina. Fishery management plans are now being developed for red drum, and must be developed for weakfish and spotted seatrout in the future. The management plans must be based upon accurate spawning stock assessments for each of these species and surveys of essential fish habitat (EFH).

Why Use Hydrophone Surveys?

Descriptions of EFH and areas of aggregation for spawning sciaenids, which can be identified using hydrophone surveys, are specifically required by the these fishery management regulations (South Atlantic Fishery Management Council, 1998). The current report will address the need for documentation of EFH-HAPC (Essential Fish Habitat-Habitat Areas of Particular Concern) for these three sciaenid fishes in North Carolina.

A hydrophone-assisted acoustic survey of spawning sciaenid fishes is a more expedient way to delineate discrete spawning sites within Pamlico Sound than traditional net capture and fishery-dependent methods. This report details the methods and the efficacy of such a passive acoustic approach to locating spawning areas of soniferous fishes. Once spawning areas have been delimited using this hydrophone method, they can be monitored using more traditional methods (fishery independent net harvests such as gill nets, haul seines, and trawling) to ascertain the size and age composition, mortality, fecundity and frequency of spawning of adult fish. Furthermore, active acoustics (using split-beam echosounders and other acoustic approaches) can be used to estimate biomass, numerical abundance, and size of fishes. Fishery management measures, such as area closures or size-specific harvest limits, can then be effectively and fairly implemented in such areas.

Objectives

Our overall objective was to record the sounds made by spawning male red drum, spotted seatrout, and weakfish (the target species) and to determine whether these species can be differentiated from one another and from other soniferous fishes. We have identified unique spectrographic signatures of the drumming sounds made by each species when in spawning aggregations.

METHODS

Captive Fish Collection and Recording

Fish were caught by hook and line methods and placed in aerated sea water transport tanks. They were taken to the Pamlico Aquaculture Field Laboratory or the East Carolina University Department of Biology to be held in tanks for recording purposes. Most fish collected called upon first capture; recordings were made immediately after capture in air or in seawater in a portable floating net pen or a cooler.

Using our spectrographic analyses (see below), published spectrographs (Fish and Mowbray 1970), and spectrographs produced from our own and other’s audio tape recordings of captive specimens (*personal communication,* Martin Connaughton, Washington College, Chestertown, MD; *personal communication,* R. G. Gilmore, Harbor Branch Oceanographic Institution. Ft. Pierce, FL), we were able to easily discriminate between the three species’ calls and other known calls of fishes.

Adult Fish Collection, Egg Collections, And Hydrophone Surveys - 1997

On a biweekly basis from May of 1997 until October 1997, we sampled ten or more stations with gill nets, plankton nets, and hydrophone surveys (Figure 1). Stations were sampled on the eastern side of Pamlico Sound near Ocracoke Inlet (Teaches Hole, Wallace Channel, Lehigh Dredge, Howard’s Reef and Royal Shoal) and near Hatteras Inlet (Hatteras Hole and Hatteras North). Four or more stations were sampled on the western side of the sound near Rose Bay (Rose Bay Creek and Rose Bay Mouth) and in the Bay River area (Fisherman's Bay East, Fisherman's Bay West, Jones Bay East, Jones Bay West, Mouth of Bay River, Brant Island Shoal) (Table 1). At all sampling locations, precise geographical positions (latitude and longitude) were determined using either a Trimble Pathfinder Basic Plus Global Positioning System (GPS) satellite receiver or a Trimble NT200 GPS chartplotter receiver with a ProBeacon MSK receiver operating in real-time differential mode (± 10 m Circular Error Probable accuracy; see Pietraszewski et. al 1993).

Table 1. Hydrophone stations in 1997 and GPS positions (latitude and longitude). These stations were selected as general locations for all sampling in each area. Plankton tows, gill net sets, and hydrophone recordings were made in the general vicinity of these stations, and the GIS maps reflect the exact GPS locations for each sample taken within these general areas.

No.	Station name	Latitude (North)			Longitude (West)		
		Degrees	Minutes	Seconds	Degrees	Minutes	Seconds
1	Jones Bay West	35	13	40.066980	76	32	17.070900
2	Jones Bay East	35	13	11.500020	76	30	47.494020
3	Fisherman's Bay West	35	10	3.847980	76	32	53.280000
4	Fisherman's Bay East	35	9	36.012000	76	32	42.120000
5	Bay River Mouth	35	10	17.824980	76	30	22.711920
6	Brant Island Shoal	35	10	59.817000	76	22	48.585000
7	Rose Bay Creek	35	27	19.763040	76	24	19.119960
8	Rose Bay Mouth	35	22	39.460020	76	25	9.993000
9	Royal Shoal	35	8	40.980000	76	4	35.346000
10	Lehigh Dredge	35	9	6.492000	76	1	3.124980
11	Howard's Reef	35	7	40.135980	75	58	51.511920
12	Teach’s Hole	35	5	53.317980	75	59	28.534020
13	Teach’s Hole Channel Marker 29	35	4	59.590980	75	59	54.459000
14	Wallace Channel	35	4	23.263020	76	3	7.794000
15	Hatteras Hole	35	11	56.074020	75	46	55.949040
16	North Hatteras Inlet	35	11	36.309000	75	45	3.056940

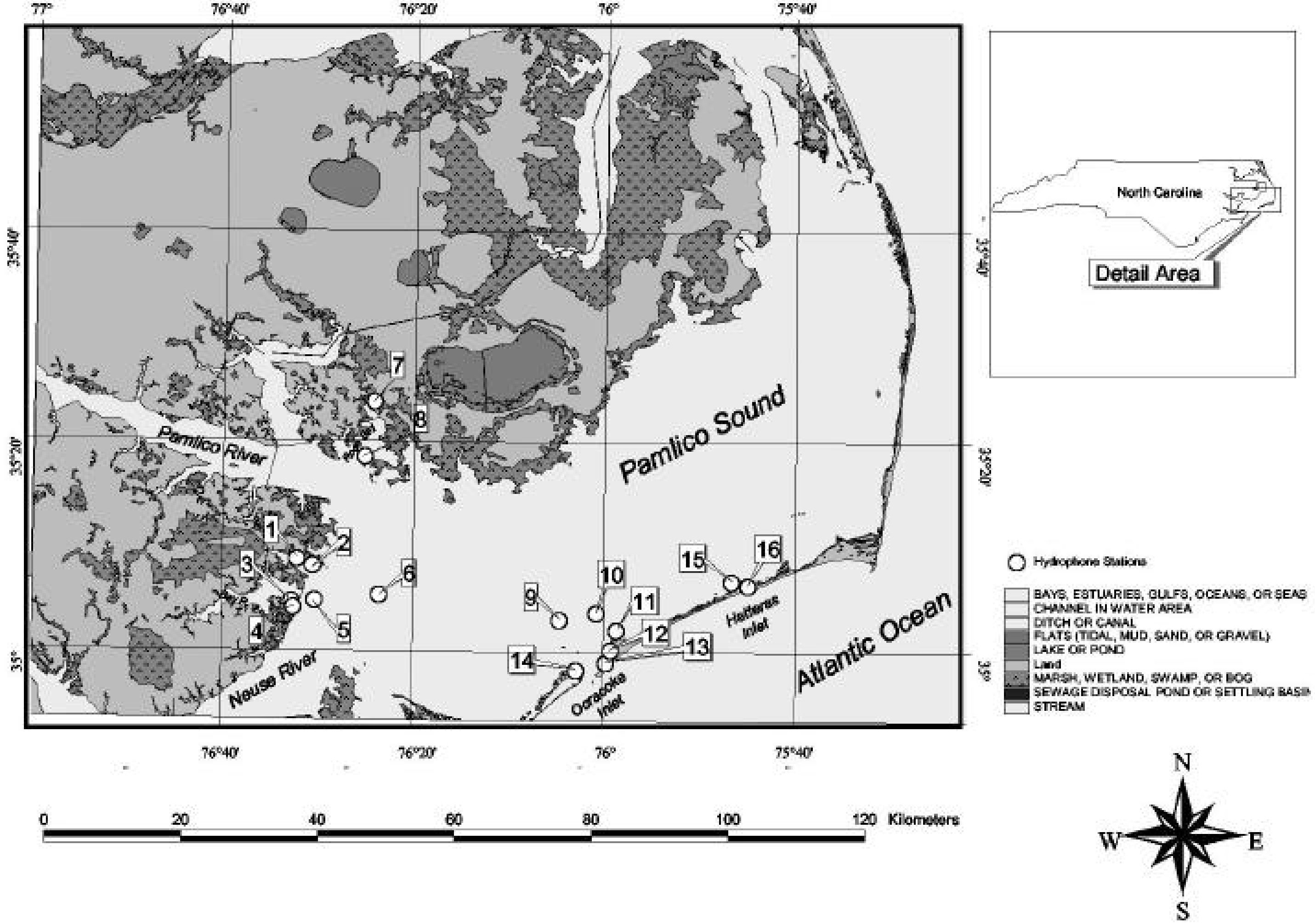

Figure 1 - A map of the hydrophone survey stations visited in 1997 in Pamlico Sound, North Carolina. See Table 1 for station names and GPS data.

At each station, we measured the salinity and temperature along a depth profile at 1.0-m intervals of depth using a Hydrolab Surveyor II probe or a YSI Model 85 probe; vertical sound speed-profiles were calculated from temperature, salinity and depth using the formula in Medwin (1975). We examined vertical sound-speed profiles for sharp changes, which may cause refraction in sound waves and could increase the propagation distance of sound waves.

Gill Net Collections

At hydrophone listening stations during 1997, experimental gill nets were deployed prior to sundown and recovered the next morning. Average soak time was 14 hours and 47 minutes for these net sets (See Appendix II). Initially in May, June, and July of 1997, we used 150-foot-long gill nets with 6" and 3" mesh panels (Gill Net Type 1). Later in August, September, and October we switched to nets that were 200 feet long with eight 25-foot panels in each net (Gill Net Type 2). Meshes on the Gill Net Type 2 panels ranged from 3” - 6.5” stretch mesh, increasing in 0.5' intervals. During the September and October of 1997, additional 12" mesh gill nets were deployed, with our intention being to capture large red drum (Gill Net Type 3). The last net type never caught large red drum, but did catch smaller weakfish on occasion. Fishes were removed each morning, identified and measured. Sciaenid fishes were collected; otolith and gonad samples were obtained and sent for processing to the NC DMF laboratory in Morehead City, NC (with the assistance of Louis Daniel and the NC DMF staff).

Plankton Net Collections

Sciaenid egg collections were taken with 25-cm diameter “bongo” plankton net frame fitted with two 1.5-m long 500 µm mesh plankton nets. The nets were pulled behind a small boat at the surface at speeds of 4-6 km/hr for 5 min. A General Oceanics flow meter (Model 2030R or 2030R2) was attached to the frame inside the mouth of one of the nets and used to calculate volumes of water filtered for each sample. Using the egg counts and the estimated volume of water filtered, egg densities per m^3 were obtained. Plankton samples were passed through a 2000 µm sieve immediately after collection in order to remove seagrasses and ctenophores that could affect egg counts. One of the bongo net samples was preserved in 5 % formalin and examined later in the laboratory for early-stage fish eggs (< 1 day old) with characteristics of the Sciaenidae (700-900 µm egg diameter, 1-3 internal oil globules) (Fahay 1983, Holt et al.,1988). The second sample was not preserved and was examined later that night within 5 hours of collection. After having been stirred, ten 10-ml subsamples were taken from each unpreserved sample, classified into groups of eggs with 0, 1-3, or > 3 oil globules, and counted. Any fish eggs that exhibited significant embryonic development were excluded from these sample categories; generally, less than 10 % of all eggs captured after dark showed any degree of embryonic development, indicating that they were mostly early stage sciaenid eggs, and were just hours old. These egg counts were used to estimate density (number/m^3) in the unpreserved samples. Later, a correlation between total egg density of the unpreserved and preserved samples was estimated to check for potential bias in subsampling.

Identification of Eggs Using Molecular Genetics

The sciaenid-type eggs collected were identified based on morphological characters (spherical shape, yolk color, egg diameter, number of oil globules) from published descriptions

(Fahay 1983, Holt et al. 1998). However, these morphological characters alone do not allow an unequivocal species identification, as they overlap to some degree among sciaenid species. Thus, a molecular genetics approach was used to identify a small number of eggs collected at these putative spawning sites. Preliminary identification of eggs from a subset of the ichthyoplankton samples has been accomplished using the mitochondrial DNA restriction fragment length polymorphism (mtDNA-RFLP) approach. From the unpreserved sample of eggs with 1-3 oil globules (none with embryonic development), we measured the diameter of a subset of eggs or reared larvae and froze them individually microcentrifuge tubes (2 ml). Samples were stored at -20 °C for mtDNA analysis. Genomic DNA was extracted using Quigen® DNA extraction kit protocol, with the following modifications: reagent volumes at each procedural step were halved and a single elution step was done using 40 µl of Millipore® filtered water. We used the molecular identification method of Jan Cordes (unpublished Ph.D. dissertation, Virginia Institute of Marine Sciences, 1999) for individual eggs. The method uses portions of the 12S/16S ribosomal RNA gene. The primers, described in protocols in Palumbi et al. (1991), are12SAL (5'- AAACTGGGATTAGATACCCCACATT-3') and 16SAH (5'-TGTTTTTGATAAACAGGCG-3'). The DNA sample was amplified via the polymerase chain reaction (PCR) using the *Taq* polymerase reagents and protocol provided by the supplier (Life Technologies/Gibco/BRL™, 9800 Medical Center Drive, Rockville, MD 20850). In a thermal cycler (MJ research Model PTC-150 Minicycler™), after an initial incubation at 95 °C for 4 min to denature the DNA template, reactions were amplified for 34 cycles at 94 °C for 1 min (denaturing), for 50 °C for 1 min (annealing), and 65 °C for 3 min (extension). After cycling, samples were incubated for 10 min at 65 °C to allow final extension. PCR product yields were examined by horizontal gel electrophoresis on a 1.5 % agarose gel (with ethydium bromide added to the gel), and visualized under UV light. If the yield was adequate for visualization, the samples were digested using *Rsa*I restriction endonuclease (Promega, 2800 Woods Hollow Road, Madison WI 53711-5399) in the mixture described in Table 2.

Table 2. The restriction endonuclease mixture

Reagent	Volume (µl)
distilled water	5.0
10x buffer	1.5
*Rsa*I	0.5
DNA	8.0
Total	15.0

Digestion products were examined with gel electrophoresis using 4 % agarose gel (3:1 ratio of NuSieve® agarose: agarose). Restriction digest gels were visualized using UV light and photographed. Cordes et al. (in prep.) have produced a catalogue of mtDNA haplotypes for sixteen species of commercially important fishes in Chesapeake Bay (Atlantic croaker, *Micropogonias undulatus*, cobia, *Rachycentron canadum*, black drum, *Pogonias cromis*, black sea bass, *Centropristis striata*, bluefish, *Pomatomas saltatrix*, northern kingfish, *Menticirrhus saxatilis*, southern kingfish, *Menticirrhus americanus*, summer flounder, *Paralichtys dentatus*, silver perch, *Bairdiella chysoura*, spanish mackerel, *Scomberomorus regalis*, spot, *Leiostomus xanthurus*, striped bass, *Morone saxatilis*, tautog, *Tautoga onitis*, and our target species weakfish, spotted seatrout, and red drum) for the forensic identification of fish fillets. We compared the results of adult tissue samples taken from the target species in Pamlico Sound with those catalogued by Cordes et al. (in prep.) to establish that there were no differences between populations in these two estuaries. Little geographic variation in mtDNA has been documented for the weakfish populations along the Atlantic coast (Graves et al. 1992). Finally, we compared our unknown eggs and larvae digest profiles with the catalogue profiles of Cordes et al. (in prep.). While this RFLP analysis will provide unique identifications based on the 16 species listed above, there is a possibility that other species with morphologically similar eggs (e.g., the silver seatrout, *Cynoscion nothus*, star drum, *Stellifer lanceolatus*, and banded croaker, *Larimus fasciatus*) may have also spawned in these waters during our study period. Because these species could have identical RFLP profiles (they have not yet been characterized using molecular genetics), their eggs may have been incorrectly identified in this analysis, although this is highly unlikely.

Hydrophone Surveys

Acoustical recordings were made from a small boat stationed over the study sites. The motor was not running during the collection of acoustical data. Recordings were made using an InterOcean Model 902 Acoustic Listening and Calibration System, (frequency range: 20 Hz to 10,000 Hz; sensitivity: 100 dB re 1 µPa RMS pressure), which consisted of an InterOcean Model T-902 hydrophone (omnidirectional with sensitivity –195 dB Nominal re 1 V/µPa) connected to an amplifier (gain adjustable from 15 dB to 95 dB in 10 dB increments plus vernier adjustment) with a rectifier-type AC meter (peak deflection within 3 dB of continuous signal for 100 ms pulse) calibrated in dB connected to the amplifier output. The hydrophone was placed at 1-2 m depth below the water surface. The sound pressure levels, both during background sound measurements during the day and during periods of fish sound production at night, were measured over the entire frequency range. The acoustical data were recorded with a portable battery-operated digital audio tape (DAT) cassette recorder (Sony TCD-D8 recorder, frequency range: 20 Hz-22,000 Hz ± 1 dB). Recordings (a minimum of 2 min in duration) were made at each site from one hour before sunset and continuing at intervals of 15 min – 60 min until two hours after sunset.

Acoustic and Spectrographic Analyses

Please consult Appendix III for explanations of the terminology and some examples of the analyses described in this section. The measured sound pressure levels (*SPL*) in decibels were converted to pressures (*p*) in µPa before statistical analysis. Averages and standard deviations were calculated using the pressures, and the results were transformed back to decibels.

The sciaenid drumming and sounds produced by other soniferous organisms at each site were recorded on digital audio tape (DAT) with 16 bits of resolution. The sampling rate was 48 kHz when sounds were recorded on the DAT. We reduced the sampling rate to 24 kHz for our spectrographic analysis to save on computational resources required. We re-sampled the data using a National Instruments NB-2150F analog-to-digital board with anti-aliasing filters in a Power Macintosh computer. Power spectra were calculated using a 1024-point Fast Fourier Transform (FFT) with a Hanning window. The frequency resolution, determined by the sampling frequency and the number of points in the FFT's in each power spectrum, is 23.4 Hz. Spectrographs were plotted using the power spectrum and time information in the sampled sounds. The relative power spectral density in each spectrograph is given such that the background level in each spectrograph (the lightest region) is 0 dB. In each of our spectrographs, only the frequencies from 0 Hz to 12000 Hz are shown, due to the limitation of the Nyquist frequency and in some cases only 0 to 2000 Hz are shown. Power spectra are shown along with each spectrograph in most cases; power spectra are calculated as described in Appendix III

The sound produced by the fish must propagate through the water to the hydrophone. In the process, the sound wave will attenuate as it spreads out and will be affected by absorption, reflection (from the bottom and surface), refraction (by temperature, current, and salinity gradients), and scattering (from bubbles, turbulence and surface roughness or waves). The energy in the sound wave spreads spherically ($1/r^2$) in deep water and cylindrically ($1/r$) in shallow water (Urick, 1983). Mann and Lobel (1997) have measured the propagation of damselfish (*Dascyllus albisella,* Pomacentridae) courtship sounds in shallow water (< 7 m) and they suggest that the spreading of the sounds was nearly cylindrical. Because all of our sampling stations are in water depths of less than 10 m, we model the sounds here as spreading cylindrically.

The sound pressure level of an acoustic signal can be accurately measured when it is above the background sound pressure level at the signal frequency (Pierce, 1988). Using Pierce's (1988) criterion for the detectability of a signal above the background and assuming cylindrical spreading, the distance $r_{\max}$ that the signal will travel before being undetectable is given by

$$r_{\max} = 10^{\left(L_s - L_{bg}\right)/10}, \tag{1}$$

where L_s is the sound pressure level of the source at a distance of 1 m; and L_{bg} is the background sound pressure level. We used $r_{\max}$ to estimate the theoretical maximum distance over which we could detect the drumming sounds of individual fish.

Passive Hydroacoustic Surveys of Spawning Areas In 1998

Based on our 1997 data, we designated two areas for detailed mapping of spawning areas. These areas were Ocracoke Inlet on the eastern side of the Pamlico Sound and the Bay River/Jones Bay area on the western side on the Sound (Figure 2). We established a system of sonobuoy listening stations within these areas to create detailed spawning habitat maps for the three sciaenid species that are the subject of this report. We did not establish areas for mapping at Hatteras Inlet or Rose Bay because we failed to detect all three species in those areas in 1997 surveys. Although Rose Bay and Hatteras Inlet stations did have spawning populations of weakfish and spotted sea trout in 1997 and may eventually prove to be spawning areas for red drum, we did not detect red drum in our 1997 surveys. Given our logistical constraints (limited travel time, equipment and human resources), we therefore limited our sonobuoy surveys in 1998 to the Ocracoke and Bay River areas. These latter two areas could be sampled throughout all seasons and showed evidence of spawning activity for all three target species in 1997 hydrophone and ichthyoplankton surveys.

Recordings Made with Sonobuoys

We designed and built sonobuoys in order to record sounds indicative of fish spawning. The sonobuoy we designed was constructed of a 30-inch (76.2-cm) section of 4 inch (10.2cm) schedule 40 PVC plumbing pipe, which acted as a waterproof housing (Figure 3). Externally,

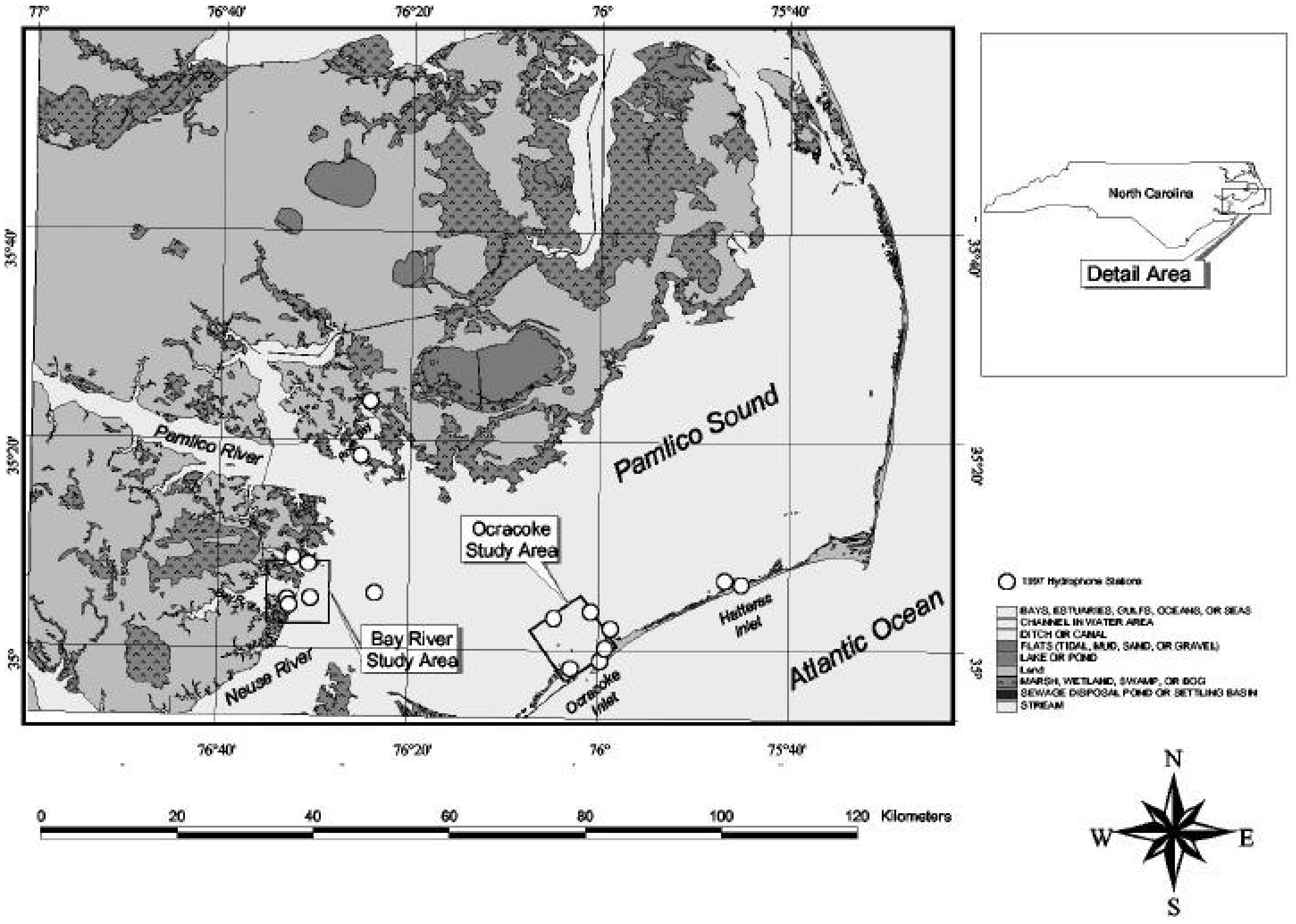

Figure 2. The two 100 km^2 areas (Ocracoke and Bay River) chosen for detailed mapping with sonobuoys in the 1998 surveys.

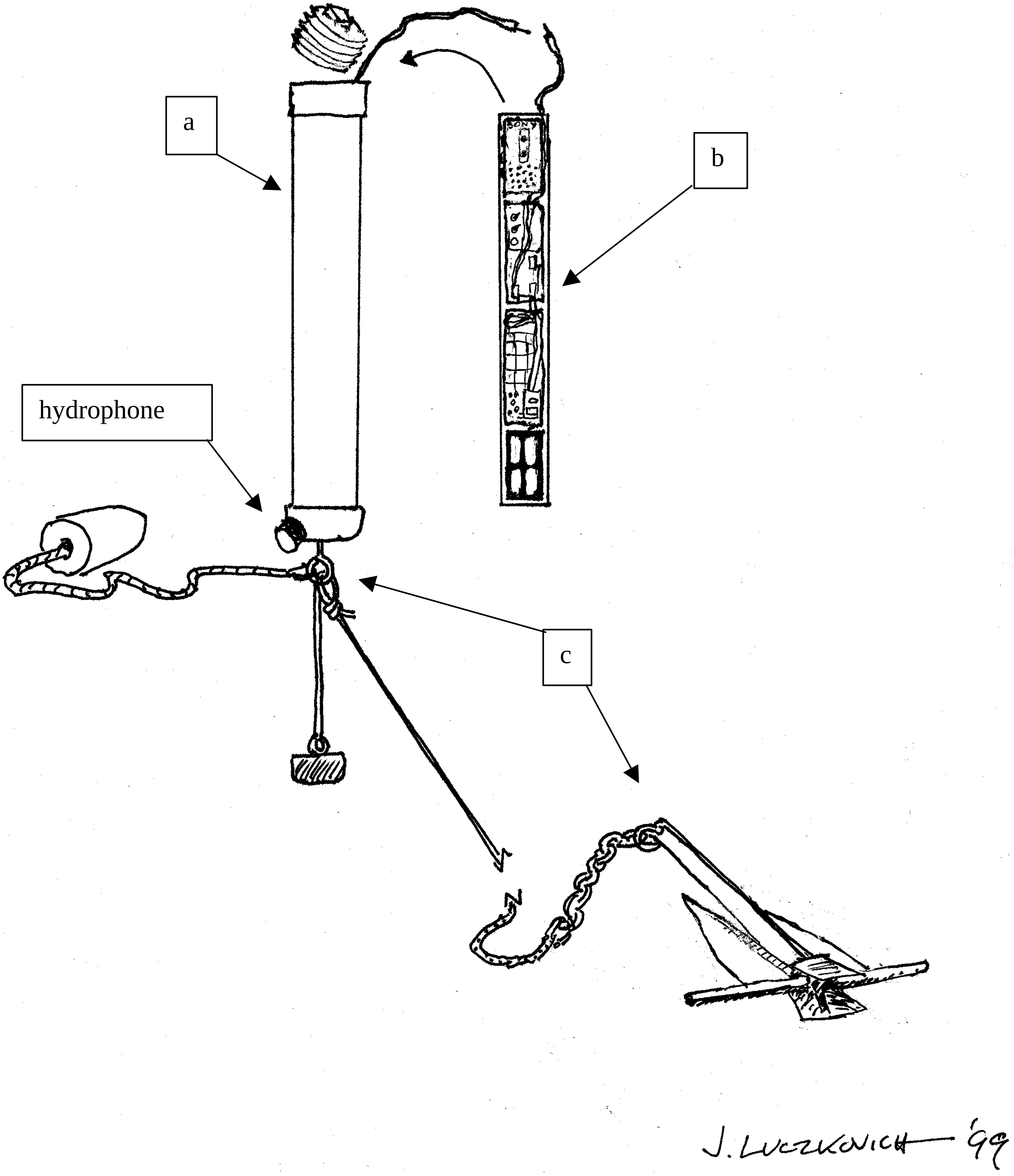

Figure 3. A diagram of a sonobuoy. a) the sonobuoy housing with the hydrophone attached to the outside, b) the aluminum frame insert with the timing circuit, tape recorder and battery supply, c) float and anchor set-up.

there was a hydrophone glued to the tube, and wired to the electronics, which were inside the waterproof PVC housing. Internally, the sonobuoy consisted of a timing circuit, a standard audio cassette tape recorder, and a power supply. A "talking clock", set to local time, announced and recorded to tape the time at the start of each sonobuoy recording. The sonobuoys were programmable and could be set to record ambient sounds through the hydrophone at 15-min, 30-min, or 60-min intervals after a start time. We used 30-min interval for standard nocturnal sonobuoy recordings, which had a 12-hour duration (2 min x 24 recordings in 12 hours = 48 min of recorded tape). We used the 60-min interval for a 24-hour sonobuoy recording.

The deployment of sonobuoys has several advantages over sampling from a boat. First, multiple sonobuoys can be made to start recording at the same time in the evening allowing temporal comparison of spawning sound activity between locations. When one records from a boat, one is limited to the area that can be covered in one evening at a given boat speed. Also, the recordings are not made simultaneously. Finally, the study area is limited by the difficulty of navigating in the dark. The sonobuoys were designed to record for an entire night on one 45 or 50 minute cassette tape. This was accomplished by sampling for a relatively short period (nominally two minutes; actual mean one minute thirty seconds) at intervals of 15, 30 or 60 minutes (hereafter called the recording period). Our recordings from 1997 suggest that a short recording can adequately characterize the number of species sounds present at a given time for a given location. A species accumulation curve on some of the 1997 recordings showed that the number of species approaches an asymptote after only a few minutes. Mok and Gilmore (1983) also used a 2-minute recording length for their automated recording equipment when studying fish sound production in Florida.

Each month, up to nine sonobuoys were deployed on four consecutive nights within the Ocracoke and Bay River study areas. One sonobuoy (24-hour sonobuoy) was set to record every 60 minutes so it could record for 24 hours on one 50-minute cassette tape. This allowed characterization of the diurnal periodicity of fish sound production. The 24-hour sonobuoy was set at one location during each week. At Ocracoke Inlet Study Area, a 24-hr sonobuoy was set near Teaches Hole channel. At the Bay River study area, a 24-hour sonobuoy was either near Boar Point in Jones Bay or in Fisherman's Bay in the Bay River. The remaining eight sonobuoys were set for 30-minute recording periods and deployed at random positions within the 100 km^2 sampling region at each study area. Four of the sonobuoys were placed in shallow water (3 to 9 feet) and four were placed in deep water (greater than 10 feet). At the Ocracoke Inlet study area, the sampling region was rotated 37 degrees to orient the side of it parallel to the barrier islands so it would more adequately cover the inlet. The Bay River sampling region was oriented with its sides parallel to true north (*i.e*. parallel to lines of longitude). Each month that sonobuoys were deployed, a set of random longitude and latitude positions within a 10,000 m x 10,000 m region was generated for each study area. These random points were printed on a transparency at the scale of the National Oceanic and Atmospheric Administration (NOAA) nautical chart for that area (1:80,000 charts: Ocracoke area: chart no. 11555; Bay River area: chart no. 11548). The transparency was then laid on the chart in the correct position and each possible deployment location was checked for suitability based on depth (*i.e*. greater than 3 feet of water) and accessibility (*i.e.* connected to navigable waters). Based on the bathymetry printed on this NOAA chart, locations were also classified into two depth strata: shallow (3- 10 feet) or deep (> 10 feet deep). We deployed sonobuoys in 16 deep locations and 16 shallow locations within each study area each month. Some positions occurred on land, in very shallow water, or in otherwise unsuitable locations; we omitted these positions and generated more positions at random before going into the field. While we were deploying sonobuoys in the field, some locations were determined to be unsuitable, and new positions nearby within 200 m of the randomly selected position were selected instead. This ad-hoc changing of random positions in the field occasionally resulted in the sonobuoys being placed outside the 100 km^2 regions.

We deployed sonobuoys on four nights each month, usually on consecutive nights, with the Ocracoke locations completed in the first week of a month and the Bay River locations on the third week of a month. During the last week of May 1998, sonobuoys were deployed only at Ocracoke; this was our "shake-down" cruise, and many malfunctions (taped that failed to record and sonobuoys programmed to power up at the wrong times) of the sonobuoys were detected on that trip and corrected prior to the next trip in June. The sonobuoys were typically set to begin recording at 1800 (1600 later in the season) and placed at the sampling locations between 1400 and 1600 in the afternoon. The following morning the sonobuoys were collected and the tapes removed. The tapes were played to detect any malfunctions and correct them before the next night. To facilitate ease of deployment and minimize navigation time, each study area was divided into four quadrants (one for each sampling night). If there were not enough points within a section for one night of sampling or if the strata were unbalanced (*e.g*. more shallow than deep), then sonobuoy locations from adjacent quadrants were used instead. We made every effort to keep the sampling balanced each night (*i.e*. 4 shallow and 4 deep) and for most deployment nights this was the case. When weather prevented our deploying sonobuoys on a given night, we returned later to complete the sampling (thus, September had two sampling periods for Ocracoke; two quadrants were completed early in the month, and only one was completed later in the month due to bad weather).

The final random sonobuoy deployment locations are shown on the maps on the following pages. For the Ocracoke study area, random location maps for sonobuoys are shown for May (Figure 4), June (Figure 5), July (Figure 6), August (Figure 7), September (Figure 8) and October (Figure 9). Another map shows the locations of all sonobuoys deployed during in the Ocracoke study area in all months (May through October 1998, Figure 10). For the Bay River study area, random location maps for sonobuoys are shown for June (Figure 11), July (Figure 12), August (Figure 13), September (Figure 14) and October (Figure 15). A final map (Figure 16) shows the locations of all sonobuoys deployed during in the Bay River study area in all months (May through October 1998)

An experienced analyst, trained to identify each target species and other soniferous species that occurred in the study area, listened to each sonobuoy tape. A drumming index was developed for quantifying the drumming activity heard on a tape. This qualitative index, ranging from 0 to 3 and representing the frequency of occurrence with which a species was detected on a segment of a sonobuoy recording, was based on a similar index developed for frogs (Heyer et al. 1994). For each 2-min track on a sonobuoy recording, a listener assigned a drumming index value according to the following relative scale: 0 = not heard; 1 = drumming heard infrequently; 2 = drumming heard frequently; 3 = aggregation chorusing. At the end of each night's sonobuoy recording, the drumming indices for each 2-minute recording were summed to get a *drumming index sum* for that station. These drumming index sums were displayed on sonobuoy maps.

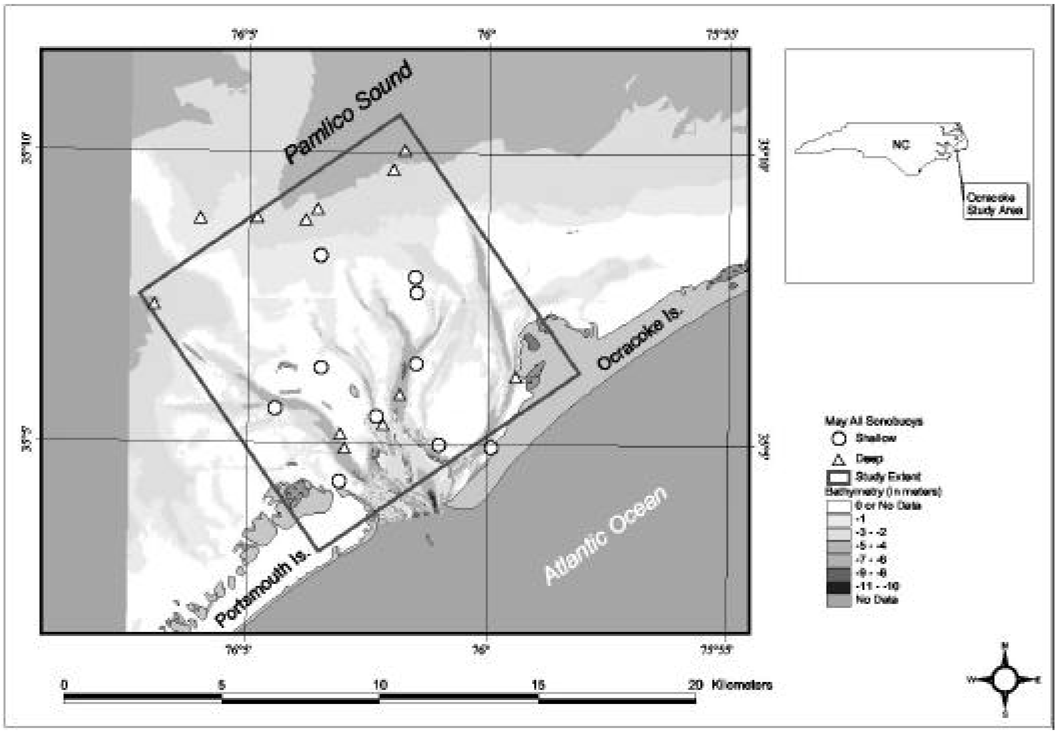

Figure 4. Random positions of all sonobuoys in May 1998 at Ocracoke. Symbols indicate deep and shallow locations.

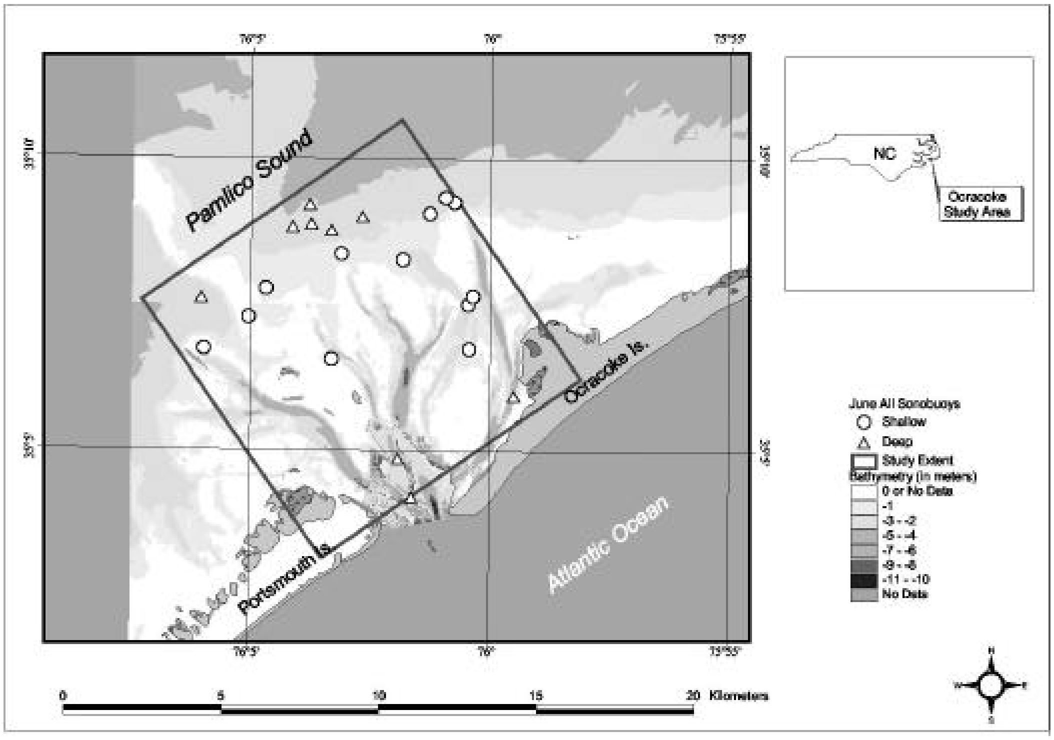

Figure 5. Random positions of all sonobuoys in June 1998 at Ocracoke. Symbols indicate deep and shallow locations.

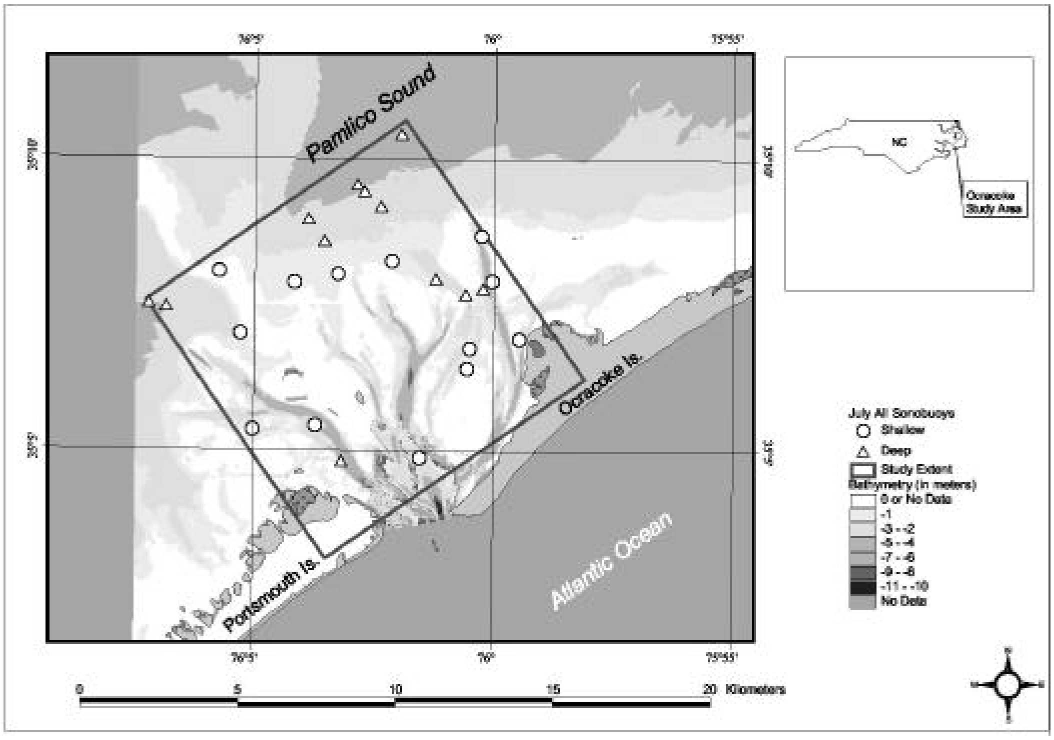

Figure 6. Random positions of all sonobuoys in July 1998 at Ocracoke. Symbols indicate deep and shallow locations.

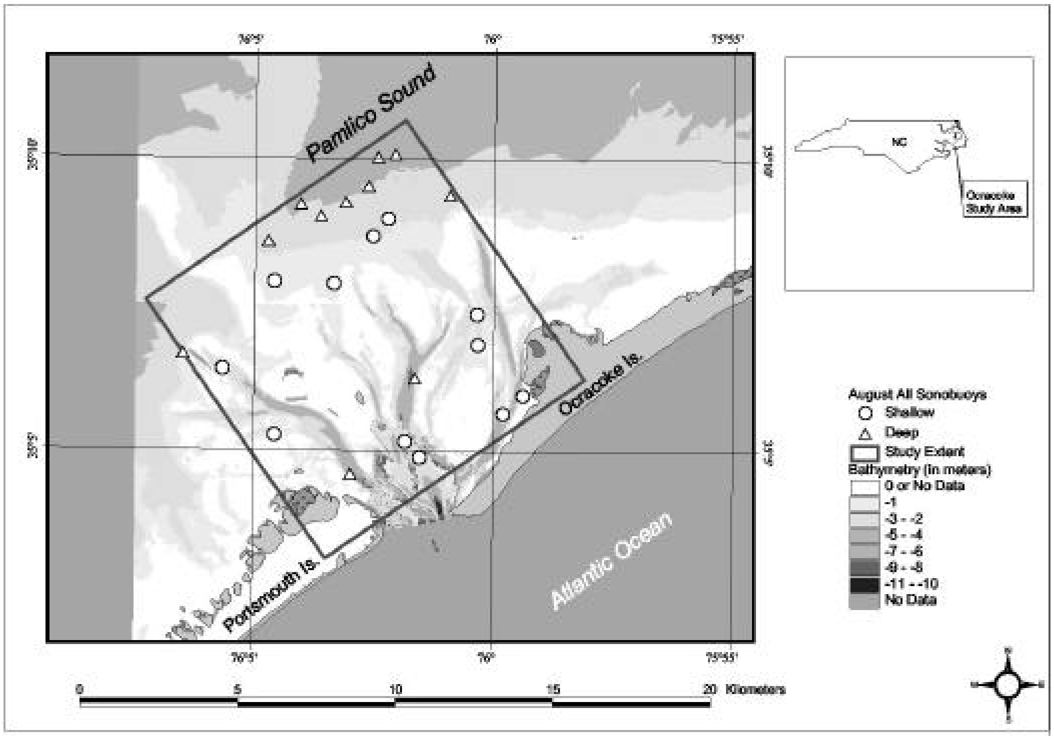

Figure 7. Random positions of all sonobuoys in August 1998 at Ocracoke. Symbols indicate deep and shallow locations

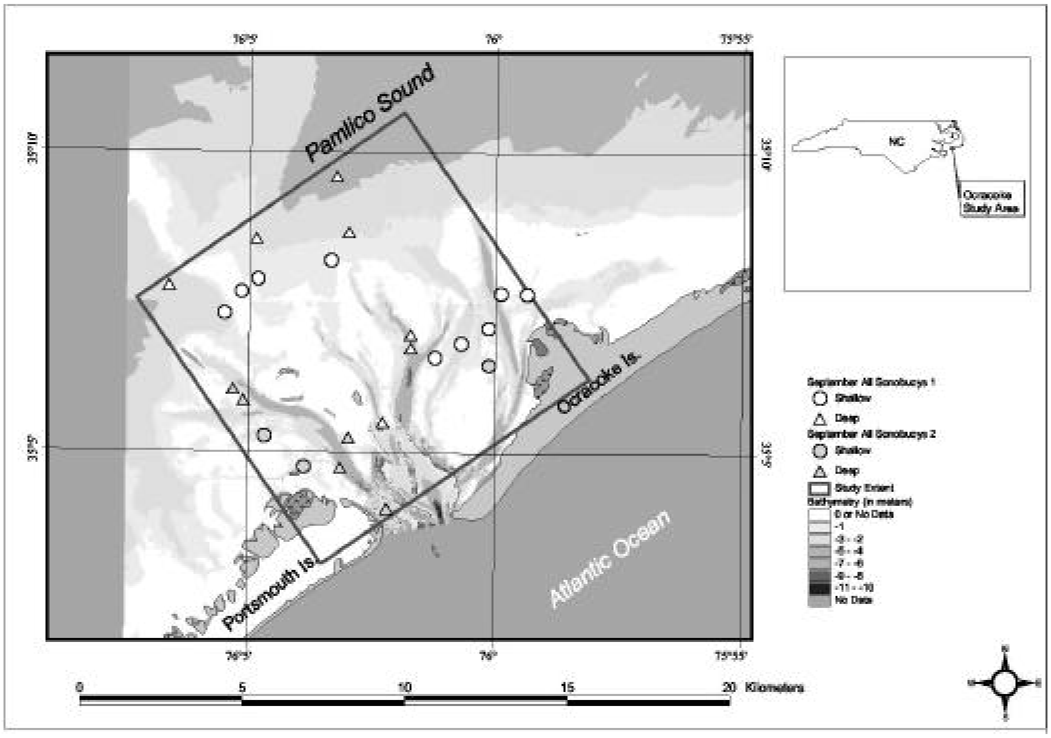

Figure 8. Random positions of all sonobuoys in September 1998 at Ocracoke. Symbols indicate deep and shallow locations.

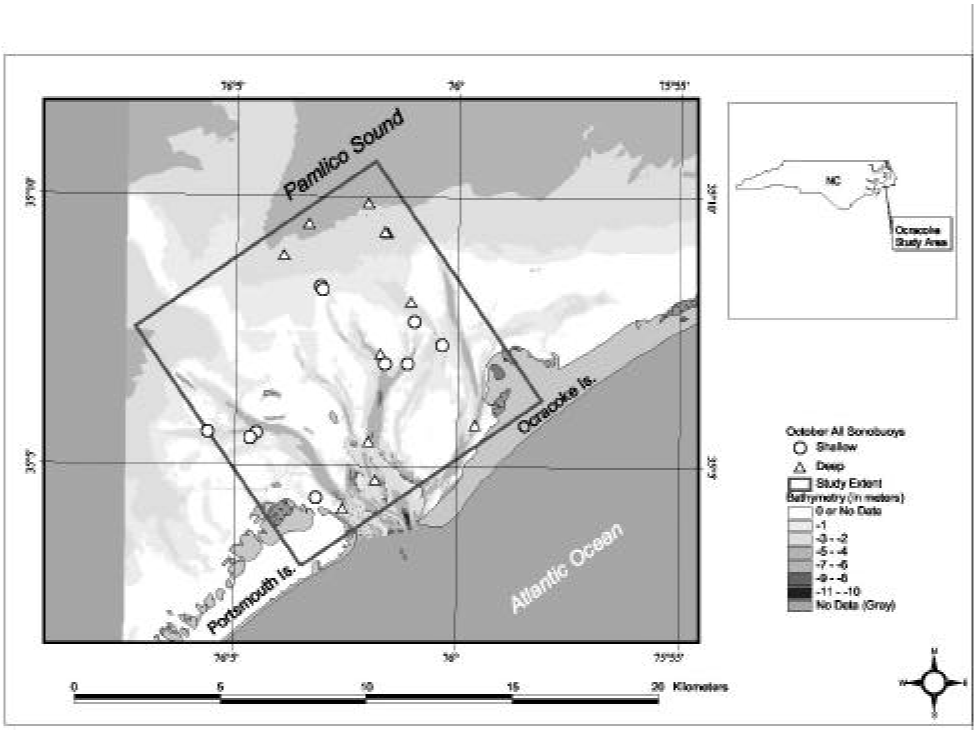

Figure 9. Random positions of all sonobuoys in October 1998 at Ocracoke. Symbols indicate deep and shallow locations

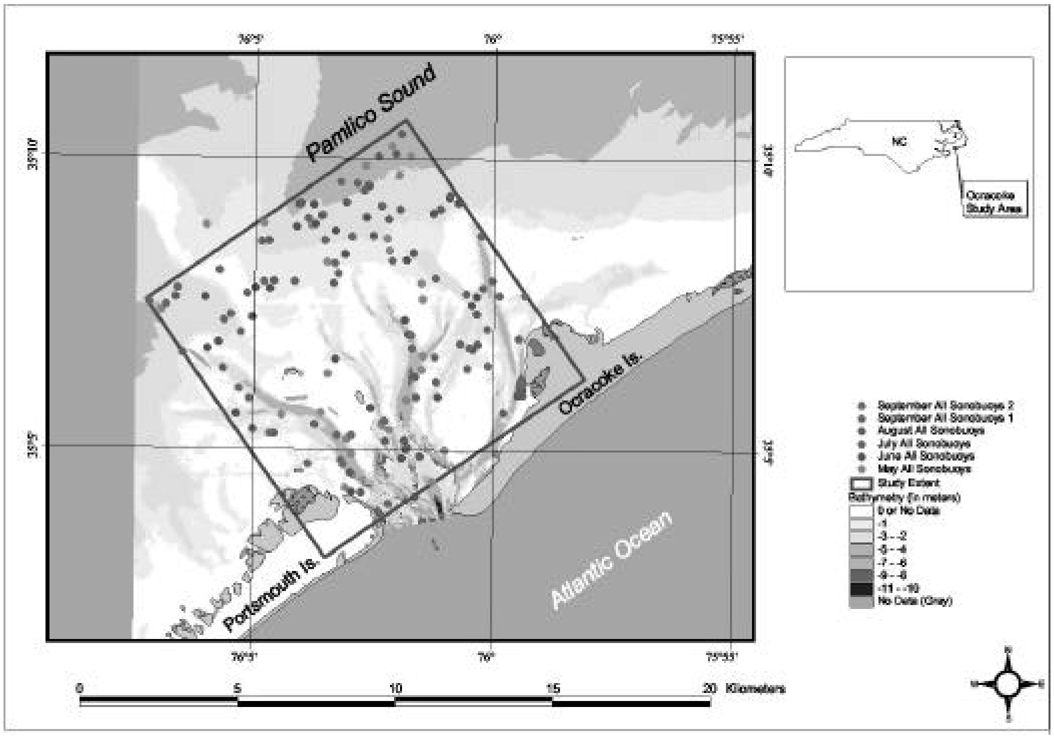

Figure 10. Random positions for all sonobuoys deployed in all months (May - October 1998) in the Ocracoke study area. Symbols indicate the sampling dates.

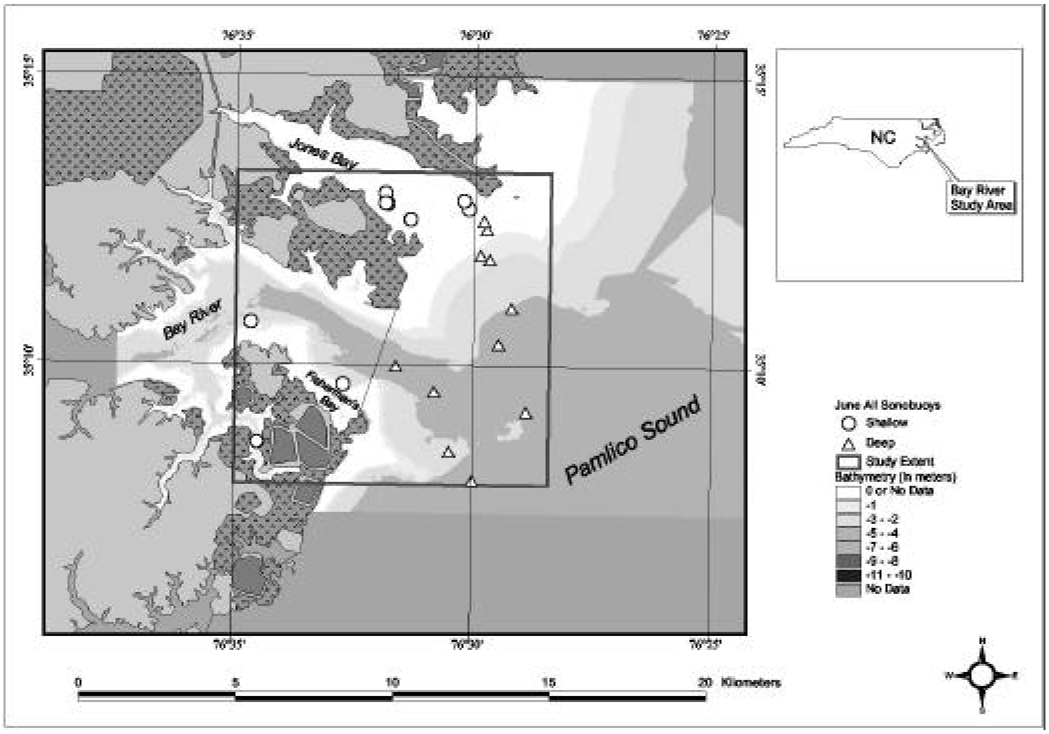

Figure 11. Random positions of all sonobuoys in June 1998 at Bay River. Symbols indicate deep and shallow locations.

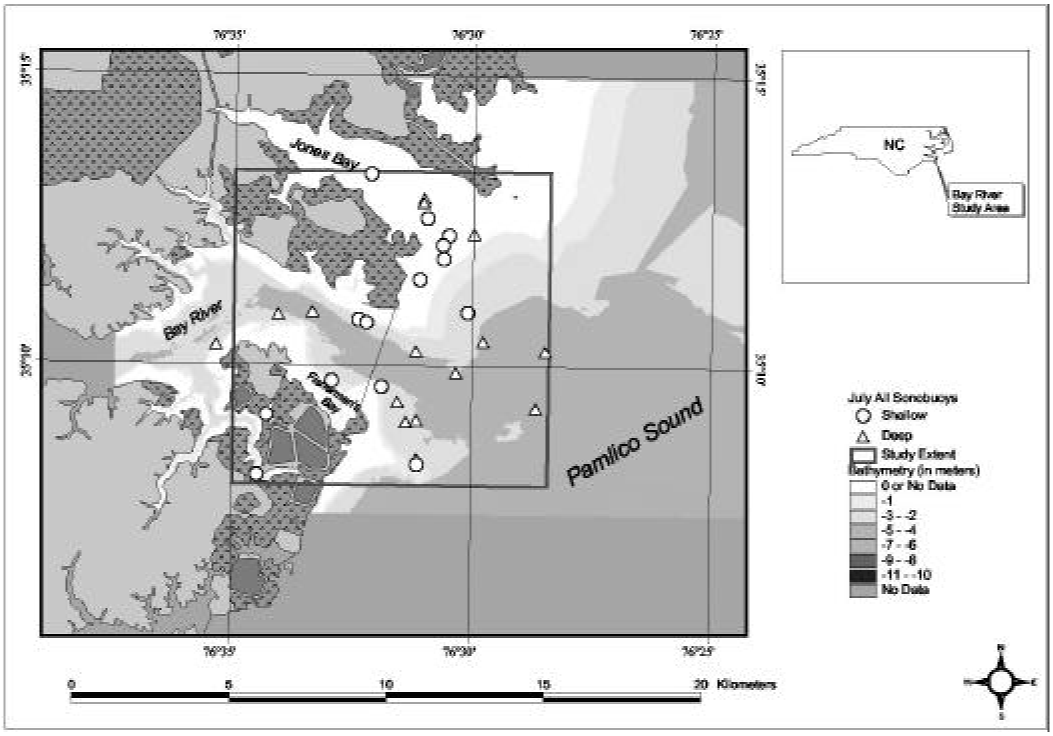

Figure 12. Random positions of all sonobuoys in July 1998 at Bay River. Symbols indicate deep and shallow locations.

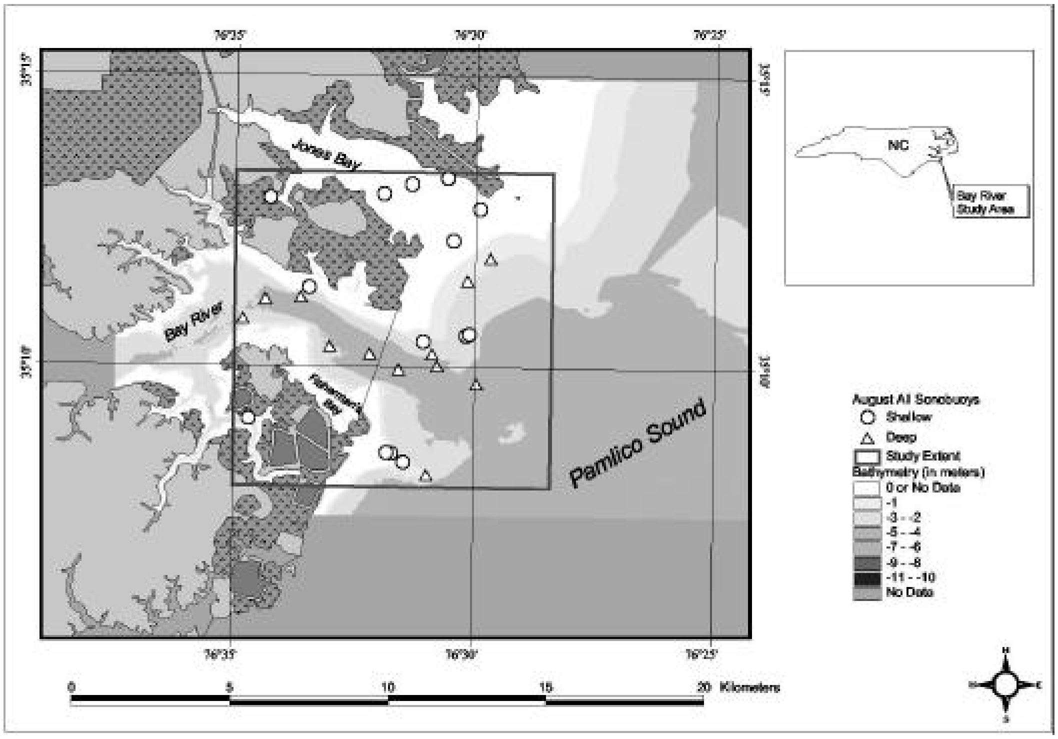

Figure 13. Random positions of all sonobuoys in August 1998 at Bay River. Symbols indicate deep and shallow locations.

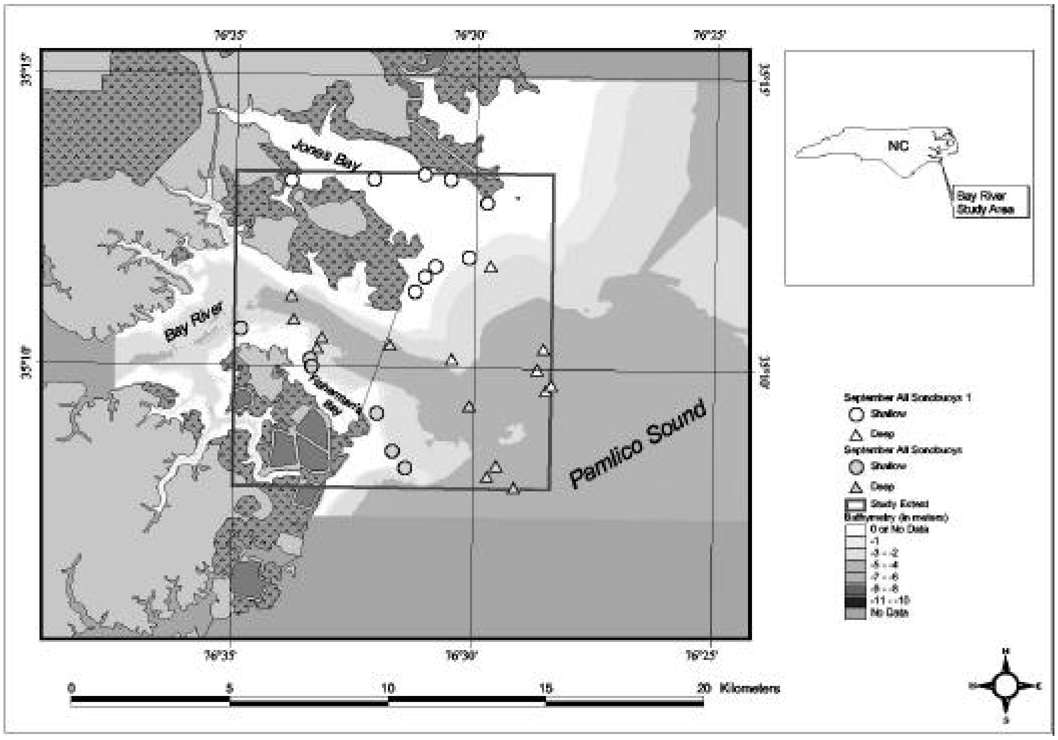

Figure 14. Random positions of all sonobuoys in September 1998 at Bay River. Symbols indicate deep and shallow locations.

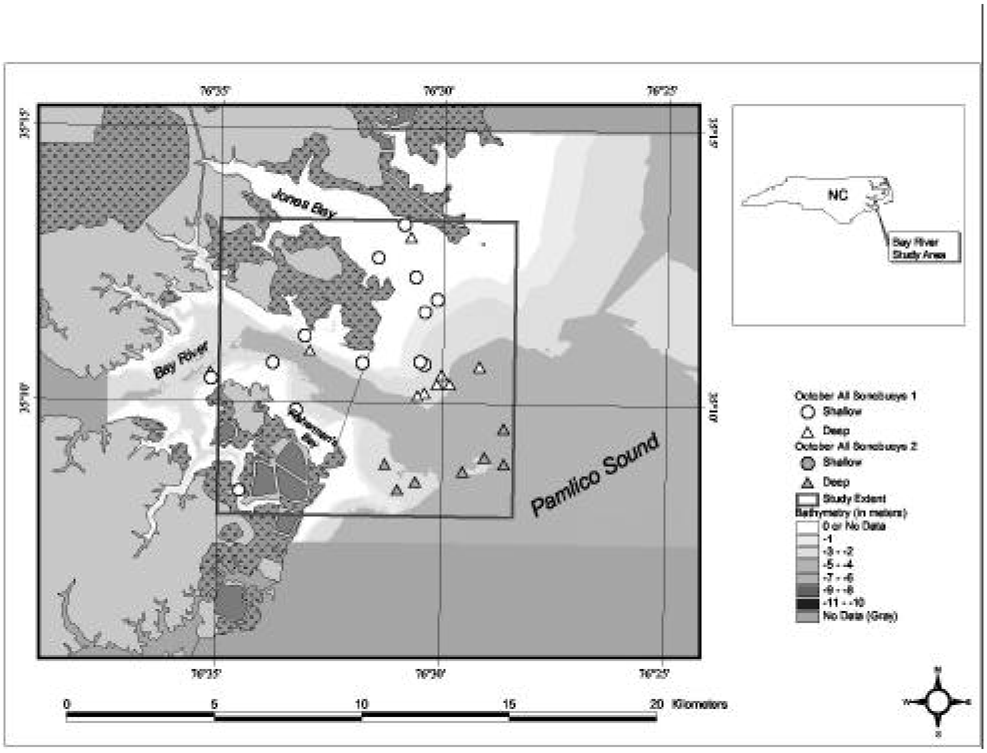

Figure 15. Random positions of all sonobuoys in October 1998 at Bay River. Symbols indicate deep and shallow locations.

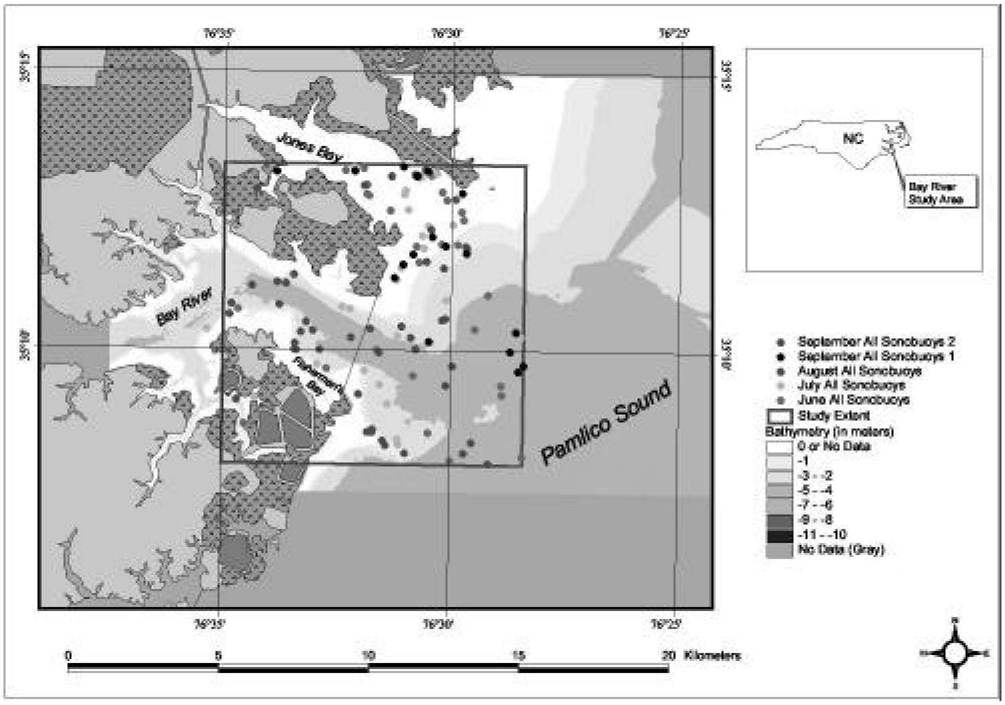

Figure 16. Random positions of all sonobuoys in May through October 1998 at Bay River. Symbols indicate sampling dates.

TASKS COMPLETED

Task 1: Recordings and spectrographs from captive sciaenid fish

Digital audio tape recordings of known species-specific drumming sounds produced by captive target sciaenids in North Carolina were made in order to have a sound call catalogue for the target species of sciaenid fishes. Spectrographs are useful in detecting the species present when two or more species are present in an area. We have included in this report a guide to fish sounds on the cassette tape labeled "Fish Sounds of North Carolina Estuaries". Additional recordings of sciaenid and other fishes can be found on Compact Disc for use in identifying spawning areas of the target species. Finally, an Internet webpage of fish sounds has been created for scientists and the public to use in the study of underwater sounds <http://croaker.physics.ecu.edu>.

This section and the accompanying tape and CD sound files have been used to identify the species in field recordings. Table 3 displays the detailed information about each of the spectrographs and power spectrum graphs in this section.

The first recording (file tankcr.wav; CD audio track no. 2) is that of a "purr" produced by a male weakfish, *Cynoscion regalis* (340 mm SL). This recording was made from weakfish captured in Teaches Hole in June 1998 using hook and line and recorded immediately after capture in a 94-quart cooler filled with seawater. The spectrograph of this recording is shown in Figure 17a. This "purr" consists of 15 bursts within a 0.5-s interval. Each burst has a broad frequency peak with maximum power spectral density between 275 Hz and 360 Hz. The average power spectrum (Figure 17b) for the entire "purr" shows the same broad peak with a maximum at 281 Hz.

The second recording (file tankcn.wav; CD audio track no. 3) is that of "burp" produced by a male spotted seatrout, *Cynoscion nebulosus* (200 mm SL). This recording was made from a spotted seatrout captured by hook-and-line in Roanoke Sound on 8 August 1998 and recorded in air 3 h after capture. The spectrograph of this recording is shown in Figure 18a. Here the fish makes three "burps." The first "burp" lasts 0.19 s, the second 0.13 s, and the third 0.11 s. The dominant frequency of each "burp" begins at a higher frequency and moves downward in time. Several peaks, or harmonics, can be seen in each "burp." The average power spectrum (Figure 18b) for the three "burps" does not show peaks that are as distinct because each "burp" begins and ends at different frequencies. The three peaks in the average power spectrum occur at 211 Hz, 281 Hz, and 352 Hz.

The third recording (file tankso.wav; CD audio track no. 4) is that of "knock" produced by a male red drum, *Sciaenops ocellatus,* recorded in a tank at the Pamlico Aquaculture Field Laboratory (PAFL), Aurora, NC (one of a group of 24 fish; $\bar{x}$ = 660 mm SL; range: 500- 780 mm SL). The spectrograph of this recording is shown in Figure 19a. The spectrograph shows three "knocks" each lasting 0.13 s (including the low frequency tail at the end). Each "knock" consists of two peaks, one at 70 Hz and another at 164 Hz as seen in both the spectrograph and the average power spectrum (Figure 19b).

Table 3. Recording and acoustical analysis data and for spectrographs and power spectrum graphs of captive fish.

Figure	**Sound file on CD**	**Audio track on CD**	**Recording date**	**Spectro-graph slide factor**	**Average Power Spectrum**			**Remarks**
					Start Time (s)	**Stop Time (s)**	**Number of Averages**	
Figure 17	tankCR.wav	2	June 1998	128	1.395	1.885	11	340 mm SL recorded in cooler in water
Figure 18	tankCN.wav	3	8 Aug 1998	128	1.700	2.700	23	200 mm SL recorded in air
Figure 19	tankSO.wav	4	3 Aug 1998	128	15.704	16.173	10	~ 660 mm SL recorded in tank at PAFL

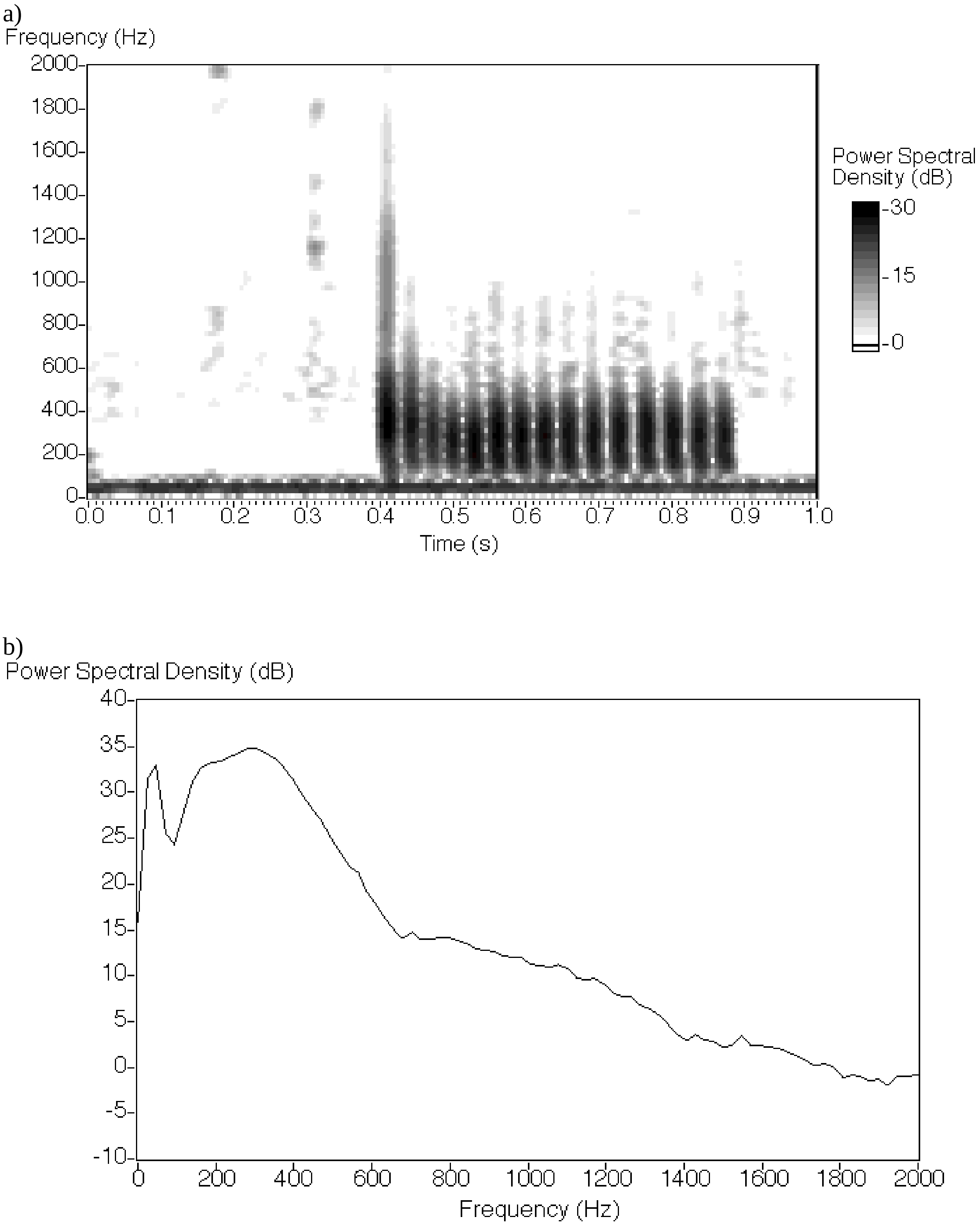

Figure 17. a) Spectrograph of a captive weakfish. This sound is termed a "purr". b) Power spectrum from the captive weakfish "purr".

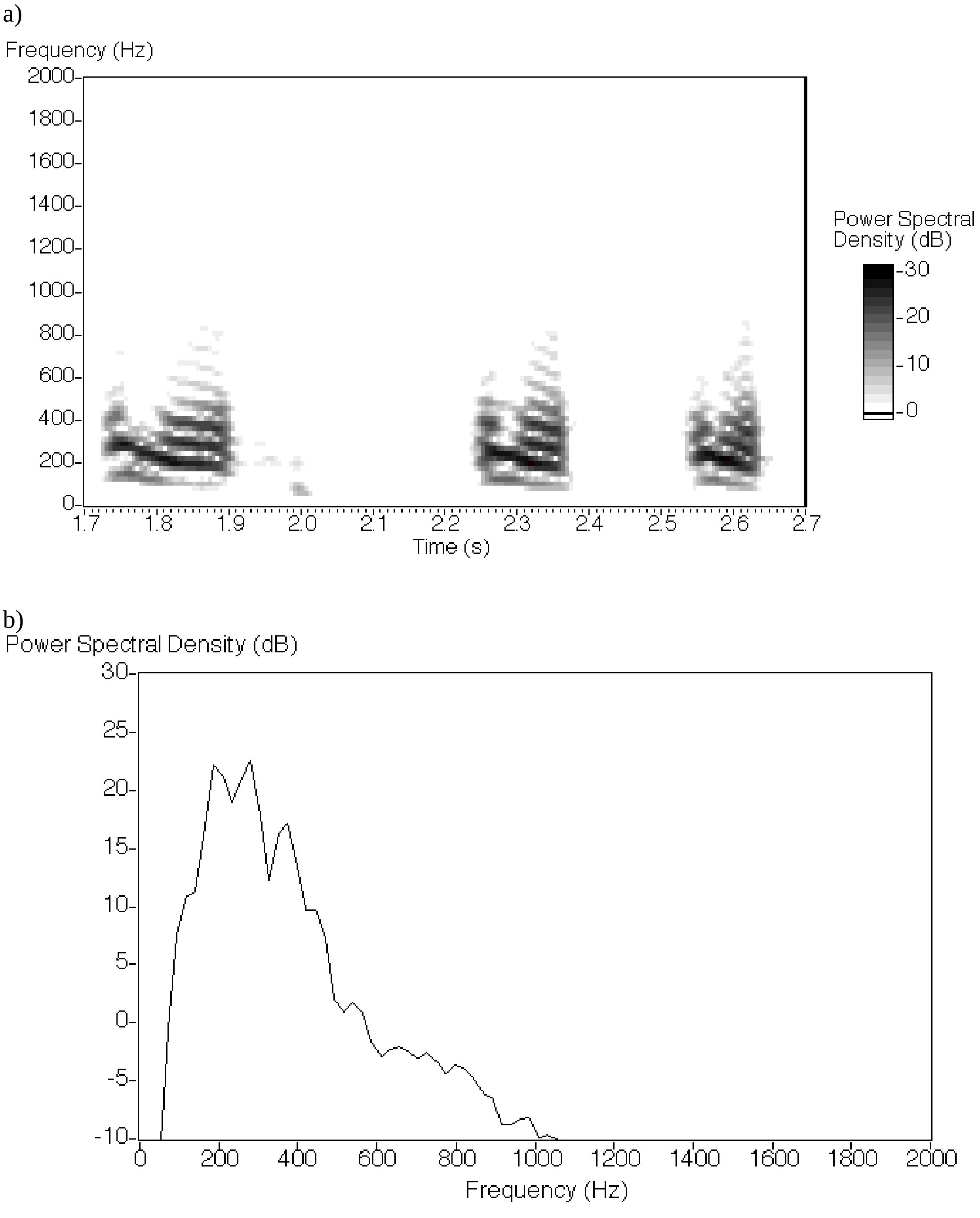

Figure 18. a) Spectrograph of a captive spotted seatrout. This sound is termed a "burp". b) Power spectrum from the captive spotted seatrout "burp".

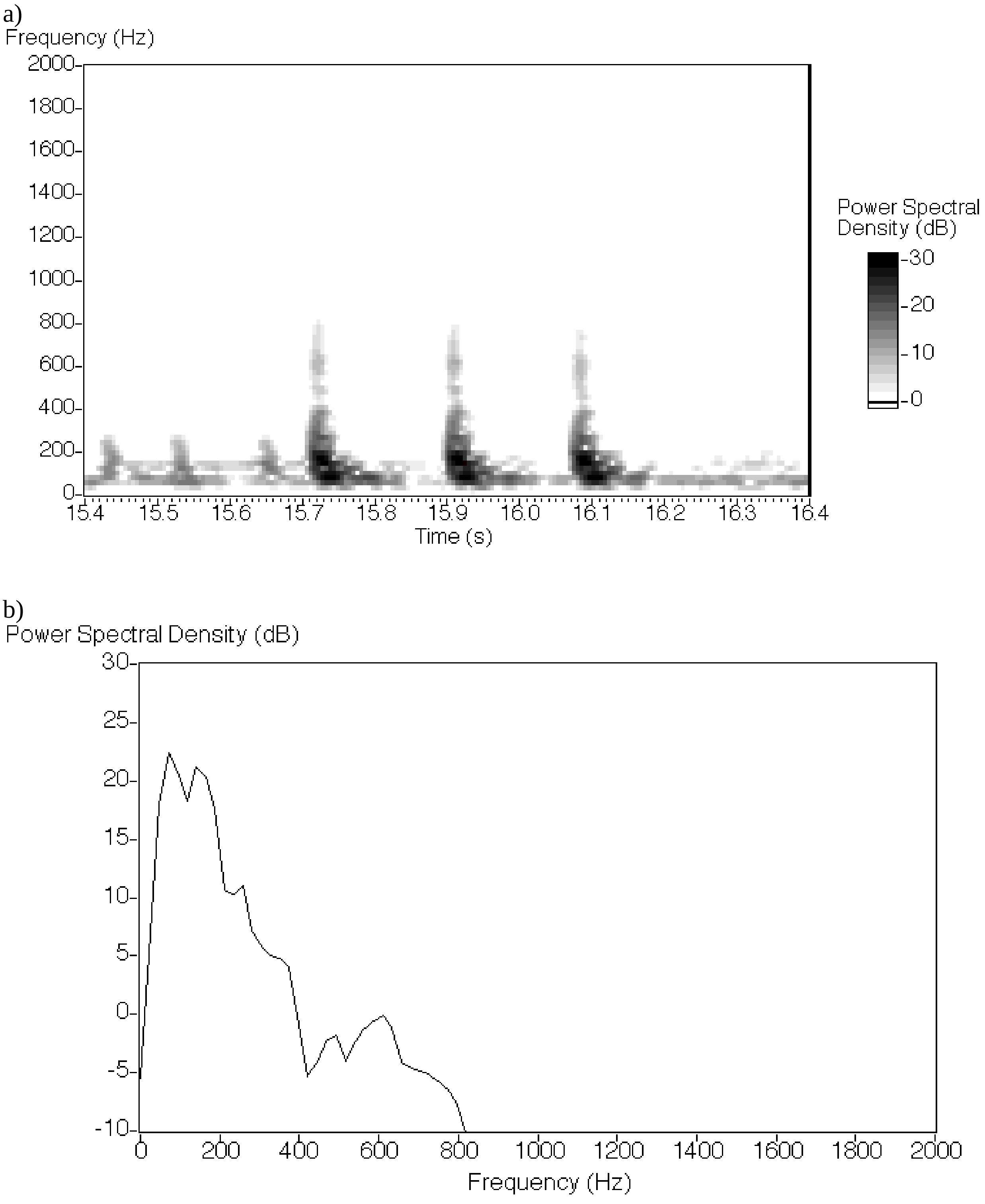

Figure 19. a) Spectrograph of a captive red drum. This sound is termed a "knock". b) Power spectrum of a captive red drum "knock".

Task 2: Sound production and egg production in spawning areas

Drumming sounds of red drum, spotted seatrout, and weakfish have been recorded at suspected spawning locations in Pamlico Sound. Global Positioning System position information was recorded along with ichthyoplankton surveys at each site where sound recordings were made.

We analyzed the drumming sound from captive fish to obtain its spectrographic characteristics for use in identification of weakfish in a location (See Task 1). Sound recordings made after sunset indicated that both individuals and groups of fish produced drumming sounds. We made 368 digital audio tape recordings with fish sounds after sunset in May through October 1997; 141 (38.3 %) of these contained weakfish "purring"; 44 (11.4 %) of these contained spotted seatrout "burps" and 11 (3.0 %) contained red drum "knocks". A map of the sites where we recorded “purring” by male weakfish (Figure 20) shows that weakfish spawning was restricted to the eastern side of Pamlico Sound; we never recorded weakfish "purring" at stations away from the inlets (Rose Bay, Jones, Bay or Bay River). Spotted seatrout were recorded in on both the Eastern and Western side of Pamlico Sound (Figure 21). Red drum were heard producing "knocking" sounds only in September of 1997, but on both sides of Pamlico Sound (Figure 22).

Other biological sources of sound contributed to the sound pressure level in some of these recordings. The soniferous silver perch (*Bairdiella chrysoura*, Sciaenidae) were recorded "clucking" on some recordings. On many inlet recordings, weakfish could be heard "purring" simultaneously with silver perch "clucking". Because these two species co-occurred at most inlet locations, we performed spectrographic analyses to identify the presence of silver perch "clucking" in recordings where weakfish were also recorded "purring" (See Task 3). After spectrographic analysis, we determined that of the 37 recordings made at the inlet sites after sunset with fish sounds, 11 recordings had silver perch “clucking” individually or in groups, 1 recording had “purring” weakfish in a group, and 25 recordings had silver perch and weakfish drumming simultaneously in groups. Thus, although silver perch and weakfish both produce sounds at the same time of year and in some of the same locations, the presence of either species can be determined from their distinctive spectrographic signatures. The locations in which silver perch were detected during 1997 and 1998 surveys are given in Appendix IV.

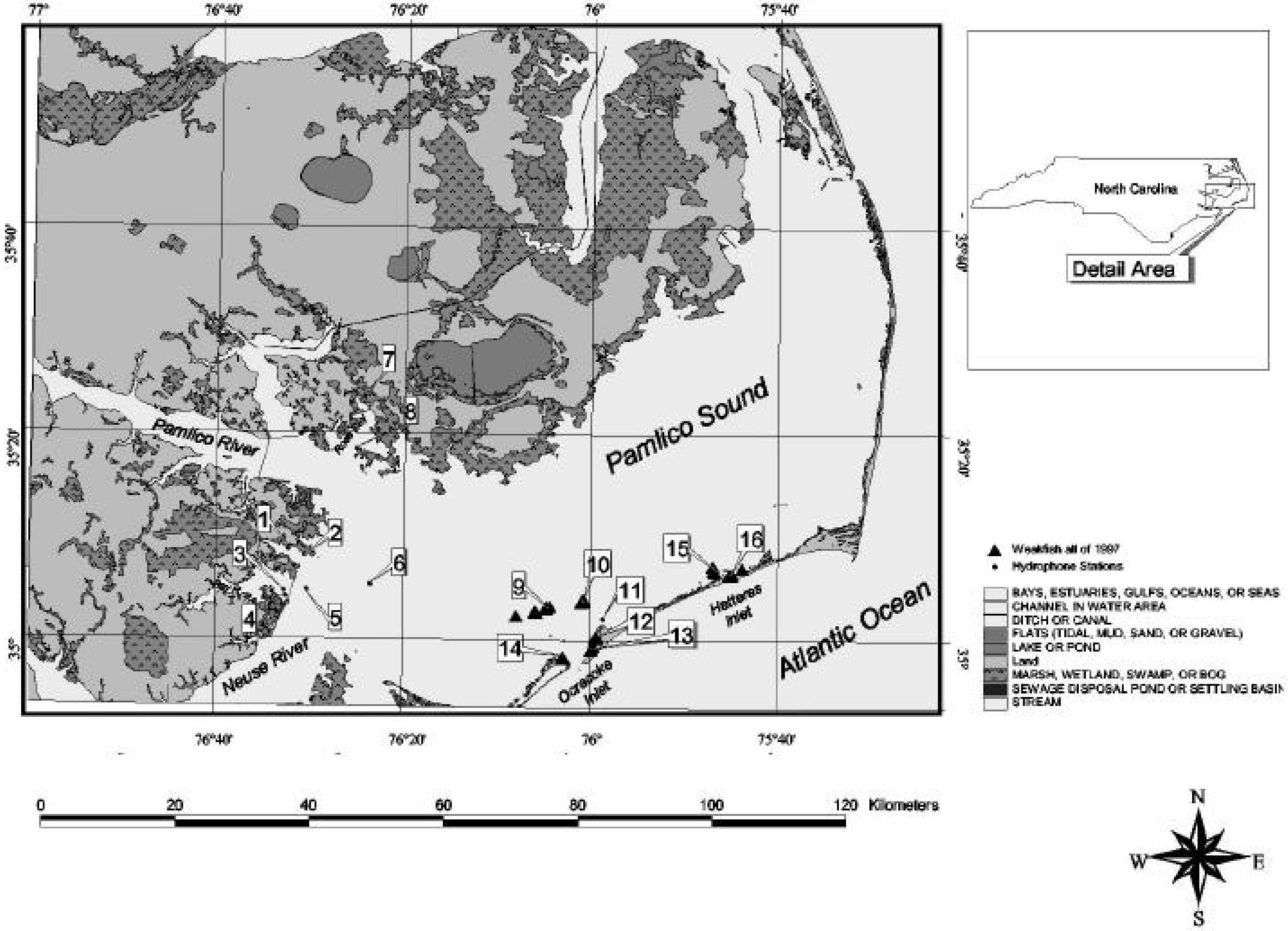

Figure 20. A map showing the locations where weakfish "purring" was recorded during the hydrophone survey in 1997.

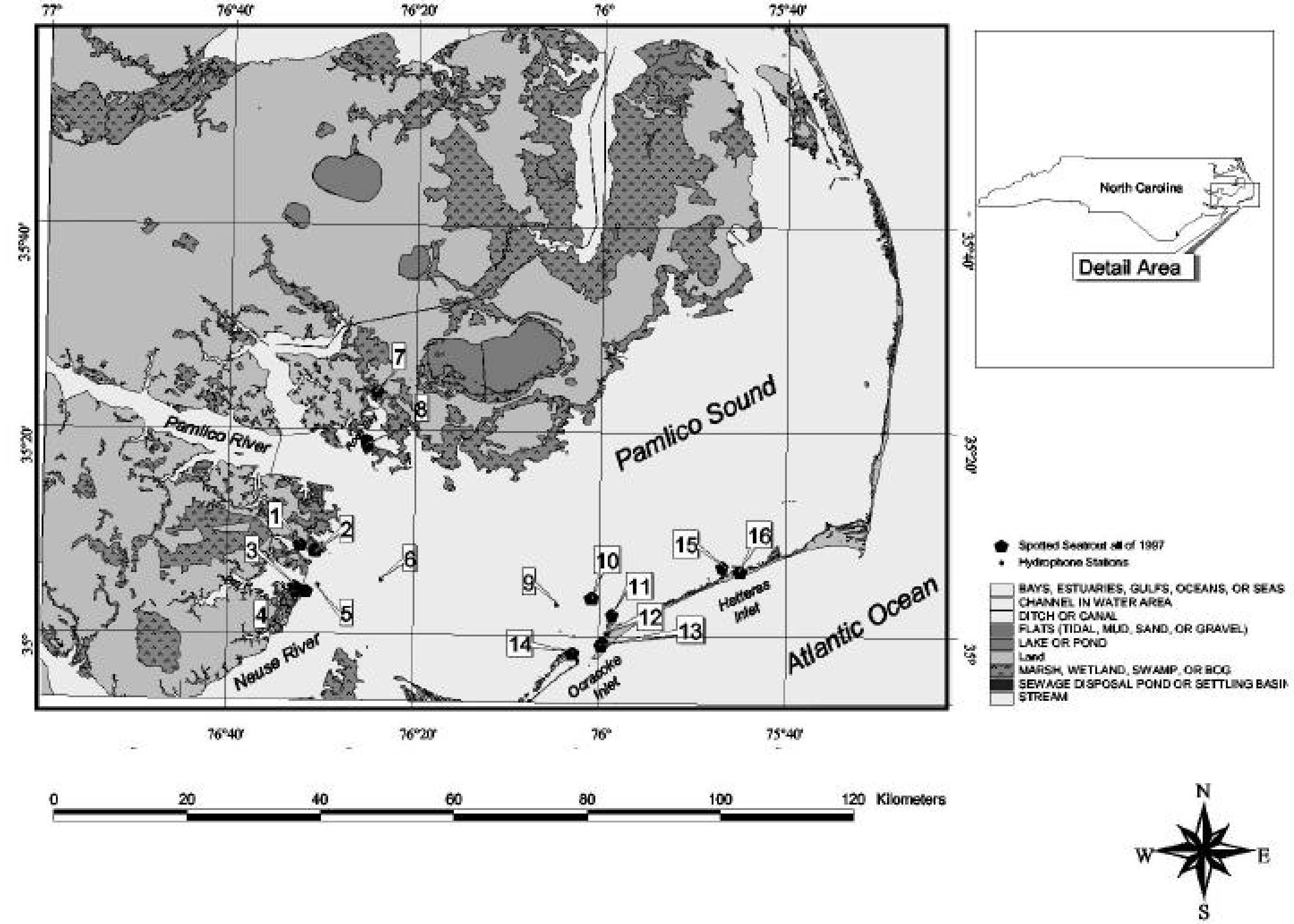

Figure 21. A map showing the locations where spotted seatrout "heartbeat, burp, and staccato" sounds were recorded in hydrophone surveys in Pamlico Sound NC 1997.

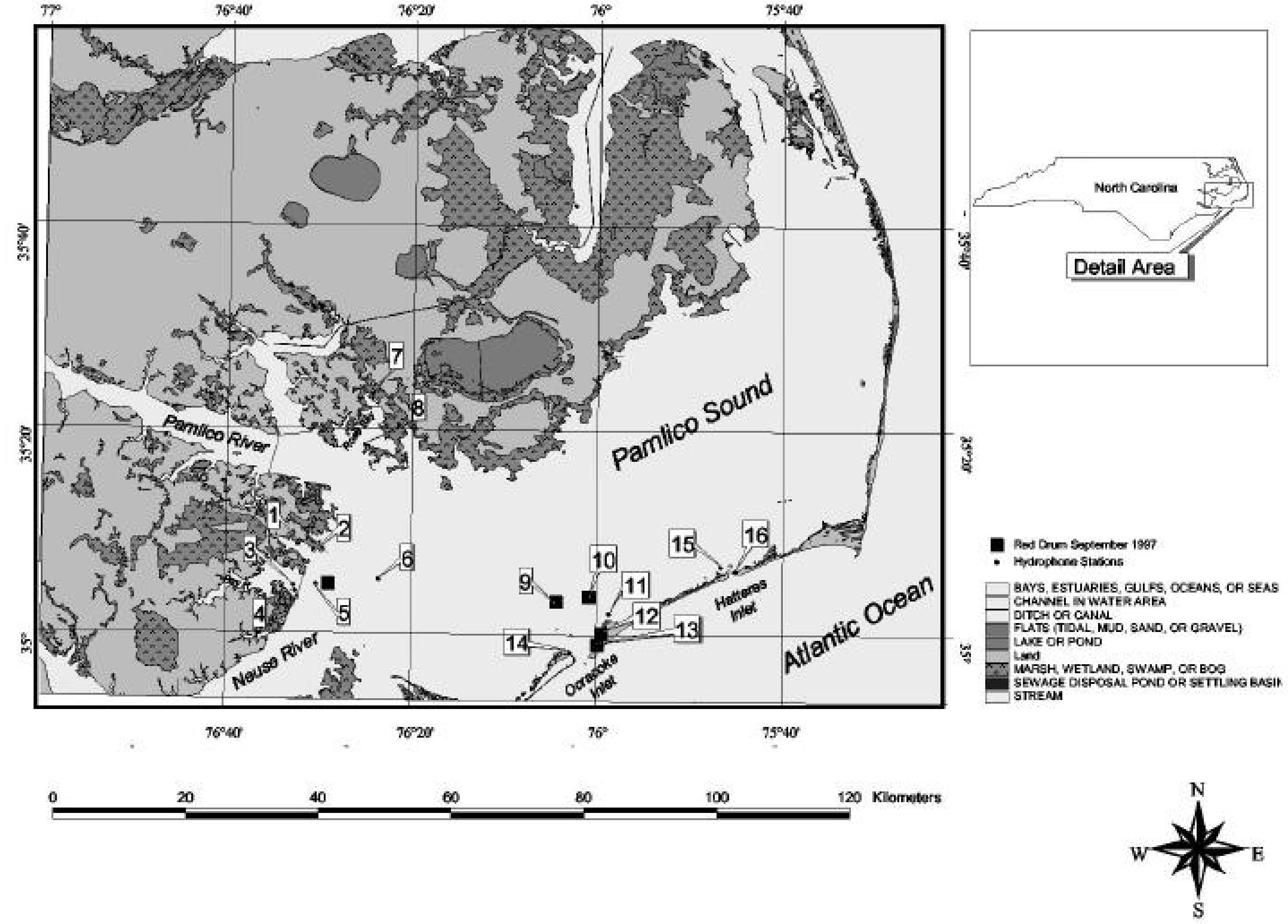

Figure 22. A map showing the locations where red drum "knocking" sounds were recorded in hydrophone surveys in Pamlico Sound NC 1997.

Comparison of Sound Production and Egg Production

The "purring" sounds of weakfish, the "clucking" sounds of silver perch, the "heartbeat, burp, and staccato" sound of spotted seatrout, and the "knocking" sounds of red drum were associated with spawning behavior, because < 1-day old sciaenid-type eggs were collected in plankton samples made at the same hydrophone stations. Sciaenid-type eggs were collected in association with fish sound production at many hydrophone stations during May through October 1997 (Figure 23). The highest egg abundances occurred near Ocracoke and Hatteras Inlets in 1997.

The spawning of weakfish appeared to peak in May 1997 in both in terms of the sound production and egg production by females. Weakfish purring was heard in May of 1997 predominantly at high-salinity stations near the inlets. Maximum sound pressure levels at stations where weakfish “purring” and silver perch “clucking” were recorded after sunset was positively correlated with $\log_{10}$-transformed sciaenid-type egg densities at those same stations (Pearson correlation coefficient, $r = 0.78$; $p = 0.002$; n = 13)(Luczkovich et al. in press). No sciaenid-type eggs were collected on the western side of Pamlico Sound during May in 1997, but high densities of sciaenid-type eggs were collected in Ocracoke and Hatteras Inlets in May of 1997 in association with weakfish "purring"(compare Figure 24 and Figure 25).

Spotted seatrout spawning appeared to peak in July of 1997 as judged by sound production. The locations in which spotted seatrout "burps" and other sounds (see Task 3) were detected in Pamlico Sound are shown in Figure 26. Spotted seatrout males appeared to drum on both the eastern and western side of Pamlico Sound. Sciaenid-type egg densities were uniformly low all over the sound in July (Figure 27), but the greatest number appeared near the Lehigh dredge station (Station 10). At that station, sciaenid-type eggs were collected in association with spotted seatrout sounds; however, both silver perch and weakfish were also detected at that time, acoustically. The spotted seatrout spawning area with the greatest likelihood of high egg production are the areas on the eastern side of Pamlico Sound, as higher egg densities were collected there in July.

Red drum egg production and sound production also coincided, but only in September 1997. No red drum were detected in other months in 1997, so the overall map of May through October sound production (Figure 22) is identical to the map of sound production for September (Figure 28). Sciaenid -type eggs were collected on 17 September 1997 in large numbers only at Station 5, Bay River Mouth (Figure 29). This station is where we collected red drum eggs, as identified by mtDNA data (see following section). These eggs were also much larger in diameter than weakfish, spotted seatrout or silver perch eggs. This location (Station # 5) appears to be an important spawning area for red drum, although areas around Ocracoke Inlet appear to have red drum "knocks" and sciaenid-type eggs present in September as well. It is also worth noting that a large 17.1 kg 1040 mm SL male red drum (29 years old) was captured in a gill net on 17 September 1997 at Station 6. This individual had developed gonads that were 1.98 % of the body mass (339 g).

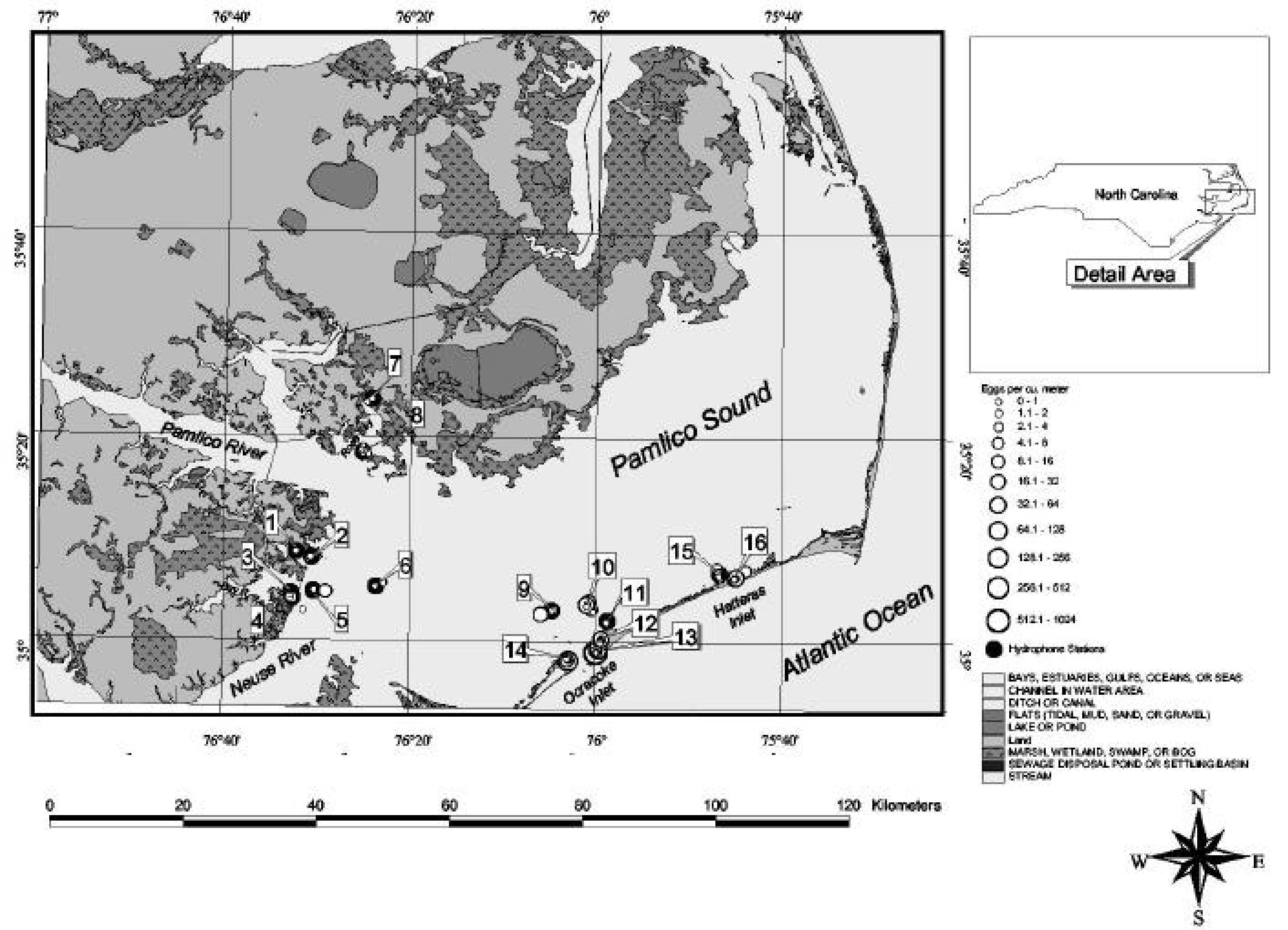

Figure 23. A map showing the hydrophone stations at which sciaenid-type eggs were collected during May through October 1997. Size of symbol represents the sciaenid-type egg density in number/m^3.

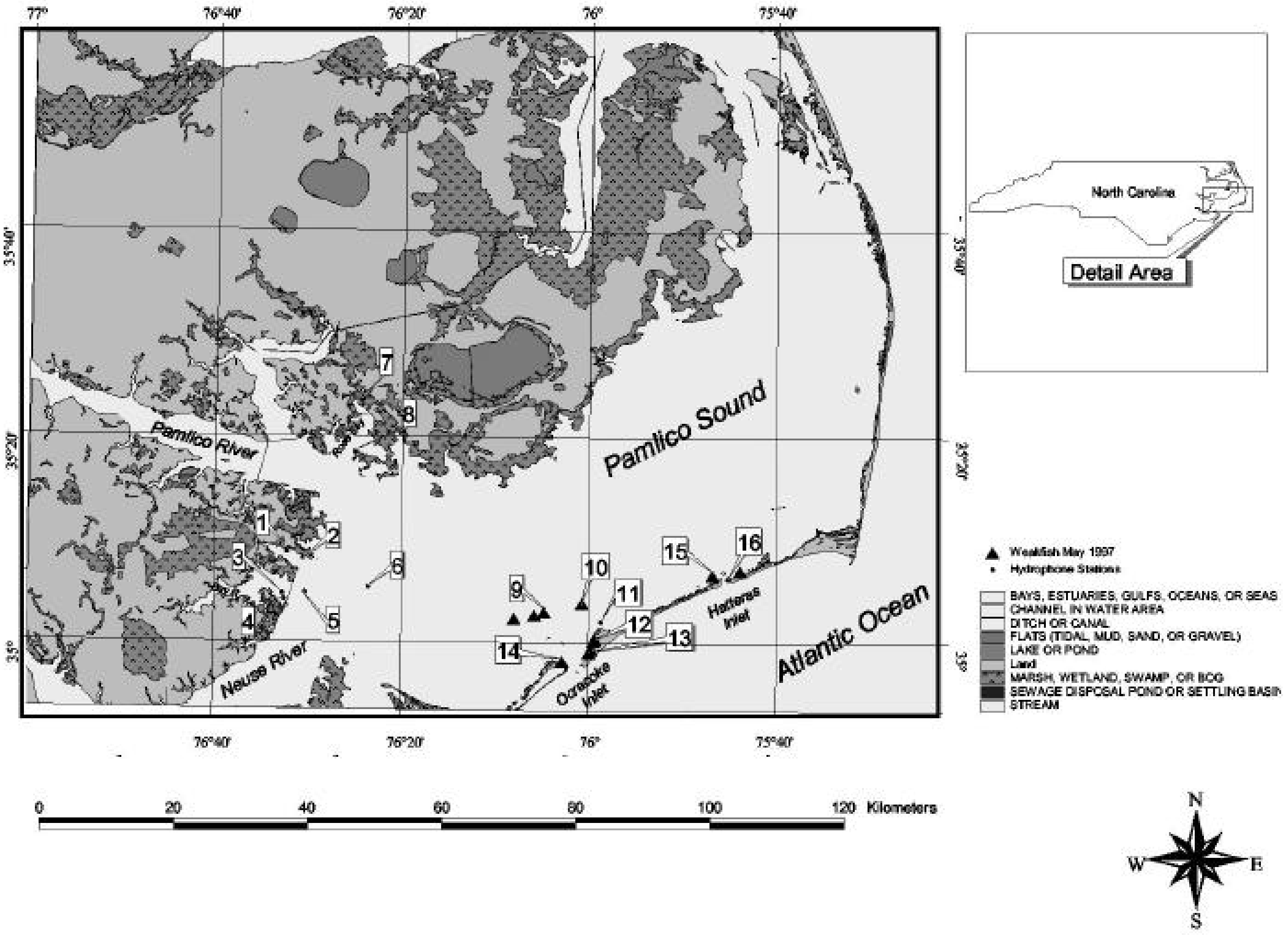

Figure 24. The locations in May 1997 where weakfish were heard purring.

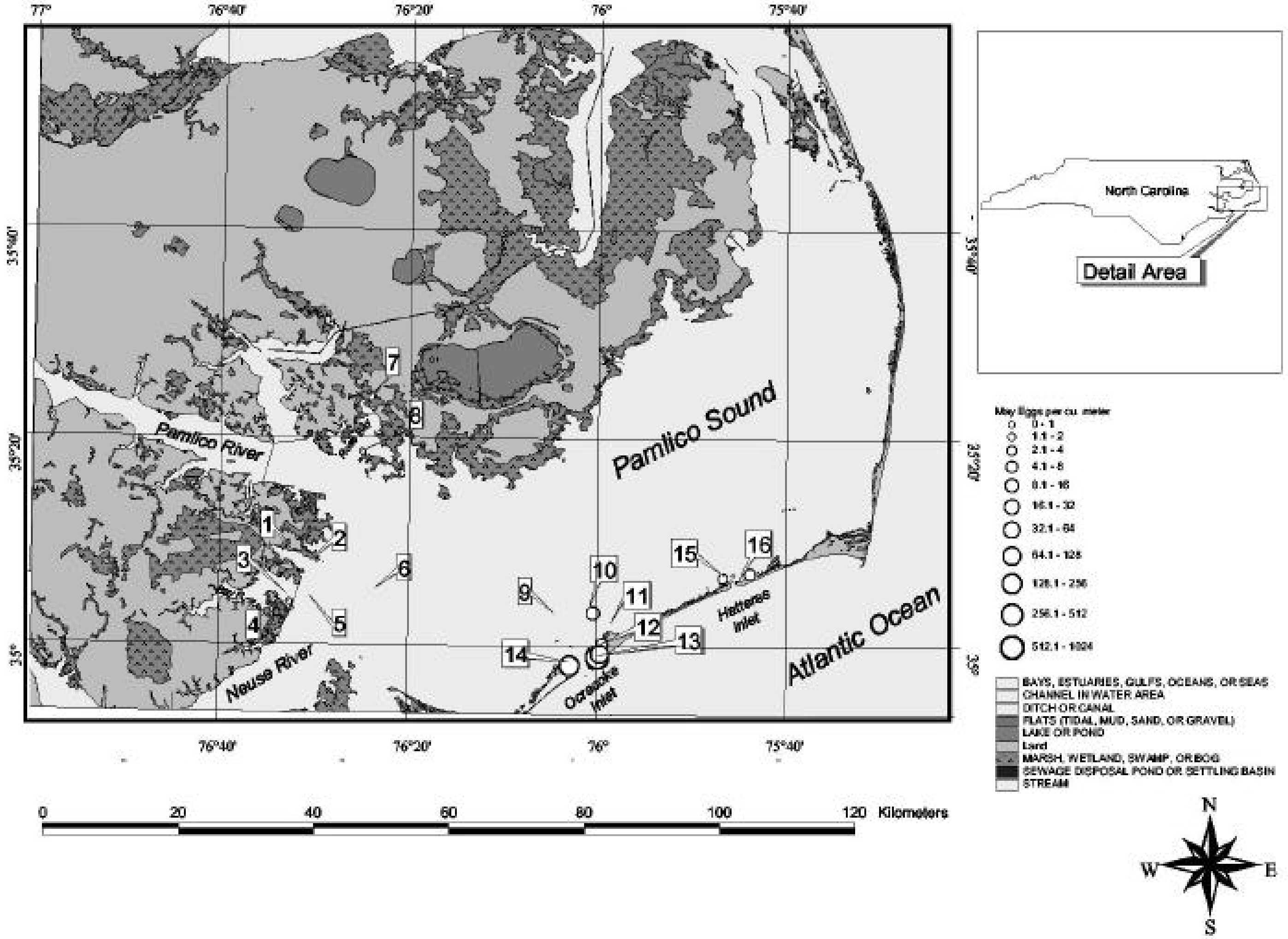

Figure 25. The locations in May 1997 where sciaenid-type eggs were collected. Size of symbol varies with egg density (number of eggs/m^3).

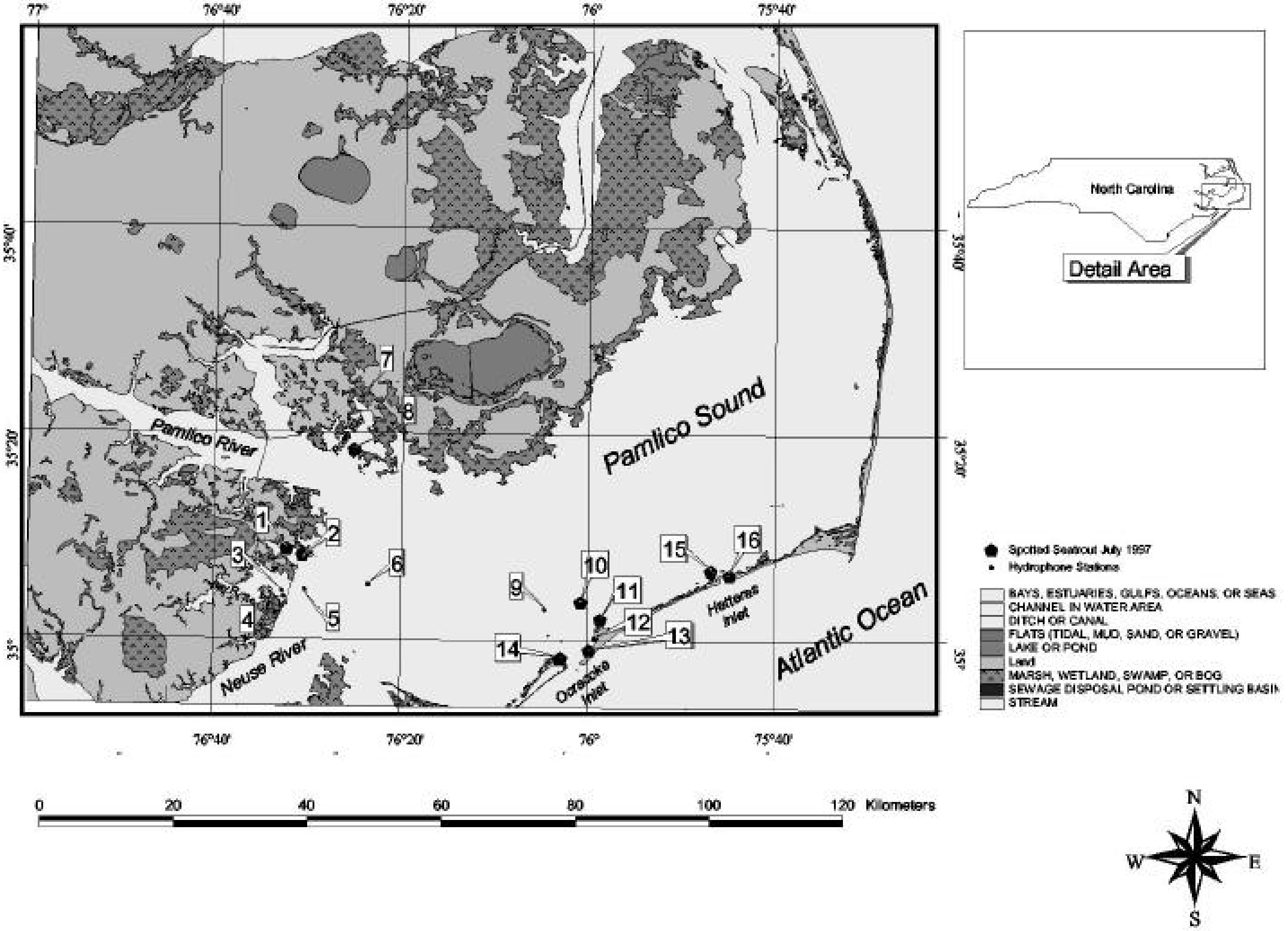

Figure 26. The locations in July 1997 where spotted seatrout were heard making "heartbeat," "burp" or "staccato" sounds.

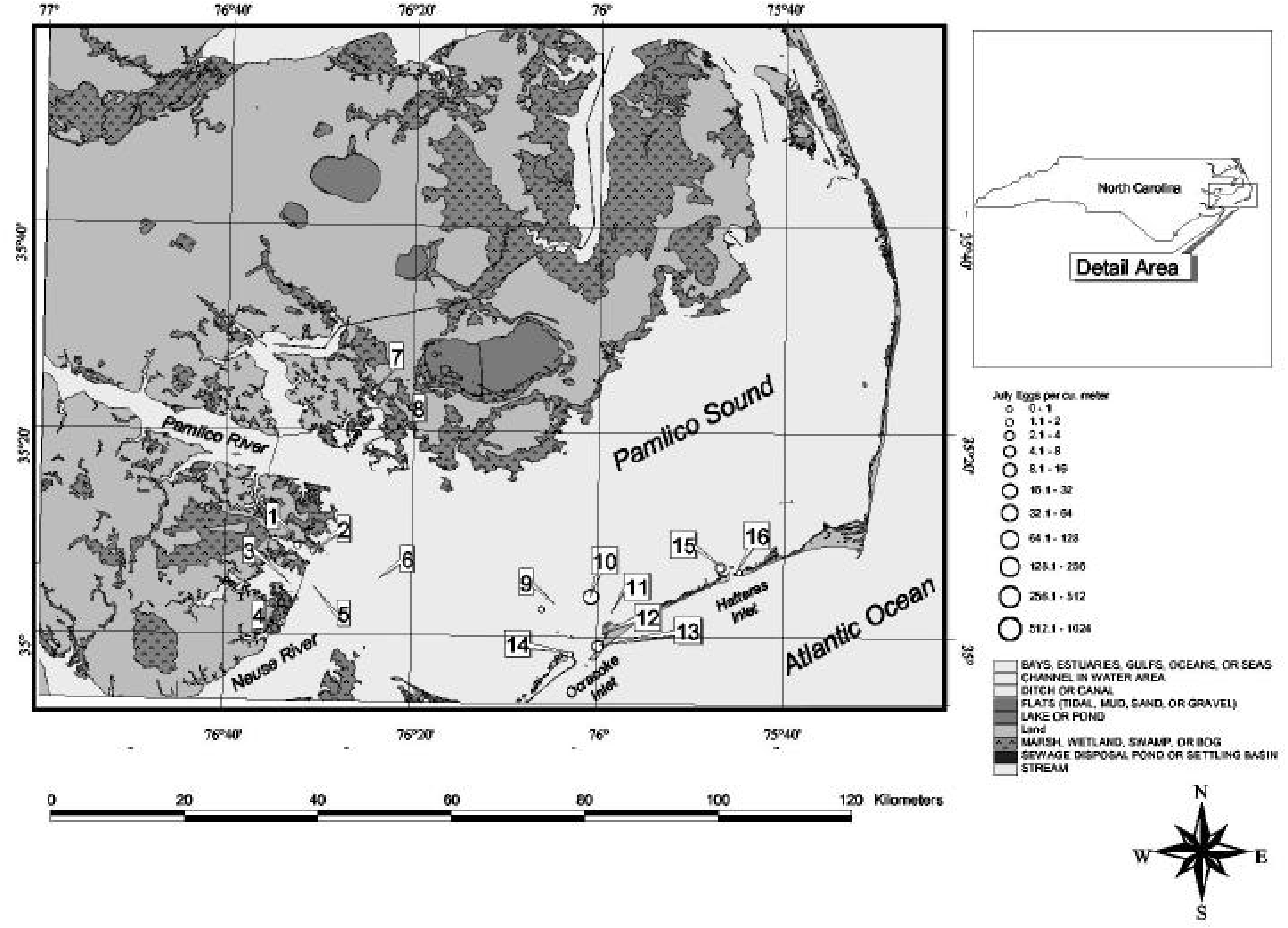

Figure 27. Locations where sciaenid-type eggs were collected in July of 1997.

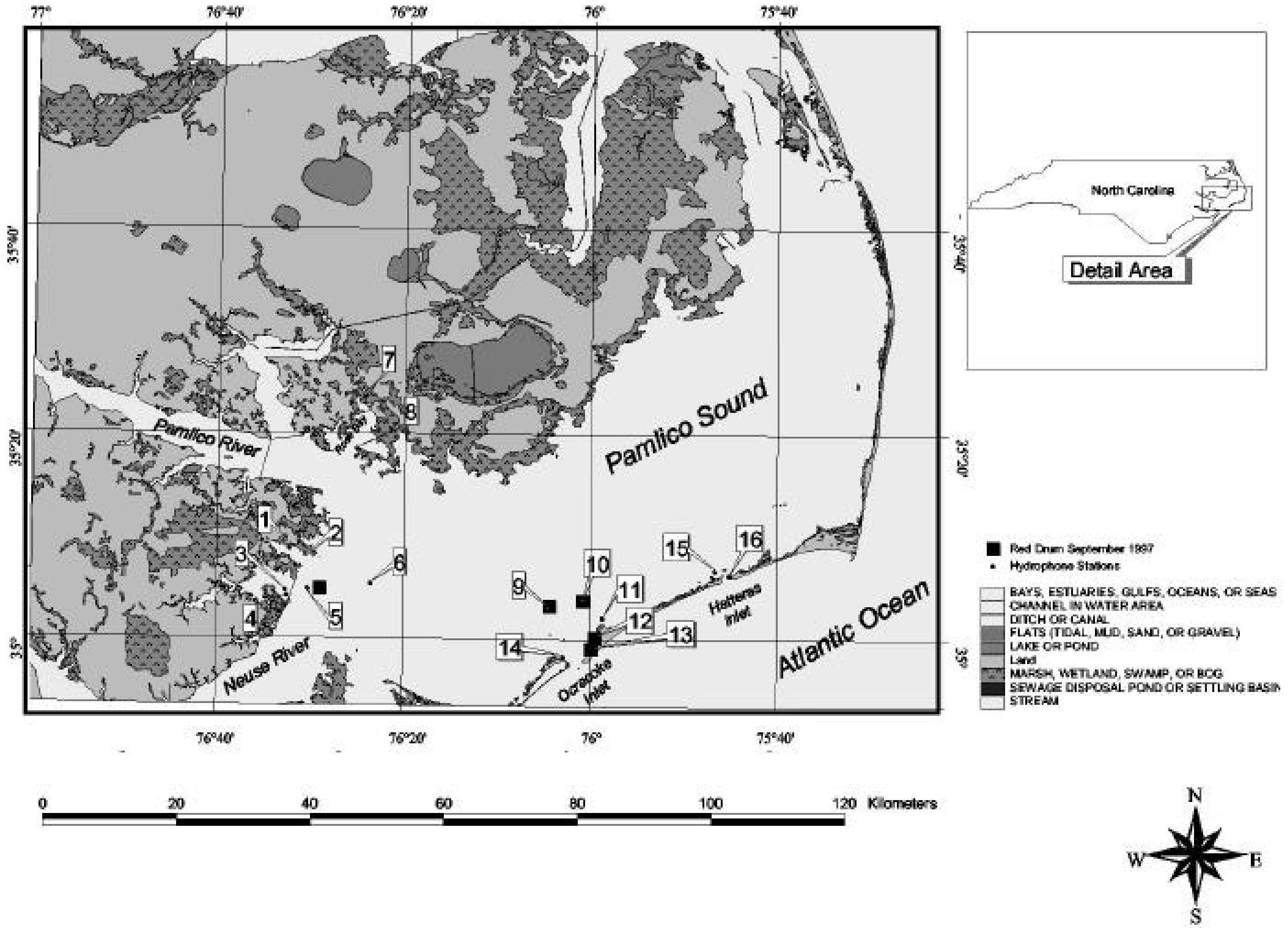

Figure 28. Locations in which red drum were heard making "knocking" sounds in September 1997.

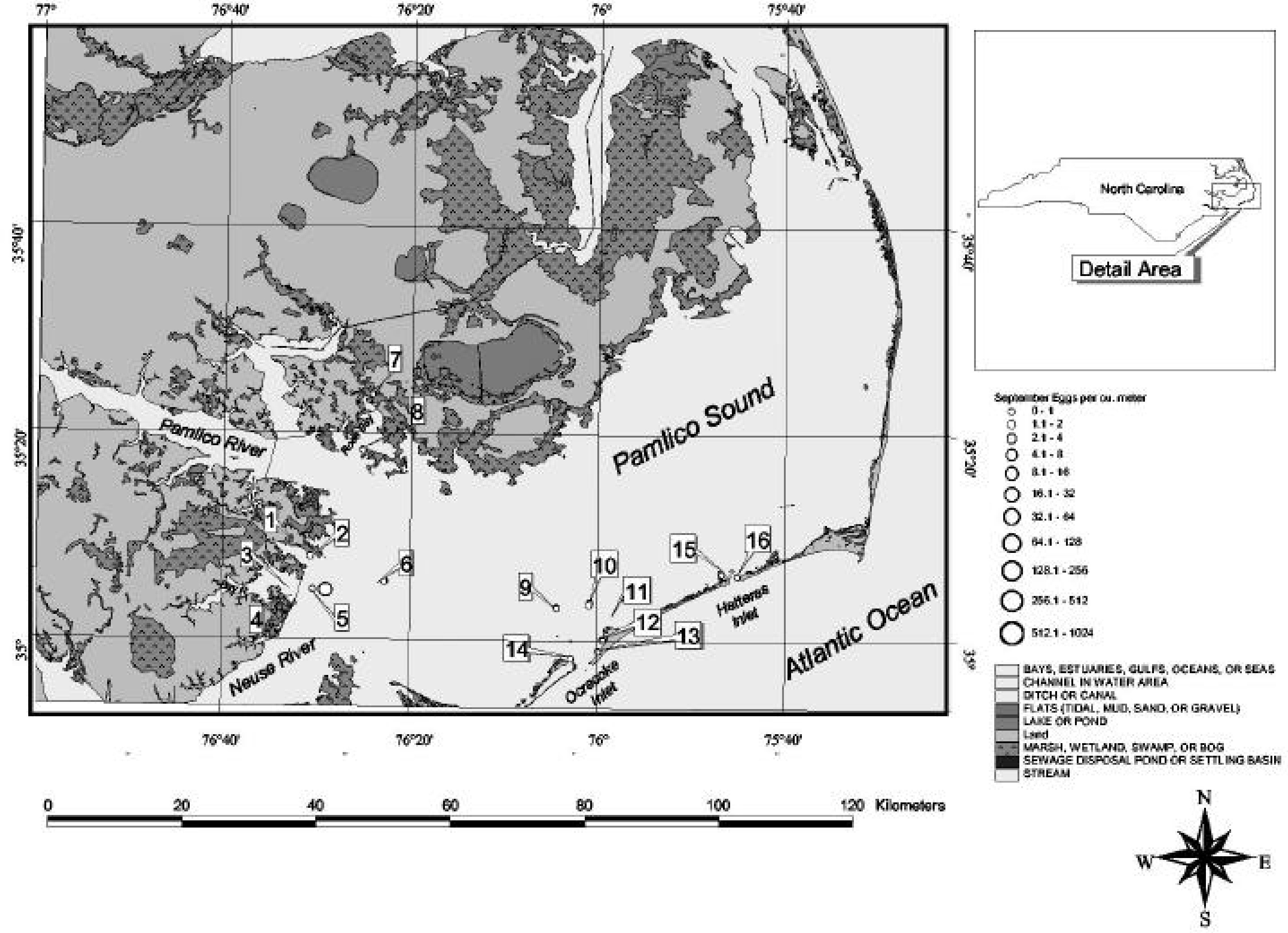

Figure 29. Locations in which sciaenid-type eggs were collected in September 1997.

Molecular identification of sciaenid type eggs

Mitochondrial DNA from tissue samples of adult weakfish, silver perch, spotted seatrout, and red drum were compared to samples of unknown sciaenid-type eggs collected in Pamlico Sound at the hydrophone listening stations in 1996,1997, and 1998 (additional collections were made in 1996 and 1998 for this molecular identification study). The egg characteristics and passive acoustic data collected in association with each of the egg collections are reported in Table 4.

Mitochondrial DNA RFLP identification of the sciaenid-eggs collected in the plankton tows matched the identity of fish producing sound in 14 of 17 mtDNA tests. Red drum aggregations were detected acoustically making the "knocking" sound on 17 September 1997 at the Bay River mouth where we collected eggs labeled U10, U11, and U12. Eggs in lanes U10, U11, and U12 have a mtDNA profile identical to that of the adult red drum in the lane labeled SO2 (Figure 30). The RFLP profile of the egg (which was reared up to a larva for identification, but not measured for egg diameter) labeled U13 indicates that it was a silver perch (Figure 31); both silver perch and weakfish were detected acoustically that evening (Table 4). In addition, the unknown egg U14 was typed as a weakfish by mtDNA analysis; both silver perch and weakfish were detected in the passive acoustic surveys. Because acoustical data suggested that both weakfish and silver perch were present and spawning at the inlets during May 1996 and 1997, silver perch and weakfish eggs could be present in the same egg collections. Spotted seatrout, weakfish and silver perch were detected acoustically at the same time at Wallace Channel on 10 June 1998 where we collected eggs labeled U36, U37, U38, and U39. Eggs U36, U37, and U38 have the RFLP profile of spotted seatrout, and egg U39 has the profile of weakfish (Figure 32).

In May of 1998, we examined the hypothesis of Daniel and Graves (1993) that the small-diameter eggs are produced by silver perch and large-diameter eggs are produced by weakfish. The mtDNA RFLP profiles obtained from unknown eggs U17, U19, and U20 are characteristic of silver perch (compare with profiles BC3 and BC4 in Figure 30); the profiles of eggs U21 and U22 are characteristic of adult weakfish (compare with profile CR2 in Figure 30). The acoustical data associated with these egg collections indicated that silver perch were detected on 18 May 1998 at Teaches Hole and both weakfish and silver perch were detected on 19 May 1998 at Wallace Channel, both stations near Ocracoke Inlet. The smaller eggs (< 800 µm) collected at that same time and at that same location were silver perch, whereas eggs > 800 µm were weakfish (Table 4 and Figure 31). These data support Daniel's and Graves' hypothesis that small-diameter eggs are silver perch and large diameter eggs are weakfish, with the cut-point occurring between 800 and 900 µm. Daniel and Graves suggest an overlap of weakfish and silver perch at 825 µm egg diameter. Unknown egg U39 (Figure 32) measures 825 µm and has the profile of a weakfish. This is consistent with Daniel's and Graves' results. Since we have only tested one egg at that diameter, we do not know if we will find silver perch eggs of that size. Our data suggest that spotted seatrout egg diameters overlap the diameters of weakfish and silver perch eggs. Unknown eggs U15, U16 (Figure 31), U36, U37, and U38 (Figure 32) have the RFLP profile of spotted seatrout and have a range in diameters of 800 µm to 925 µm which overlaps both weakfish and silver perch egg diameters. Daniel and Graves did not address spotted seatrout egg diameters because that species was not present in their samples.

There were three cases where the identity of eggs based on mtDNA data and the identity of spawners based on passive acoustical data were inconsistent. Although unknown eggs U15 and U16 were identified as spotted seatrout based on the mtDNA profiles, no spotted seatrout were heard at that the hydrophone listening station on that night. In addition, egg U18 was identified as a weakfish egg based on the mtDNA profile (Figure 31), but acoustic data collected in the same place at that same time indicated that no weakfish were drumming. These latter two results are mis-matches between the acoustic and the molecular data; they suggest that the drumming by male spotted seatrout and weakfish and the production of eggs by females of these species may not always be correlated. Such eggs could have been produced by fish spawning in a location more than 50 m away from our hydrophone listening station, and the eggs carried in the currents to us. Such spawning fish would not be detected acoustically by our instruments, because of the sound attenuation below the background sound level occurs at distances greater than 50 m away. Additionally, spotted seatrout tend to produce drumming sounds just after sunset (see 24-hour sound production data in Task 4). We may have missed these drumming males on the night when we collected the seatrout eggs in an area, because the plankton tows were normally done several hours after dark in our survey protocol, and thus after sound production had ceased by spotted seatrout.

Based on the results of this preliminary comparison of mtDNA RFLP methods and passive acoustic methods for establishing spawning areas, we conclude that there is good agreement between the two methods. The acoustic method is rapid and cost effective, but it may not be able to establish spawning activity per se, only that males are drumming within a 50 m radius of the sampling area and thus are likely to be spawning in that vicinity at some time in the future. The egg collection method with the mtDNA identification unequivocally establishes the identity of species spawning in general area near the collection site, but it is time-consuming and more expensive when processing on a large number of samples.

Table 4. Sciaenid-type egg mtDNA egg identifications as compared with passive acoustic data. Sample number of unknown eggs, collection date and location for eggs and passive acoustic data, and species identification of spawning fishes based on passive acoustical data associated with

Sample number	Egg Diameter (μm)	No. of Oil Globules	Collection Date	Collection Location	Species detected acoustically
U10	950	1	17 September 1997	Bay River Mouth	red drum
U11	950	1	17 September 1997	Bay River Mouth	red drum
U12	900	1	17 September 1997	Bay River Mouth	red drum
U13	NR[1]	1-3	16 May 1996	Wallace Channel	weakfish, silver perch
U14	NR[2]	1-3	22 May 1997	Hatteras Hole	weakfish, silver perch
U15	850	1	19 August 1997	Fisherman's Bay	none
U16	800	1	19 August 1997	Fisherman's Bay	none
U17	800	1	18 May 1998	Teaches Hole	silver perch
U18	900	1	18 May 1998	Teaches Hole	silver perch
U19	750	1	19 May 1998	Wallace Channel	weakfish, silver perch
U20	800	1	19 May 1998	Wallace Channel	weakfish, silver perch
U21	950	1	19 May 1998	Wallace Channel	weakfish, silver perch
U22	1000	1	19 May 1998	Wallace Channel	weakfish, silver perch
U36	850	1-3	10 June 1998	Wallace Channel	silver perch, weakfish, spotted seatrout
U37	925	1-3	10 June 1998	Wallace Channel	silver perch, weakfish, spotted seatrout
U38	900	1-3	10 June 1998	Wallace Channel	silver perch, weakfish, spotted seatrout
U39	825	1-3	10 June 1998	Wallace Channel	silver perch, weakfish, spotted seatrout

[1] Not recorded; issue from a larval fish reared from a sciaenid egg collected at that place and date

[2] Not recorded

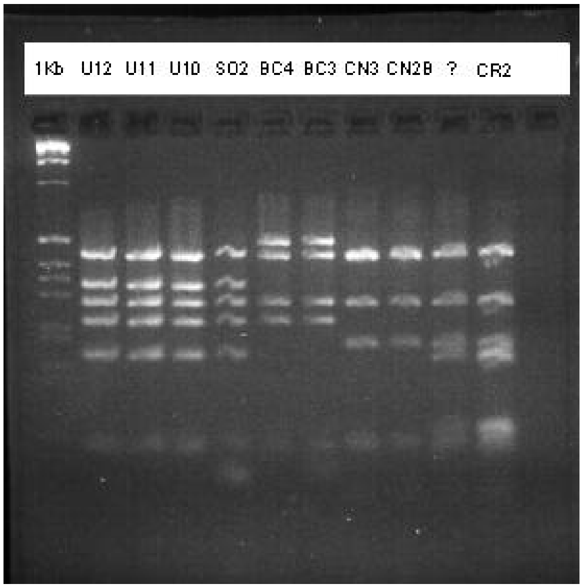

Figure 30. Restriction enzyme digest profile for adult sciaenid fishes and unknown sciaenid type eggs collected in Pamlico Sound. The lanes are as follows (left to right): 1 kb, a 1 kilobase pair DNA ladder (0.3 µg; Life Technologies, Inc.); U10 - U12, unknown eggs collected in Pamlico Sound (See Table); SO2, adult red drum, *Sciaenops ocellatus*, tissue; BC4, adult silver perch, *Bairdiella chrysoura*, tissue; BC3, adult silver perch, *B. chrysoura*, tissue; CN3, adult spotted seatrout, *Cynoscion nebulosus*, tissue; CN2B, adult spotted seatrout, *Cynoscion nebulosus*, tissue; ? unknown adult specimen tissue; CR2, adult weakfish, *Cynoscion regalis*, tissue.

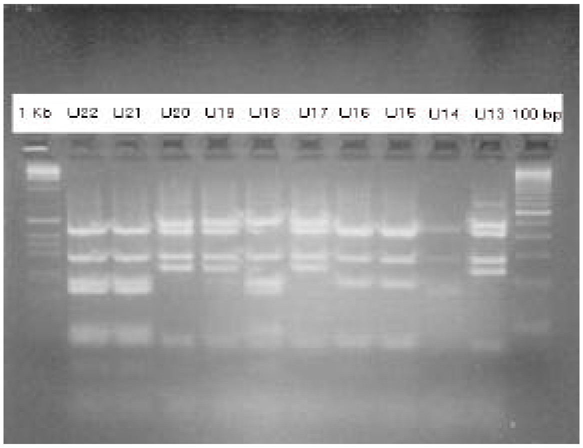

Figure 31. Restriction enzyme digest profile for additional unknown sciaenid-type eggs collected in Pamlico Sound. The lanes are as follows (left to right): 1 kb, a 1 kilobase pair DNA ladder (0.3 µg; Life Technologies, Inc.); U13 - U22, unknown eggs collected in Pamlico Sound (See Table 4); 100 bp, a 100 base pair DNA ladder (0.3 µg; Life Technologies, Inc.)

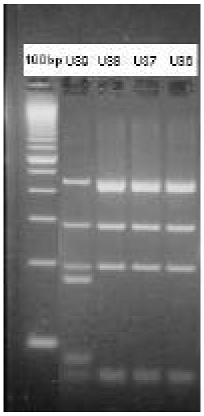

Figure 32. Restriction enzyme digest profile for additional unknown sciaenid-type eggs collected in Pamlico Sound. The lanes are as follows (left to right): 100 bp, a 100 base pair DNA ladder (0.3 µg; Life Technologies, Inc.); U36 - U39, unknown eggs collected in Pamlico Sound (See Table 4).

Can Sound Production Predict Egg Production?

In a word: yes. We have demonstrated previously that overall sound pressure levels associated with fish drumming are correlated with the abundance of sciaenid-type eggs, which in May in Pamlico Sound are either weakfish or silver perch (Luczkovich, et al. in press). Sound production varies as weakfish and other sciaenids aggregate to spawn. More males making sound may indicate that there is more spawning occurring. To test this idea for each sciaenid species separately, we regressed the egg abundance ($\log_{10}$ transformed) against relative sound pressure levels (in dB) from the field recordings of digital audio tapes made at the same locations as the plankton tows. We obtained sound pressure levels from frequencies specific to weakfish (~ 350 Hz; range: 304- 375 Hz) and those specific to silver perch (~ 1000 Hz; range: 984-1078 Hz) by integrating the sound pressure within those frequency ranges. Spectrographs from field recordings were made using Labview software and sound levels recorded in the field at each location were corrected for the contribution by each species. The egg abundances for each fish species were obtained by measuring egg diameter for a sample of eggs collected at each location. We assumed, based on the data in the previous section and that of Daniel and Graves (1993), that eggs < 0.8 μm were those of silver perch and those > 0.85 μm were those of weakfish. The plot of the relationship is shown in Figure 33 for weakfish and Figure 34 for silver perch. The regression relationship after log-transforming is nearly linear in both cases. These data should provide the basis for predicting the egg production for each of these species from sound levels in the future.

Comparison of Gill Net Catches and Passive Acoustic Data

In general, target species (weakfish, spotted seatrout, and red drum) were caught infrequently in gill nets set at hydrophone listening stations in 1997 (See Appendix II). Of 119 gill nets set at the hydrophone stations between 13 May and 30 October 1997, weakfish were caught in 23 net sets (20% of net nets); spotted seatrout were caught in 6 net sets (5.2 % of net sets); and red drum were caught in only 2 net sets (1.7 % of net sets). Weakfish captured were all mature (23 females and 4 males), with an average Gonadal Somatic Index (GSI) of 0.98 % of body mass. However, weakfish caught in July and August had GSI average of 1.80 % of body mass; those captured in September and October had much lower GSI (0.87 %), indicating that spawning had largely ceased by then. Spotted seatrout captured were also mature fish, with 3 females and 1 male (we were unable to sex at least 2 more fish because they were in poor shape upon recovery, having been eaten by crabs), with a GSI of 2.53 % of body mass. We captured 11 immature red drum and 1 large mature male red drum (GSI = 1.98 % of body mass). The male red drum was captured 18 September 1997, the morning following our best recordings of red drum "knocking" and the capture of red drum eggs in the Bay River area.

In general, there was a poor correlation between adult fish captured in gill nets and hydrophone identification of the spawning fishes. Often, when we detected the target species with hydrophones, we failed to confirm the presence of these same species in the area using gill net collections. This lack of correlation between gill net collections and hydrophone surveys may be due to: 1) the avoidance of the gill nets by adult fish, or 2) by the adult fish being heard from a great distance away using the hydrophone (and thus being unavailable for net capture), or some combination of these two factors.

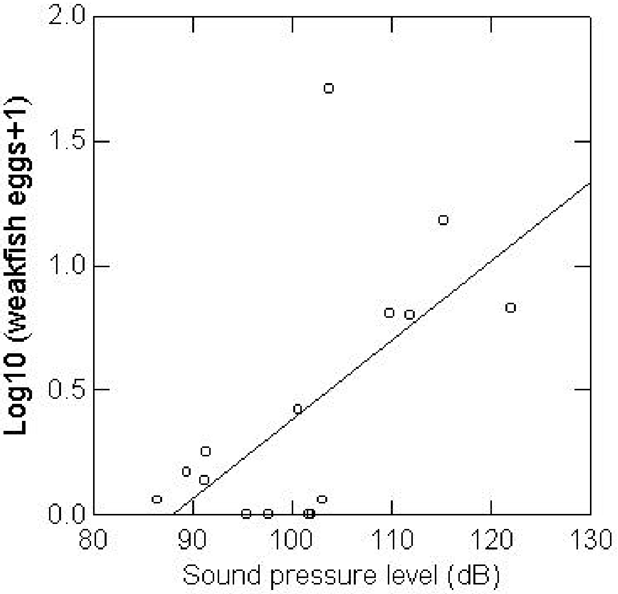

Figure 33. The $\log_{10}$ transformed abundance of weakfish eggs (sciaenid-type eggs > 0.85 μ) regressed on the sound pressure level specific to weakfish (at frequency of 304 - 375 Hz). The regression relationship ($y = 0.032x - 2.80$) has an R^2 of 0.375

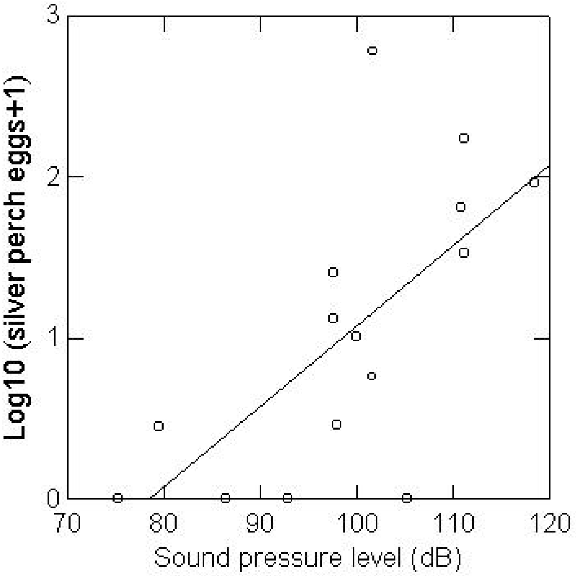

Figure 34. The $\log_{10}$ transformed abundance of silver perch eggs (sciaenid-type eggs < 0.80 μ) regressed on the sound pressure level specific to silver perch (at frequency of 984 - 1078 Hz). The regression relationship ($y = 0.050x - 3.933$) has an R^2 of 0.443.

Task 3: Spectrographic analyses of sounds from the field

Sounds produced by the fishes on the recordings obtained in Task 2 above were analyzed using power spectra and spectrographs derived from Fast Fourier Transforms (FFT's) (See Table 5 for parameters used during spectrographic analysis). The characteristic frequency spectra produced by different species were identified, allowing a discrimination of the species by their calls. Representative spectrographs and power spectra have been included in this report and on the compact disc (CD).

A weakfish making "purrs" was recorded 15 July 1997 at Hatteras Inlet, Hatteras Hole station (file jul97a16.wav; CD audio track 5). Nine distinct "purrs" can be seen in the spectrograph (Figure 35a), but there is some background noise from other "purring" weakfish in the vicinity. As in the captive weakfish recording, each "purr" consists of many short bursts of sound energy between 250 Hz and 515 Hz. The peak power spectral density in the average power spectrum (Figure 35b) occurs at 305 Hz.

A weakfish making "chattering" sounds was recorded on 25 August 1997 at Hatteras Inlet, Hatteras Hole station (file aug97d02.wav; CD audio track 6). The "chatter" sound was identified as a weakfish sound by comparisons with published spectrographs in Fish and Mowbray (1970). The "chatter" consists of a large number (about 50) of rapid, broad band clicks with dominant frequency near 1300 Hz (Figure 36a). The average power spectrum has distinct peaks at 1312 Hz and 1921 Hz (Figure 36b).

A small aggregation of weakfish making "purring" sounds was recorded 17 June 1997 at Hatteras Inlet, Hatteras Hole station (file jun97a15.wav; CD track 7). The sound level fluctuates as each individual makes its "purr." Most of the sound energy is in the characteristic broad peak near 300 Hz (Figure 37a). The dominant frequency in this recording is 281 Hz (Figure 37b).

A recording of a chorus of "purring" weakfish in a large aggregation was recorded on 6 August 1998 near Ocracoke Inlet at the Teaches Hole station (file: choraug98a11.wav; CD track 8). The aggregation produced a rumble in which individual "purrs" cannot be distinguished in the spectrograph (Figure 38a), but the power-spectral density in the spectrograph fluctuates in pattern that is similar to power-spectral density fluctuations in spectrographs of individual weakfish "purrs." The average power spectrum shows the same broad peak as an individual "purr." The dominant frequency of this aggregation is 305 Hz (Figure 38b).

A field recording of an individual silver perch producing a "cluck" was recorded 18 April 1998 at the National Marine Fisheries Service, Beaufort Laboratory, Beaufort, NC dock (file: Apr9820.wav; CD audio track 9). Each "cluck" is a single burst of sound with dominant frequency near 1000 Hz in the spectrograph (Figure 39a) and sound energy extending from 650 Hz to 3200 Hz as can be seen in the power spectrum (Figure 39b). Other species are also present in this recording including snapping shrimp (*Alpheus* sp.) producing broad band "clicks", which extend to a much higher frequency than the silver perch "clucks" and oyster toadfish producing their characteristic "boop" sound with harmonics near 175 Hz and 350 Hz.

A field recording of an aggregation of silver perch producing their characteristic "clucks" in a chorus was made 8 June 1998 near Ocracoke Inlet at the Teaches Hole station (file: jun98a07.wav; CD audio track 10). The power spectral density in the spectrograph fluctuates as the individual silver perch synchronize their clucks (Figure 40a). This group has a dominant frequency of 938 Hz. The average power spectrum has a peak between 950 Hz and 1100 Hz (Figure 40b).

Table 5. A list of the parameters used during the spectrographic analysis of the sciaenid sound recordings described in this section. The sound recordings were digitized, and a slide factor (number of digital sample points analyzed between the start of consecutive FFT's) used to create the spectrographs. The average power spectrum was done on a portion of the each sound recording as indicated by the start and stop times and number of averages.

Figure	**A. Spectrograph**	**B. Average Power Spectrum**		
	Slide Factor	**Start Time (s)**	**Stop Time (s)**	**Number of Averages**
Figure 35	512	1.500	14.500	304
Figure 36	256	1.152	2.987	43
Figure 37.	1024	0.000	16.500	386
Figure 38	1024	0.000	19.000	445
Figure 39	256	11.576	13.763	51
Figure 40	512	1.000	15.000	328
Figure 41	512	0.000	11.000	257
Figure 42	256	N/A	N/A	N/A
Figure 43	256	N/A	N/A	N/A
Figure 44	128	3.565	3.832	6
Figure 45	512	0.000	13.500	316
Figure 46	128	N/A	N/A	N/A
Figure 47	128	0.133	0.869	17

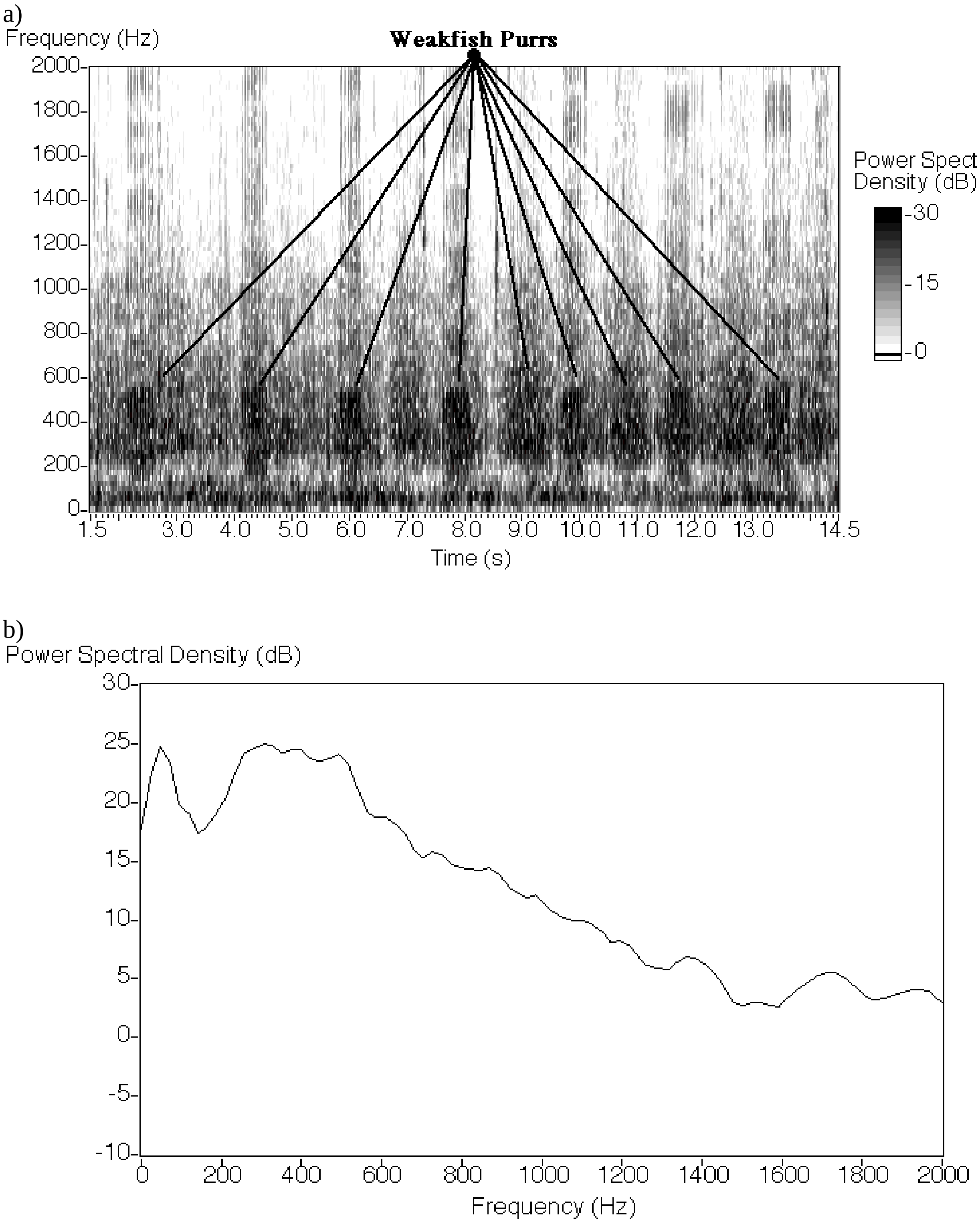

Figure 35. a) Spectrograph of weakfish "purring"; b) power spectrum of weakfish "purring"

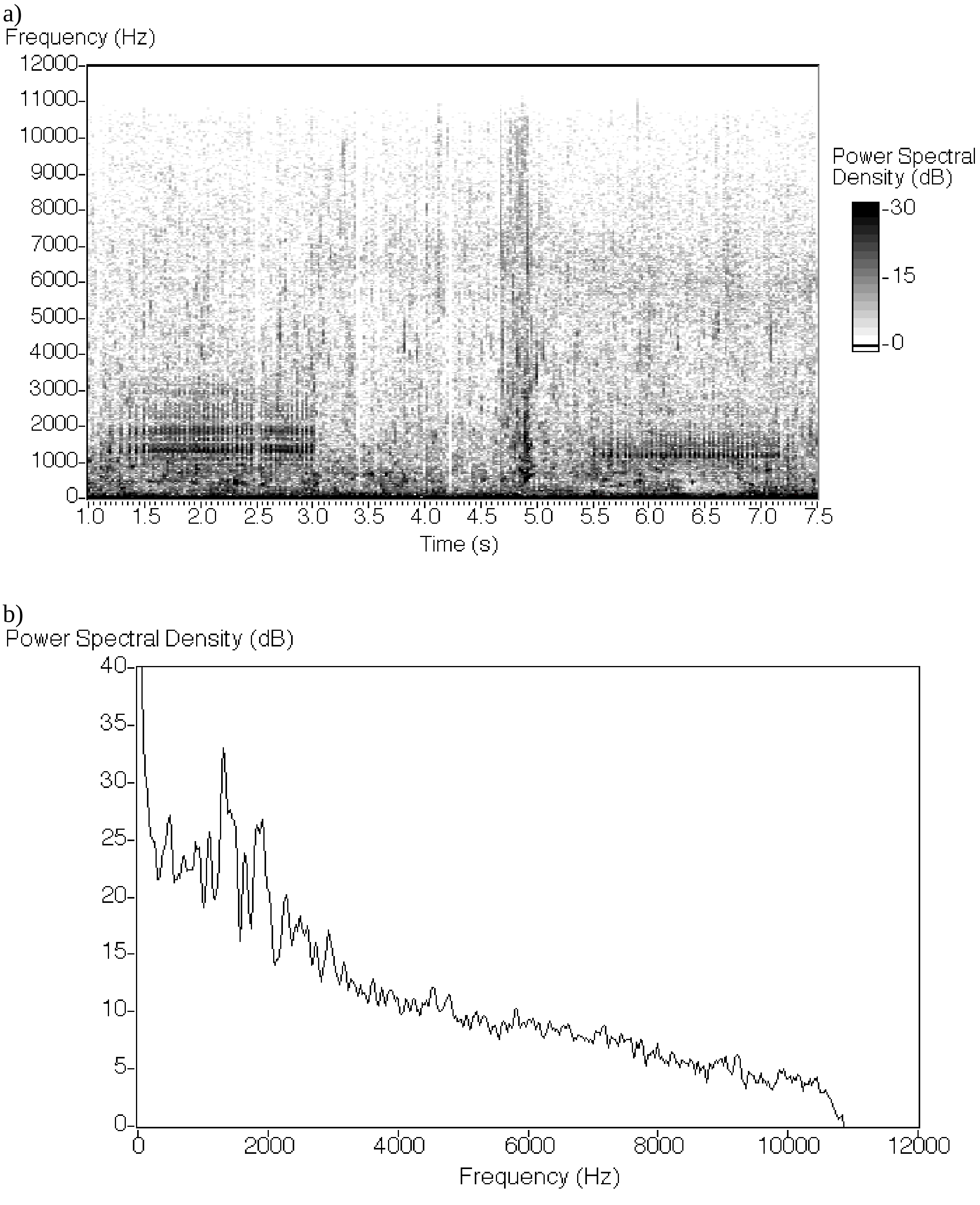

Figure 36 a) Spectrograph of weakfish "chattering"; b) power spectrum of weakfish "chattering".

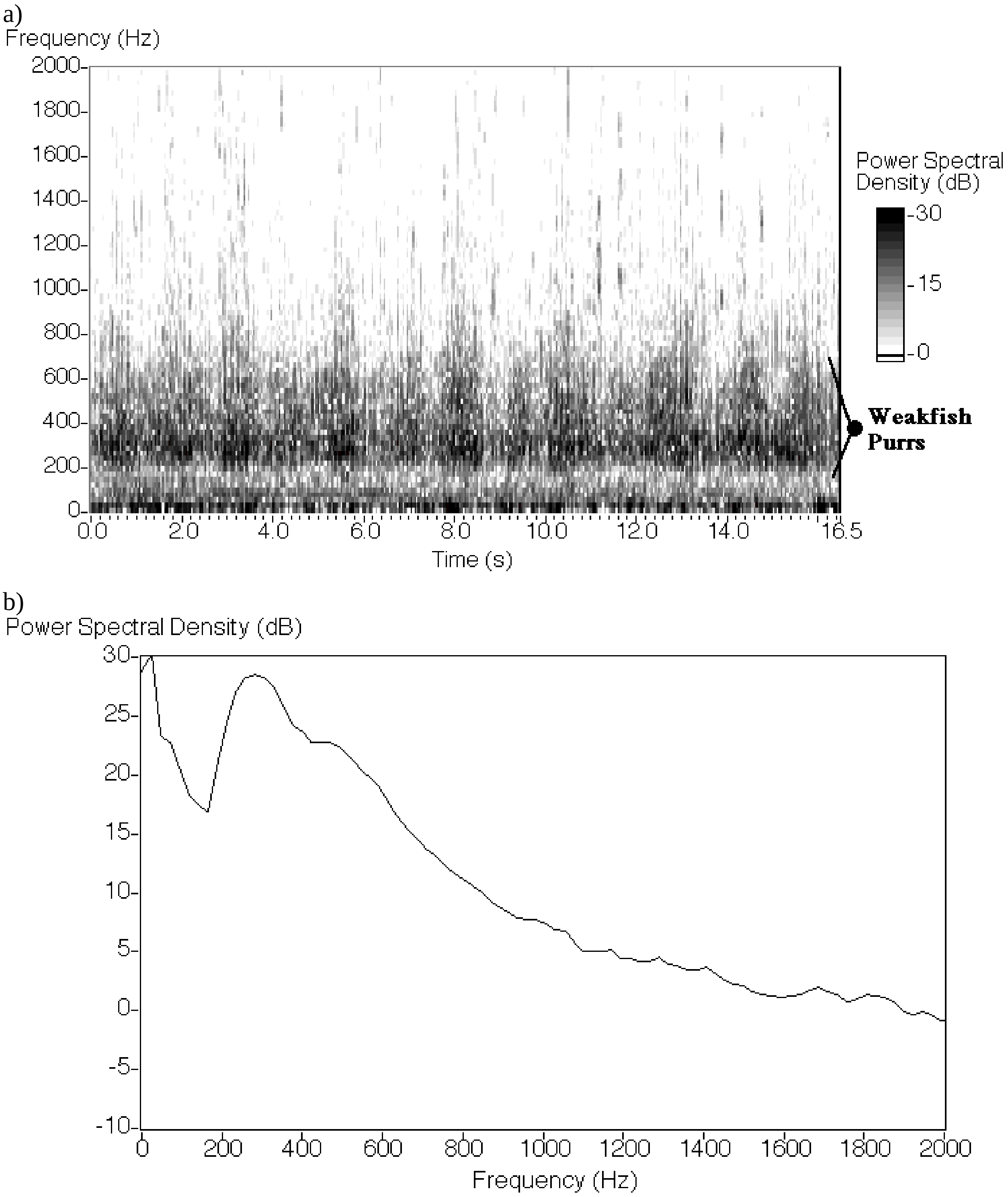

Figure 37. a) A spectrograph of a small group of weakfish making "purrs" - b) a power spectrum of the same recording

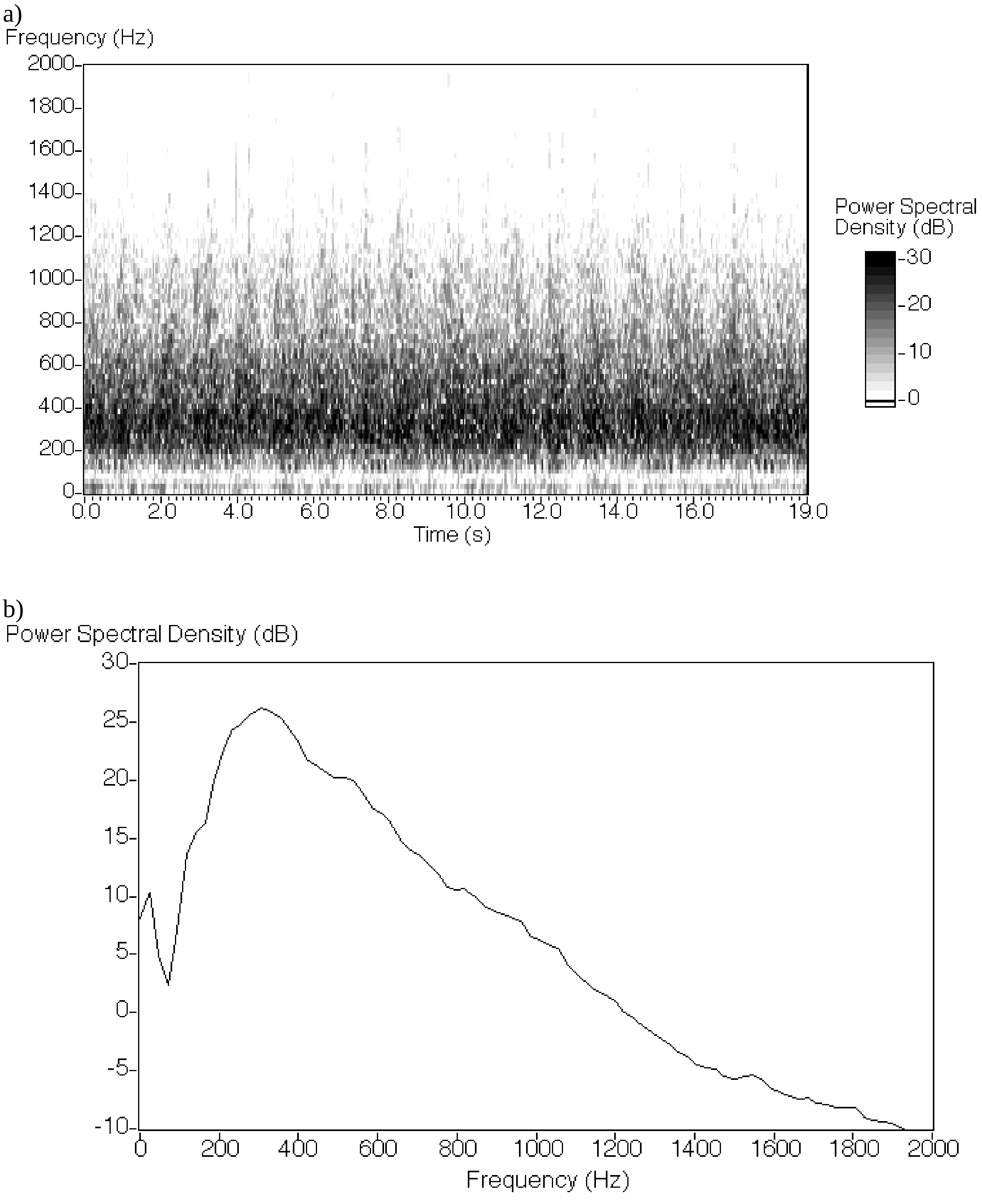

Figure 38. a) Spectrograph of weakfish large aggregation "purring"; b) power spectrum of the same recording.

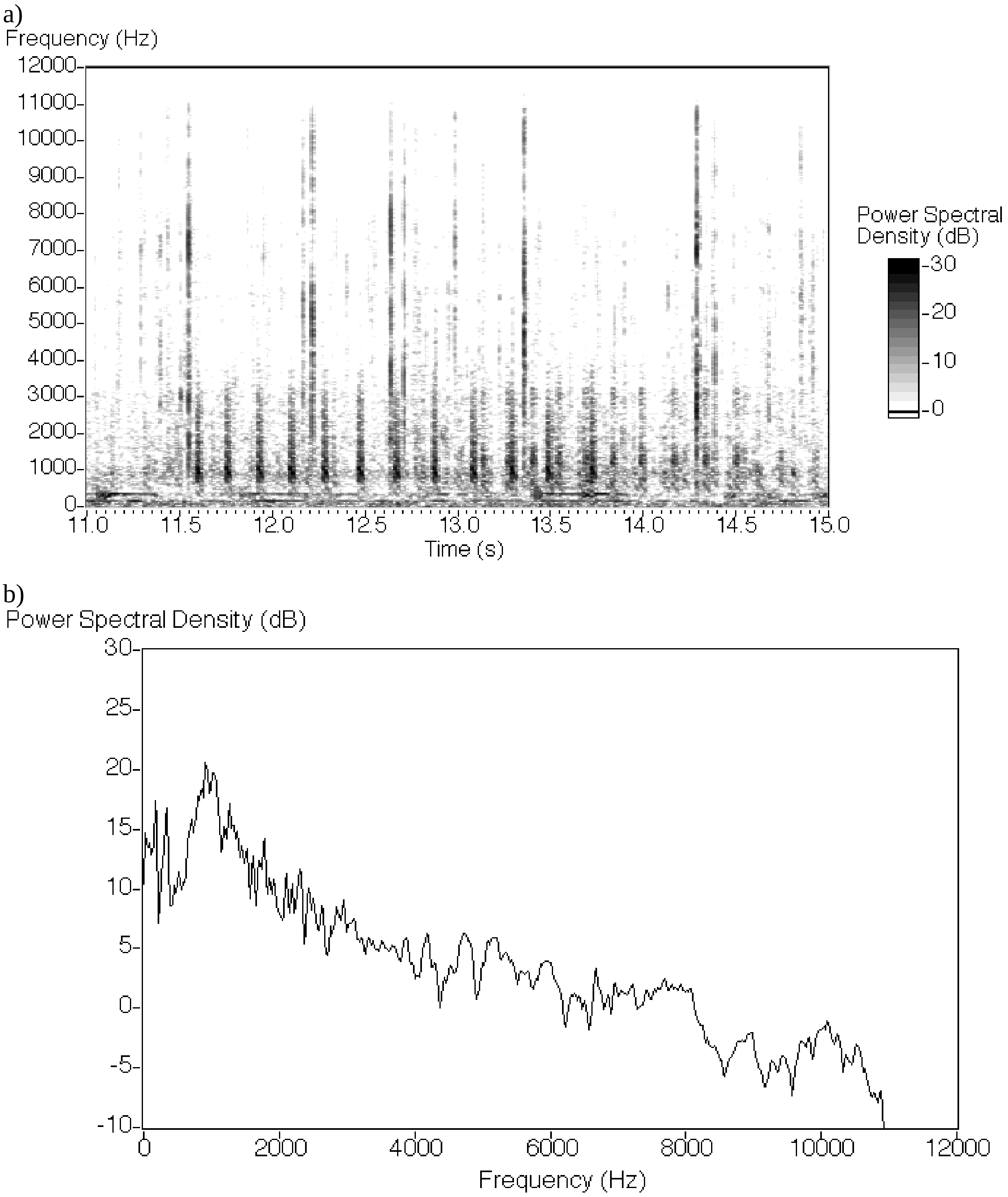

Figure 39. a) Spectrograph of silver perch "clucking" -b) power spectrum of the same recording.

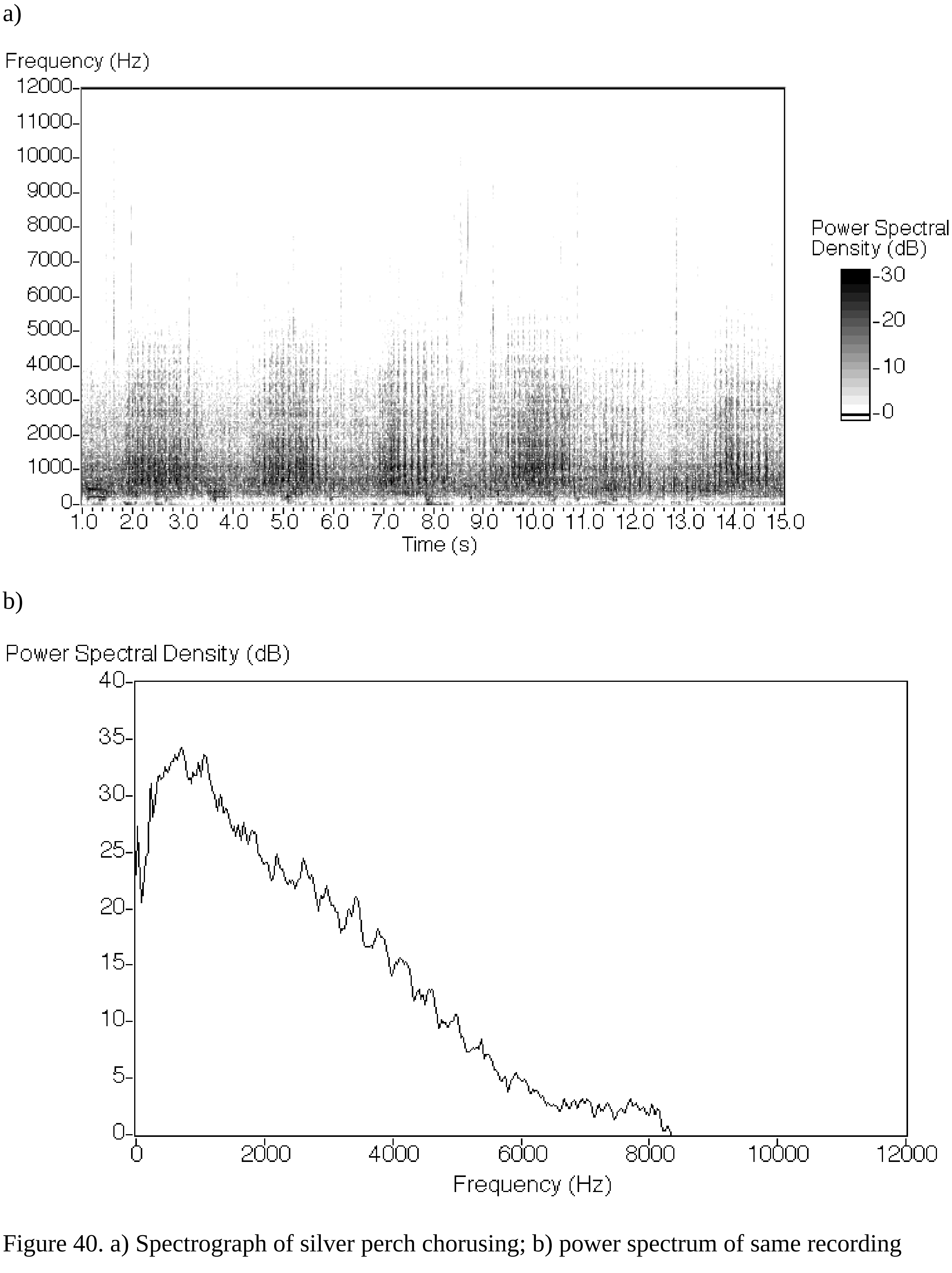

Figure 40. a) Spectrograph of silver perch chorusing; b) power spectrum of same recording

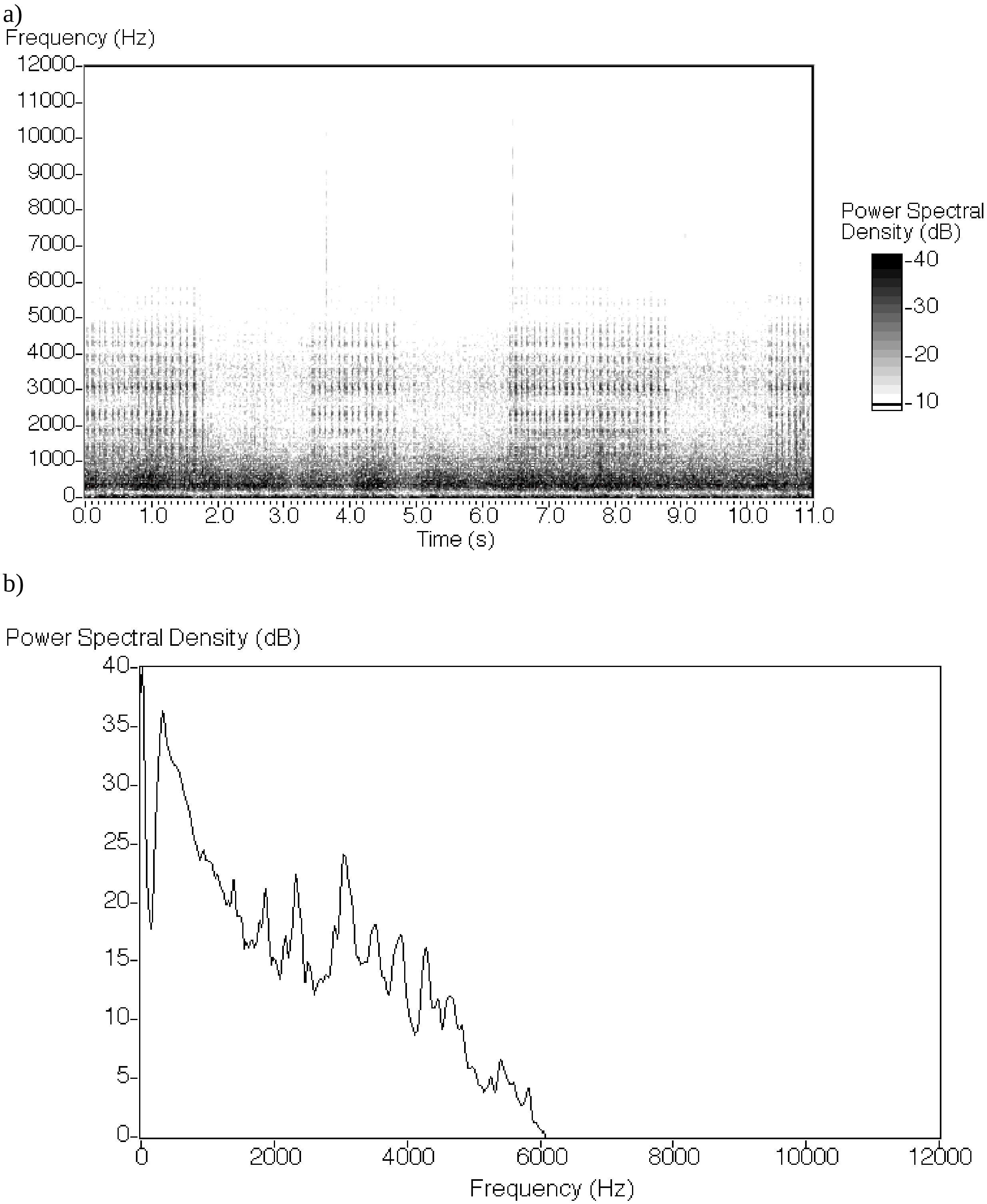

Figure 41. a) Spectrograph of weakfish and silver perch together; b) power spectrum of same recording

An individual spotted seatrout, making two of the three spotted seatrout sounds,(the "burp" and the "heartbeat"), was recorded at the Lehigh Dredge on 12 August 1997 (file: aug97a02x.wav; CD track 12). In the spectrograph, the "burp" appears as two harmonic peaks that slide down in frequency by several Hertz as it continues (Figure 42). The lower peak is usually in the 230 Hz - 260 Hz range and the upper peak is usually in the 350 Hz - 380 Hz range. The "heartbeat" is a sequence of between two and four rapid pulses in which the first four harmonics of a fundamental frequency near 120 Hz can be seen. The dominant frequency in the "heartbeat" is near 400 Hz.

Another individual spotted seatrout, making two of the three spotted seatrout sounds (the "staccato" and the "burp"), was recorded at Wallace Channel on 14 July 1997 (file: jul97a02x.wav; CD track 13). The "staccato" consists of a large number of clicks in rapid succession (Figure 43). In this example, there are 35 clicks in a 1.72-s interval. The clicks have a dominant fundamental frequency of 258 Hz, and the first three harmonics are present. The "burp" is similar to the example described in the previous sound.

In order to view a "burp" in greater detail in the spectrographs, a 1-second segment of the sound recording above (original file: aug97a02x.wav; CD track 12) was created. When a spectrograph was created from this 1-second sound clip (Figure 44a), a magnification of the "burp" in the spectrograph in Figure 42 can be observed. The average power spectrum (Figure 44b) of this sound clip shows two distinct peaks at 234 Hz and 352 Hz. The fundamental frequency is near 119 Hz, and a very small peak can be seen near that frequency.

An aggregation of spotted seatrout was recorded at Marker 29 in Teaches Hole Channel on 11 June 1998 (file: jun98a23.wav; CD track 14). Individual "heartbeats," "burps," and "staccatos" are difficult to resolve in the spectrograph (Figure 45a). The average power spectrum shows a broad peak from 234 Hz to 421 Hz with a peak at 305 Hz (Figure 45b). A "burp" occurs in this spectrograph, but is difficult to resolve. To better view the seatrout drumming and the "burp" in this aggregation, a 1-second clip was analyzed as described above to magnify the "burp". The spectrograph of this 1-second clip clearly shows a "burp" between 6.0 s and 6.2 s (Figure 46).

Red drum making their characteristic "knock" sound were recorded at Bay River Mouth station on 17 September 1997. In this spectrograph, four successive "knocks" occur in a 0.74-s interval (Figure 47a). The average power spectrum (Figure 47b) shows four distinct frequency peaks at 141 Hz, 304 Hz, 445 Hz, and 539 Hz indicating a fundamental frequency of 153 ± 12 Hz. The dominant frequency of this short interval is 141 Hz.

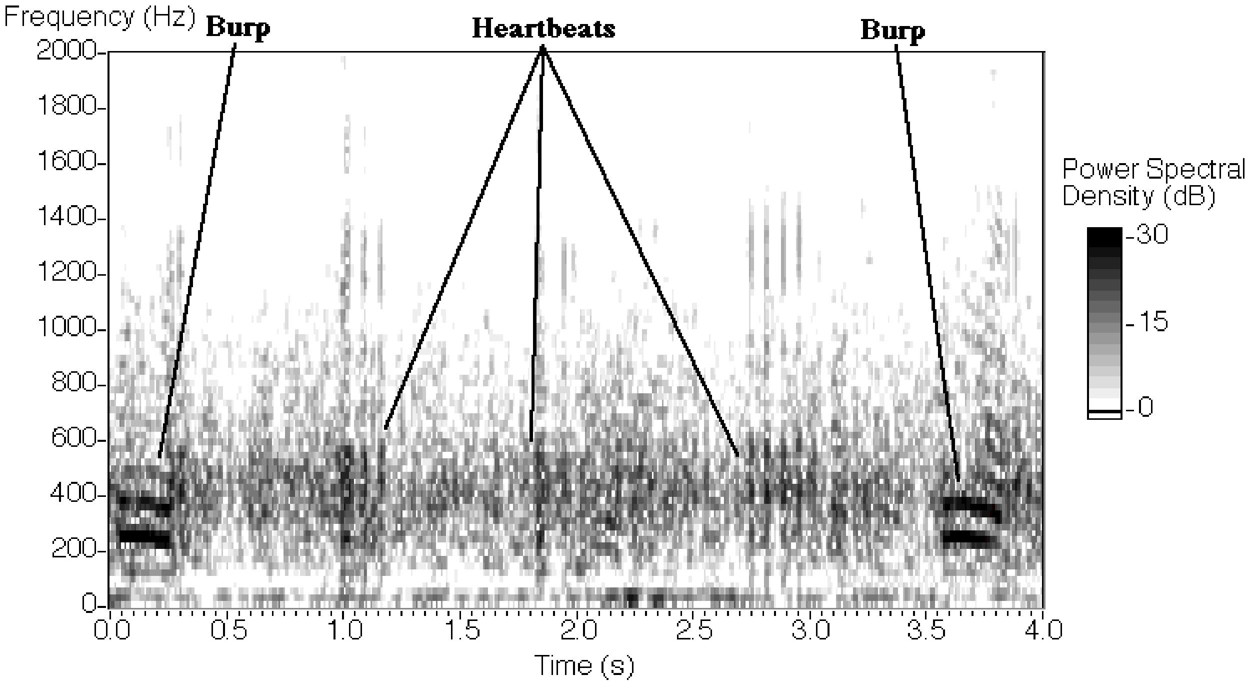

Figure 42. Spotted seatrout spectrograph showing "burp" and "heartbeat" calls

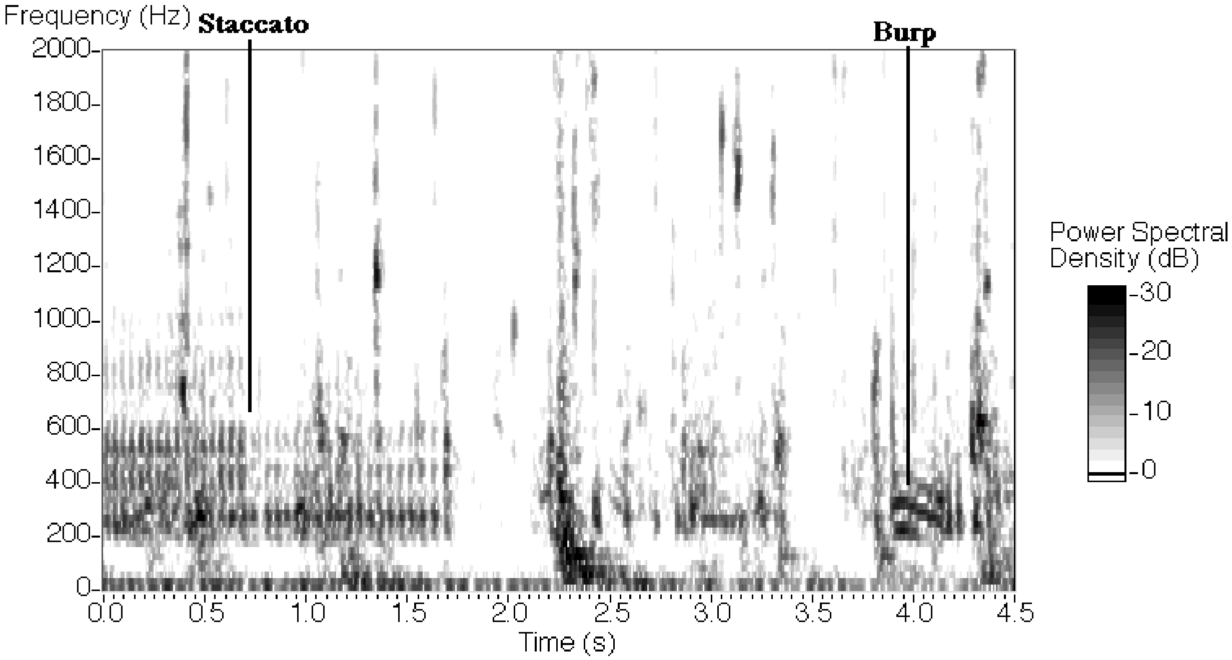

Figure 43. Spotted seatrout spectrograph showing "staccato" and "burp" calls

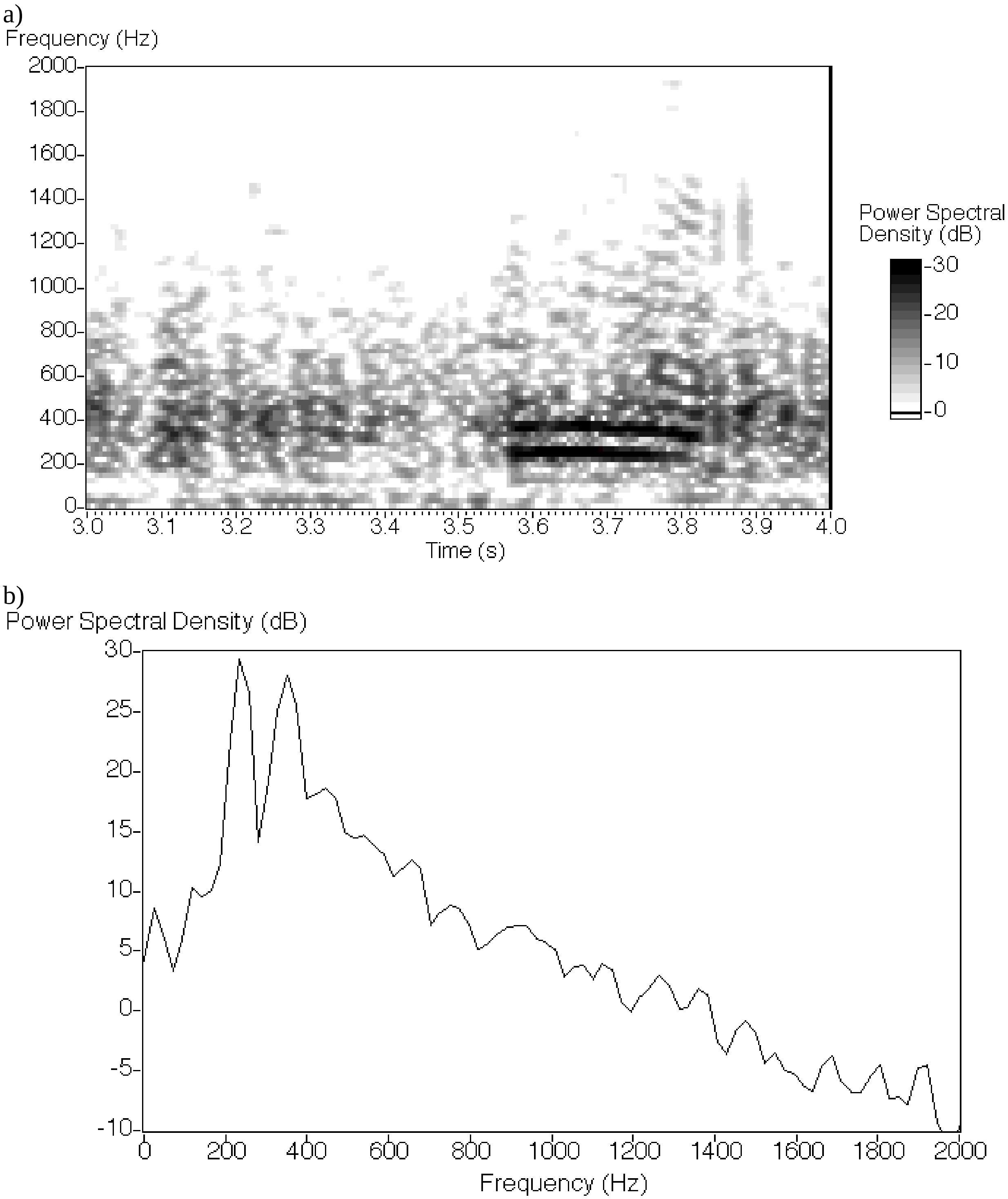

Figure 44. a) Spectrograph of a 1-second section of the spotted seatrout call in Figure 42 showing a "burp"; b) power spectrum of the 1-second section.

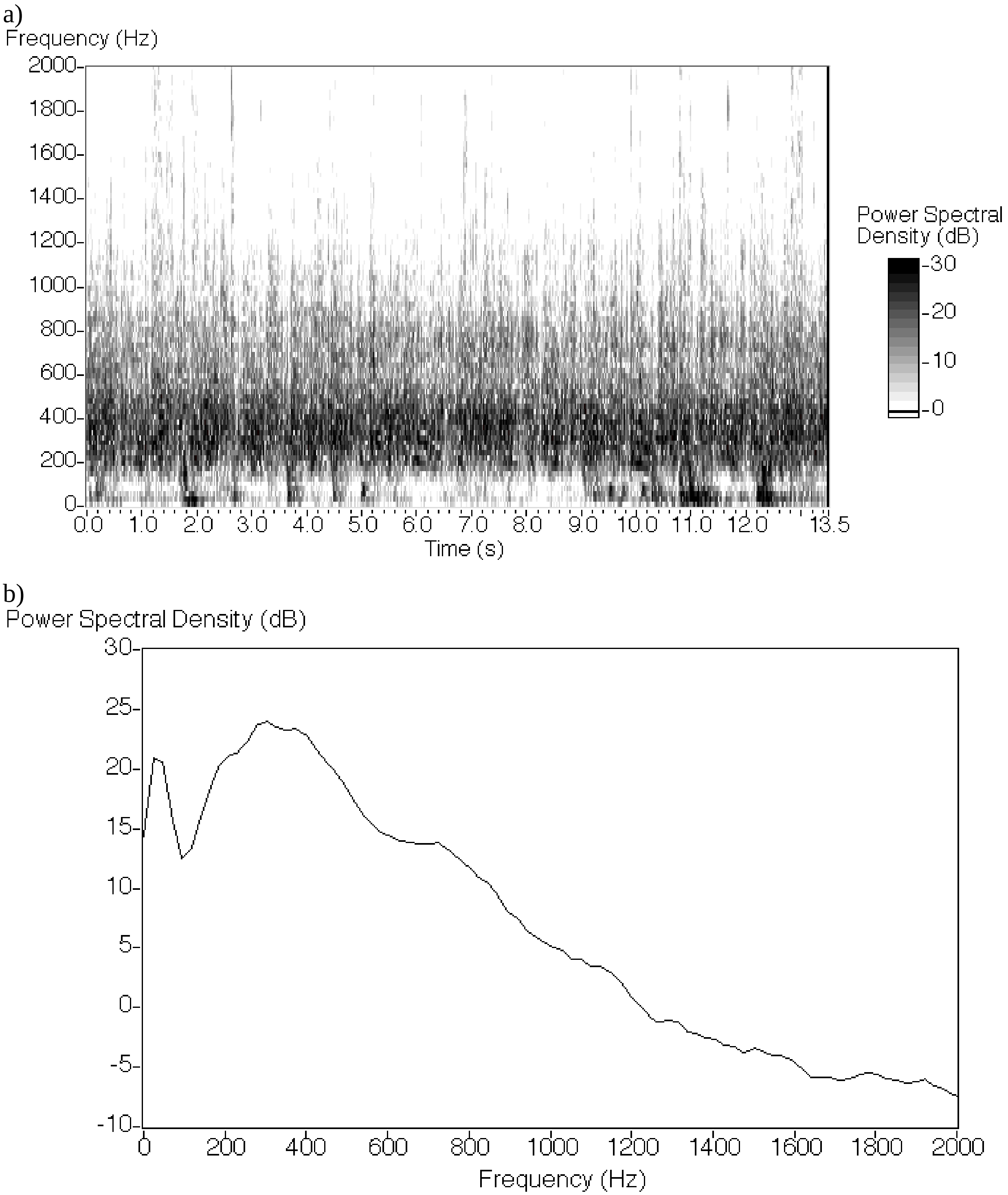

Figure 45. a) Spectrograph of a spotted sea trout aggregation; b) power spectrum of the same recording.

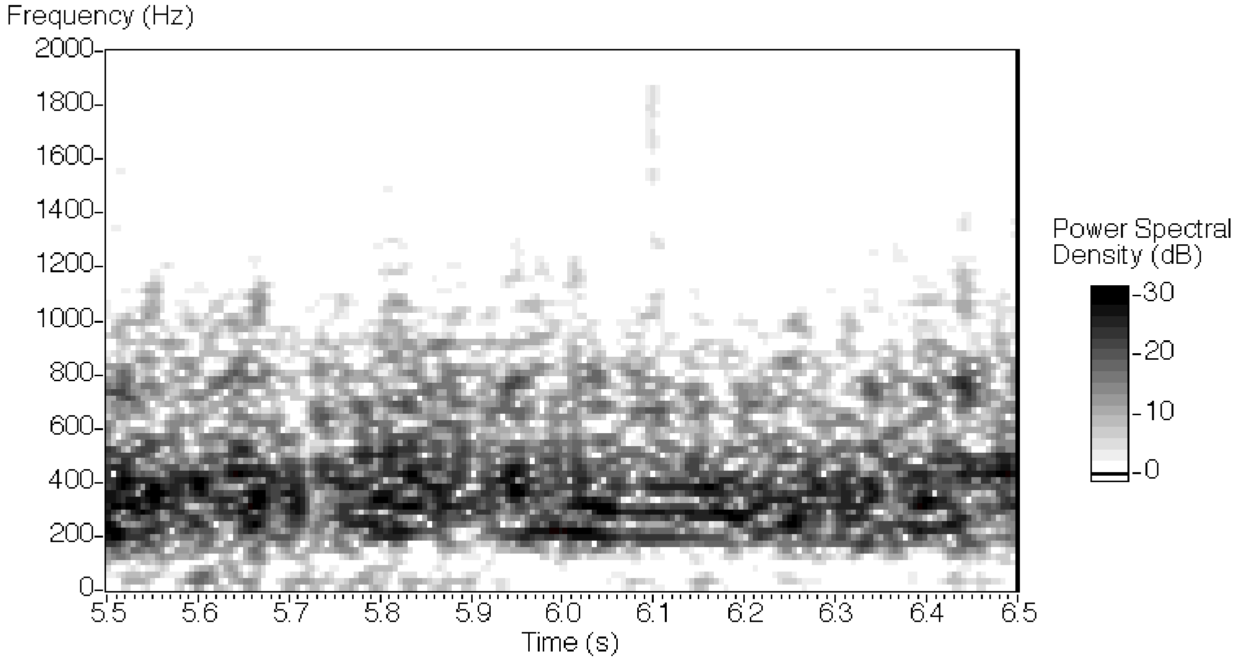

Figure 46. Spectrograph of a 1-second section of the spotted seatrout aggregation shown in Figure 45.

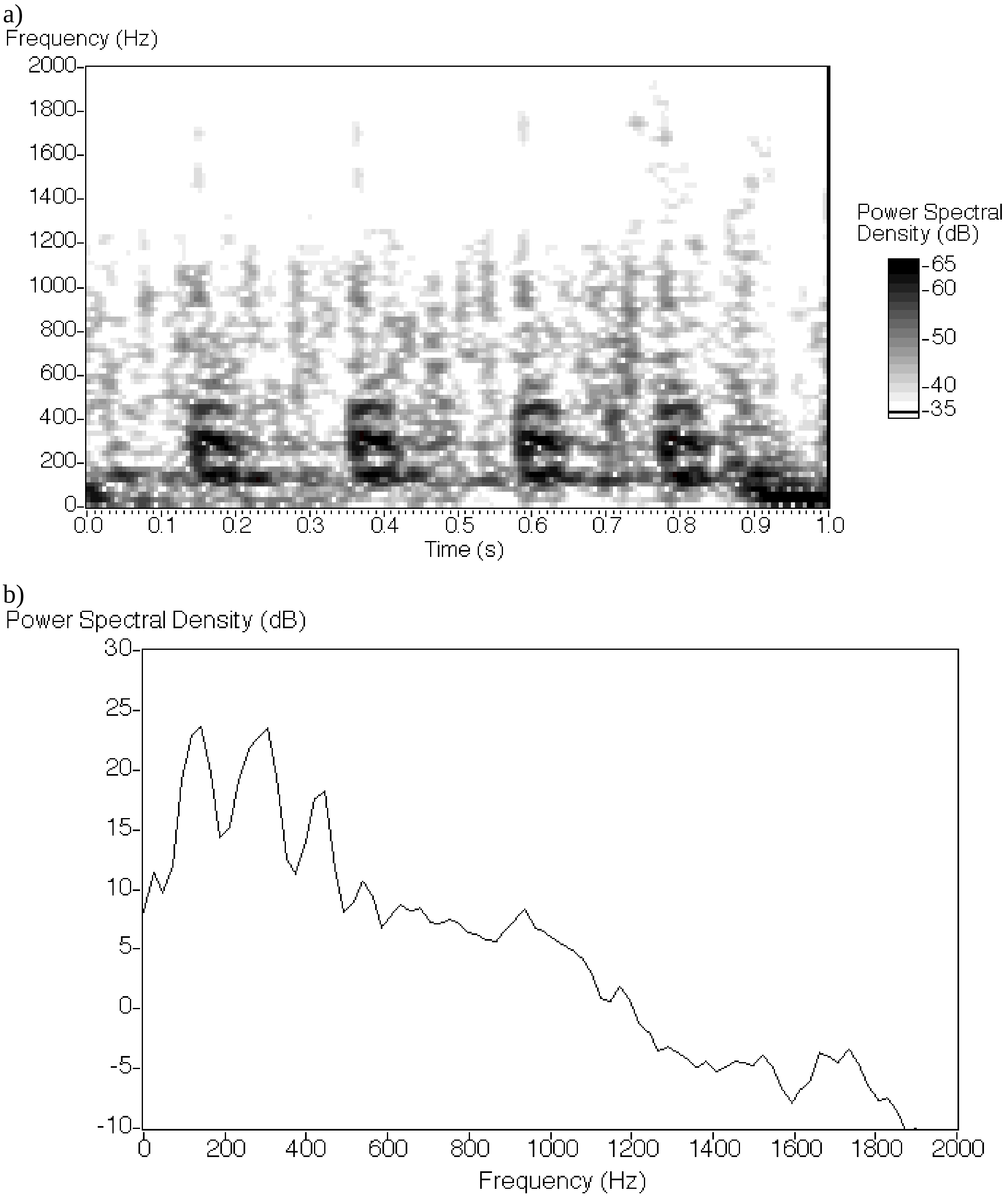

Figure 47. a) Spectrograph of red drum "knock"; b) power spectrum of red drum "knock"

Task 4: GIS maps of spawning areas based on sound and egg production

Geographical information system (GIS) maps of spawning habitats for these species in Pamlico Sound have been produced and are included on the compact disc as Joint Photography Group image files (*.jpg). Base maps throughout this section are from United States Geological Survey 1:100,000 scale hydrography Digital Line Graphs (available on the Internet at: http://edc.www.cr.usgs.gov/doc/edchome/ndcdb/ndcdb.html). Bathymetric coverages were provided by the National Oceanic and Atmospheric Administration, National Ocean Survey (NOAA/NOS) data files, which are available at for download at the NOS Estuarine Bathymetry world wide web page (http://seaserver.nos.noaa.gov/bathy/index.html). The Environmental Systems Research Institute (ESRI) data files were used for all other maps. Maps of the locations where we detected drumming sciaenids are included in the printed of this report as well.

Sonobuoy Surveys and the Drumming Index

The sonobuoys were deployed monthly from May through October 1998 in two 100 km^2 regions at Ocracoke and the Bay River. Sonobuoys were programmed two make recordings at two time intervals: one sonobuoy (24-hour sonobuoy) was set to record hourly (24 recordings at 2 min each for a 48 minute total); eight others (normal sonobuoys) were set to record every 0.5 hr from ~1800 until the tape ran out the next morning (~0600; 24 recordings at 2 min each for a 48 min total). Because the recordings were often less than 2 min, the tape often did not run out until 0800 or later, depending on the sonobuoy. The 24-hour sonobuoy made recordings even during the daytime, but we expected to hear few fish then, based on previous work (Mok and Gilmore 1983). Thus, we only devoted one sonobuoy to this time period, and programmed the others to obtain most recordings during the night when fish were actively drumming.

Based on the 24-hour sonobuoy recordings, sciaenid fishes did not drum during daylight with only one exception (Figure 48). Weakfish began drumming as early as 1800 EDT and increased their drumming activity after sunset (which occurred at ~2000 EDT); the greatest drumming index values were at 2200 EDT. Weakfish could be heard "purring" until 0200 EDT the next morning. Spotted seatrout had a much more restricted period during which they drummed: they did not begin until after sunset (2000 EDT) and had ceased calling by 2300 EDT. Spotted seatrout had peak in drumming activity at 2200 EDT. No red drum were ever detected using the 24-hour sonobuoys, probably because there was just one 24-hour sonobuoy set out per day in a single location and red drum were sparsely distributed. Thus, we can only use the regular sonobuoys that recorded at before sunset and stopped in the morning to examine their diurnal drumming activity patterns. Red drum began drumming as early as 1800 and reached a peak of drumming activity at 2200. Red drum drumming had ceased by midnight in most cases. However, in one case a red drum "knock" was heard at 0806 (25 Sep 1998).

Drumming index values varied from 0 through 3 during any given 2-min recording, and thus it was possible to reach a drumming index sum value of 72 for the night, if every recording on a tape were scored as a 3 (continuous drumming by an aggregation). In the following maps (Figure 49 through Figure 74) the geographic position of sonobuoys that recorded drumming activity along with the drumming index sum for each sonobuoy location are plotted for the three target species in the two sampling regions from May through October 1998. Weakfish had the overall highest drumming index sums (Figure 49 through Figure 58), with 69 being the highest recorded index sum for a night's recordings (weakfish in May 1998 sonobuoy recordings at Ocracoke; Figure 50). Spotted seatrout was the species with the next highest drumming index sums (Figure 59 through Figure 68). Red drum had the lowest drumming index values of all sciaenids heard, which suggests their relative scarcity in the study area (Figure 69 through Figure 74). In annual drumming index maps for each area, which summarize the drumming index sum values for each region for the entire spawning season, the drumming index sum has been plotted using symbols of different sizes that correspond to geometric classes of the index (0-2, 2-4, 4-8, …). Weakfish "purring" heard in all months of sampling is displayed as a drumming index sum for the Ocracoke (Figure 49), and Bay River (Figure 55) study areas; spotted seatrout "heartbeat, burp, and staccato" heard in all months of sampling is displayed as a drumming index sum for the Ocracoke (Figure 59), and Bay River (Figure 65) study areas; and red drum "knocks" heard in all months of sampling as a drumming index sum in the Ocracoke (Figure 69), and Bay River (Figure 72) study areas. These summary maps represent the overall most significant spawning areas for each species.

In summary, it is clear that high-salinity inlet stations are the most significant areas for weakfish spawning, especially in May of each year because the greatest drumming index values occurred at that time. Weakfish began "purring" in May and this spawning behavior lasted until September 1998. Western Pamlico Sound (low salinity) areas were not used much by drumming male weakfish, only being detected "purring" in the Bay River a few times in 1998, even with our greater sonobuoy sampling effort. Spotted seatrout, in contrast, used habitats in shallow water on both sides of the Pamlico Sound, both in high salinity and low salinity areas. The spotted seatrout drumming index was greatest in July of 1998 on both sides of Pamlico Sound, although spawning may begin as early as May and end as late as September for this species. Red drum were only detected in August, September, and October of 1998 with the sonobuoys. The highest values of the drumming index sum occurred in September in both Ocracoke and the Bay River areas, with the highest values at the mouth of the Bay River. These high values of the drumming index sum coincide with the locations in which red drum eggs identified using mtDNA data were collected in association with drumming in 1997. The low-salinity areas on the western side of Pamlico Sound near the mouth of the Bay River appear to be the most critical areas for spawning of red drum.

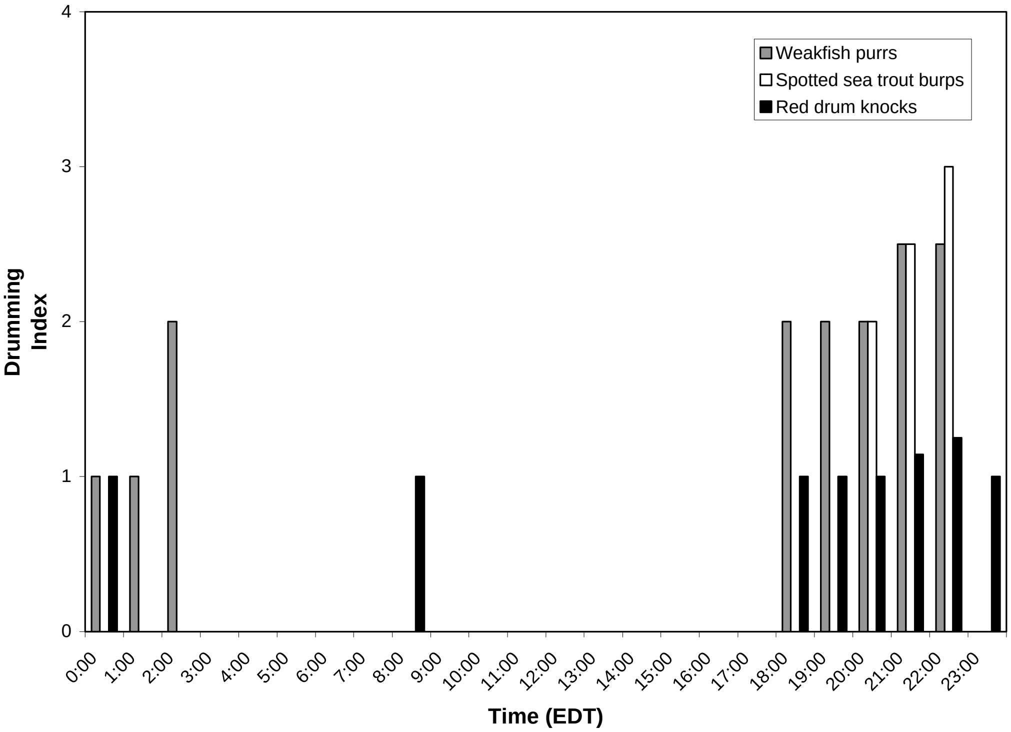

Figure 48. Average drumming index for weakfish, spotted seatrout and red drum as it varied during the course of a day as recorded by the sonobuoys. The weakfish and spotted seatrout data are averaged from drumming index values during 2-min recordings from 24-hour sonobuoys set during June and July of 1998 at Ocracoke and Bay River areas. As no red drum were recorded on 24 hour sonobuoys, the red drum data are averaged from hourly recordings on normal sonobuoys (recordings were made at 0.5 intervals from 18:00 until 0800), deployed during September 1998 at Ocracoke and Bay River areas.

Weakfish Spawning Areas

The following pages display maps of the weakfish spawning areas as determined by plots of the drumming index at each sonobuoy location in the Bay River and Ocracoke study areas May through October 1998.

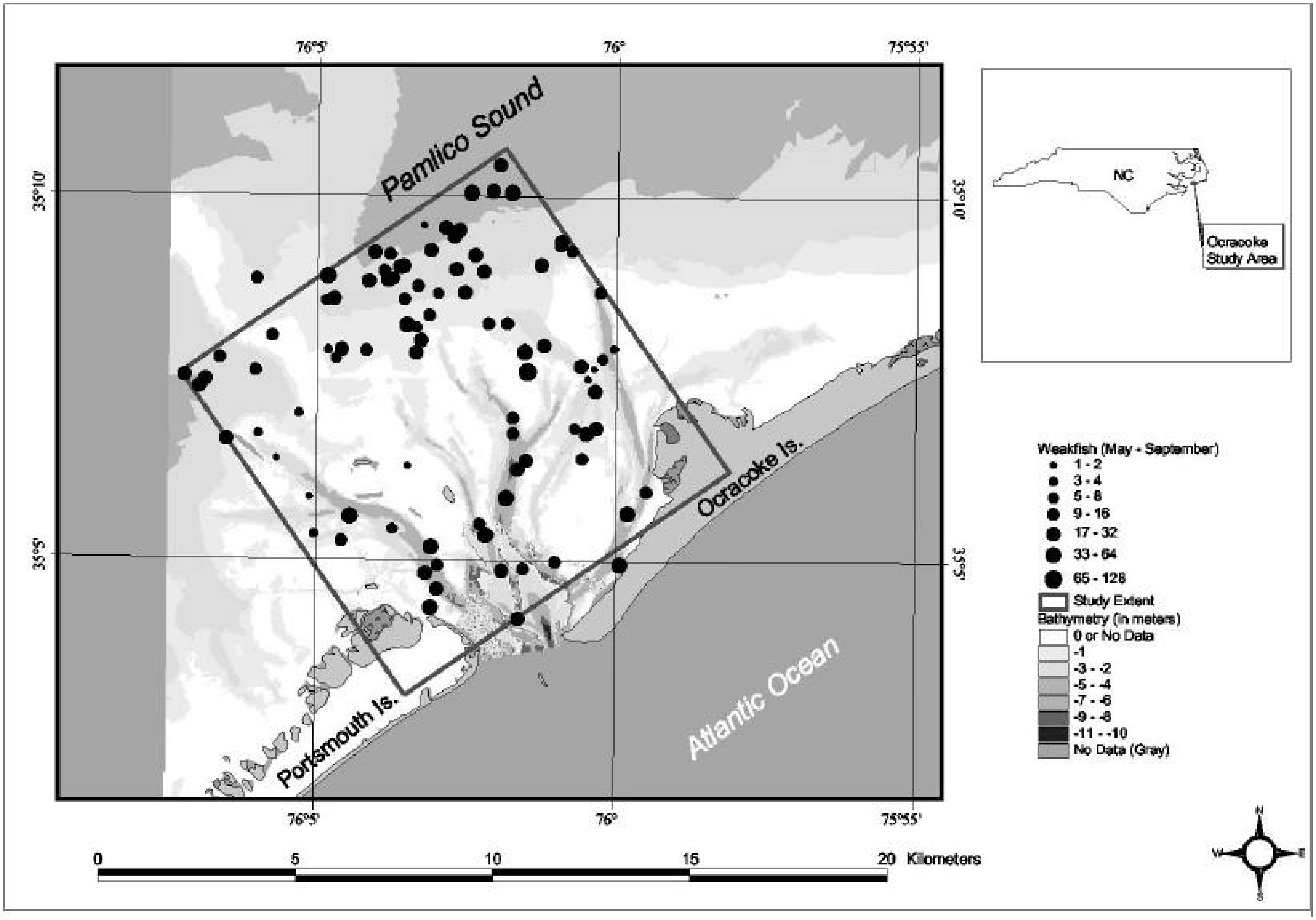

Figure 49. Weakfish drumming index sum from all sonobuoys deployed at Ocracoke, May - October 1998.

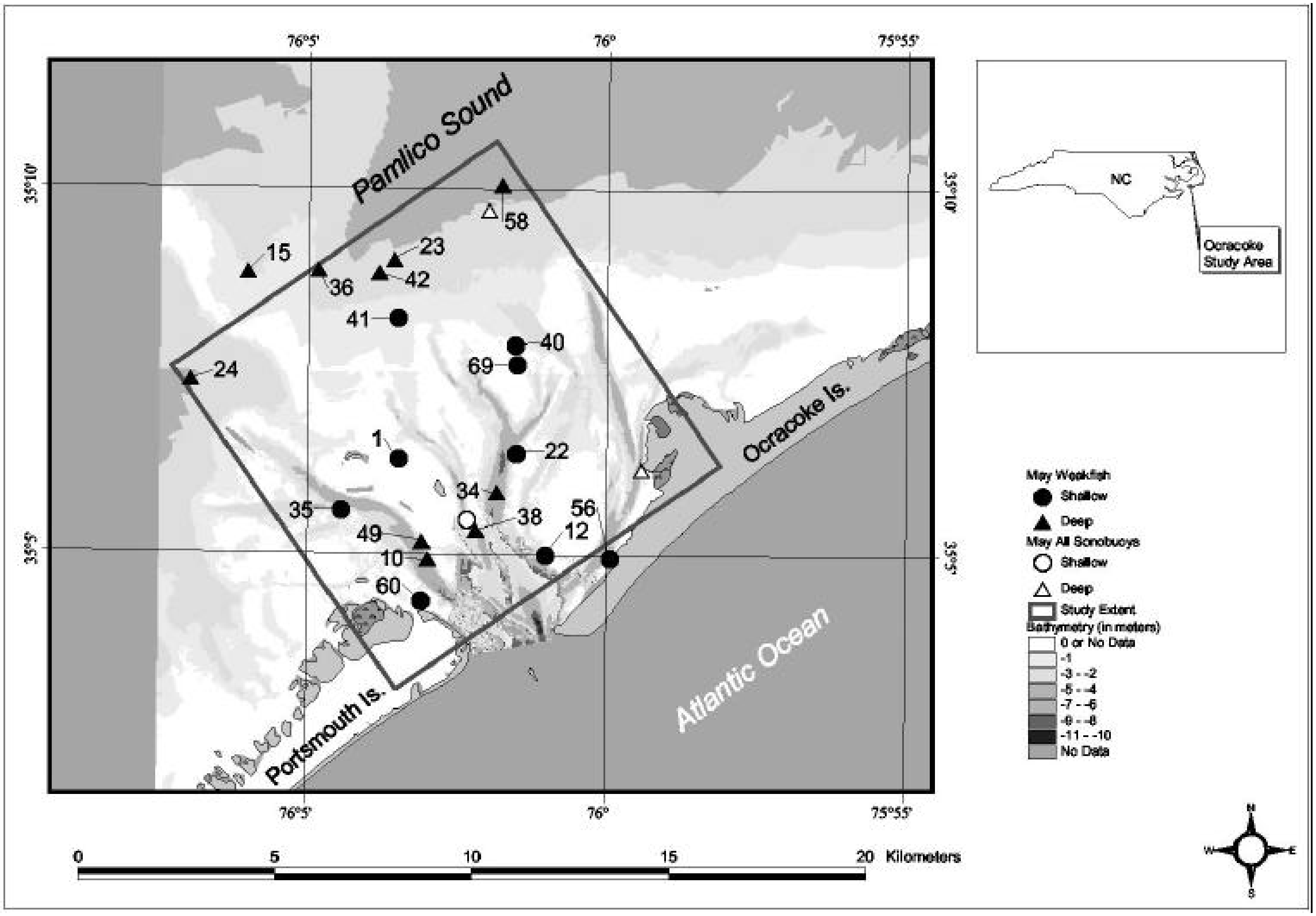

Figure 50. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in May 1998. Filled symbols represent stations at which weakfish "purring" was detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

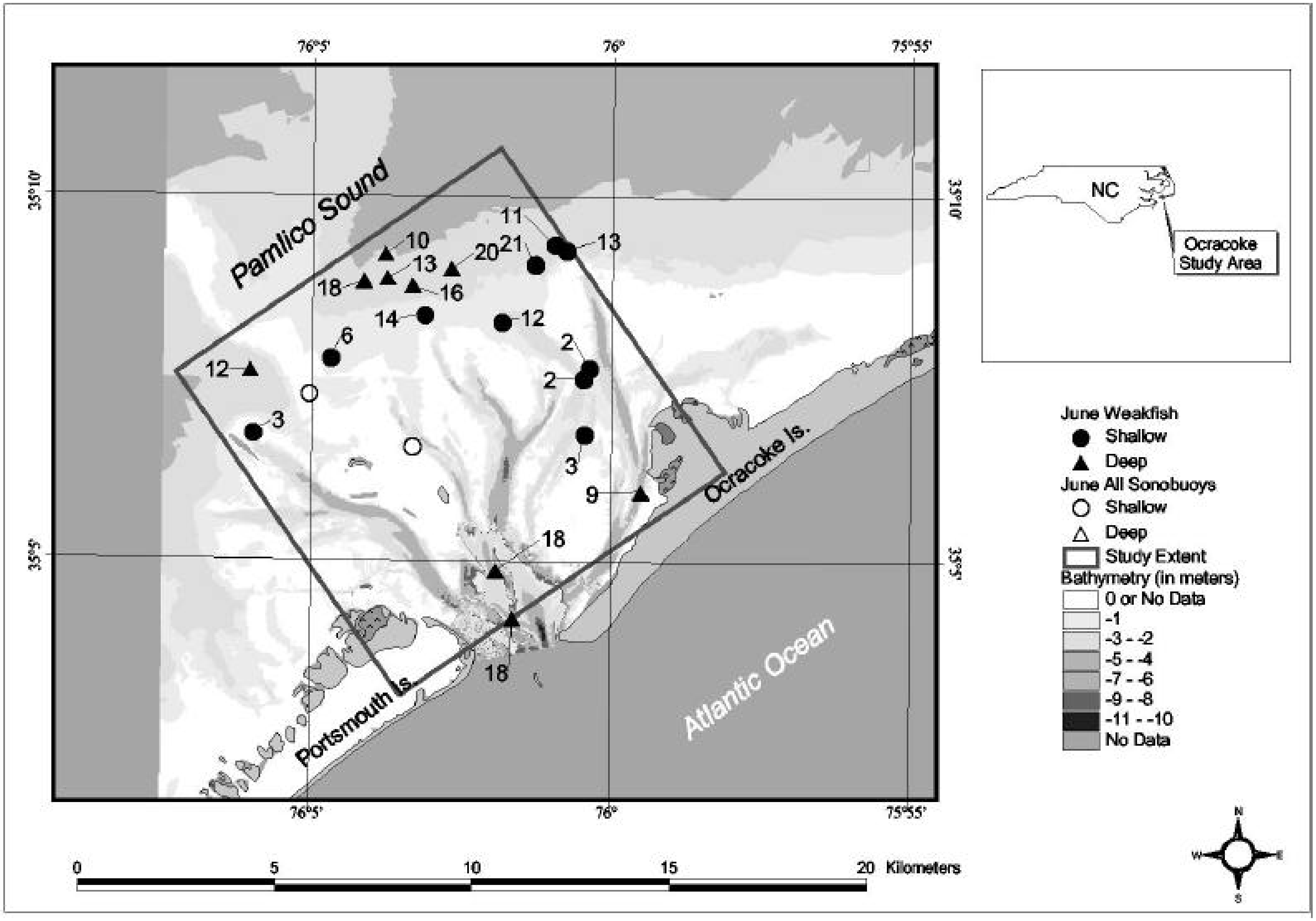

Figure 51. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in June 1998. Filled symbols represent stations at which weakfish "purring" was detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

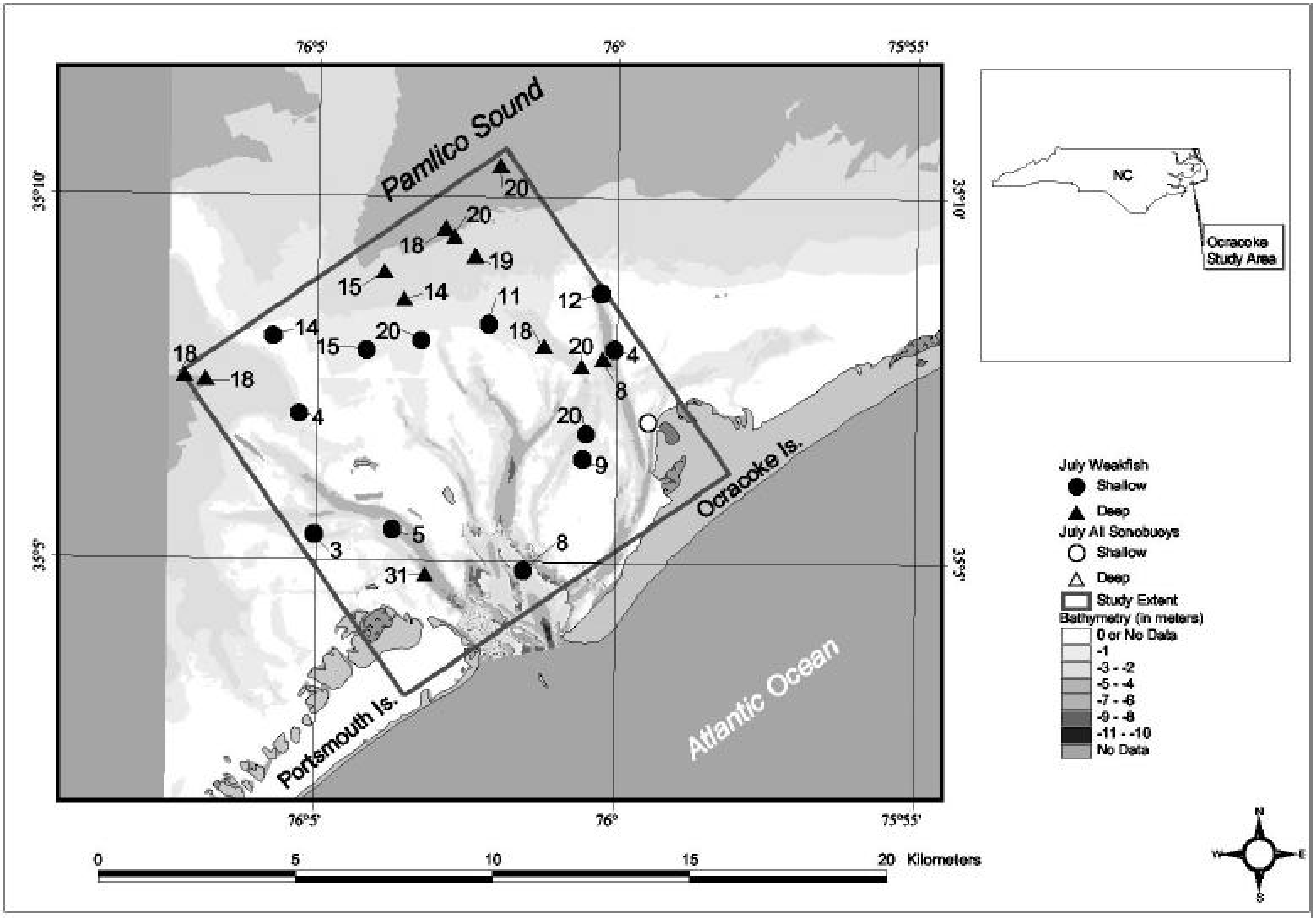

Figure 52. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in July 1998. Filled symbols represent stations at which weakfish "purring" was detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

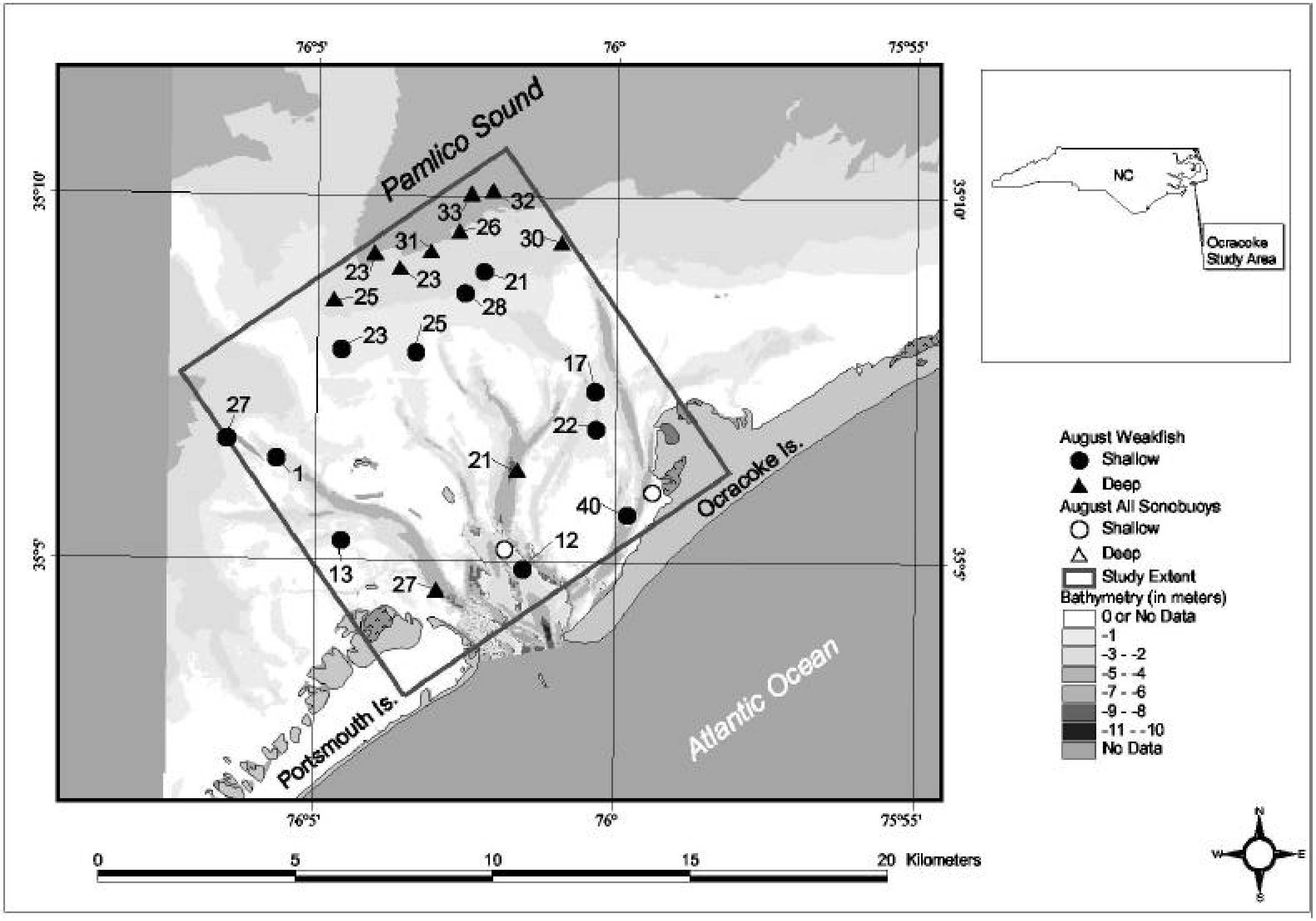

Figure 53. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in August 1998. Filled symbols represent stations at which weakfish "purring" was detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

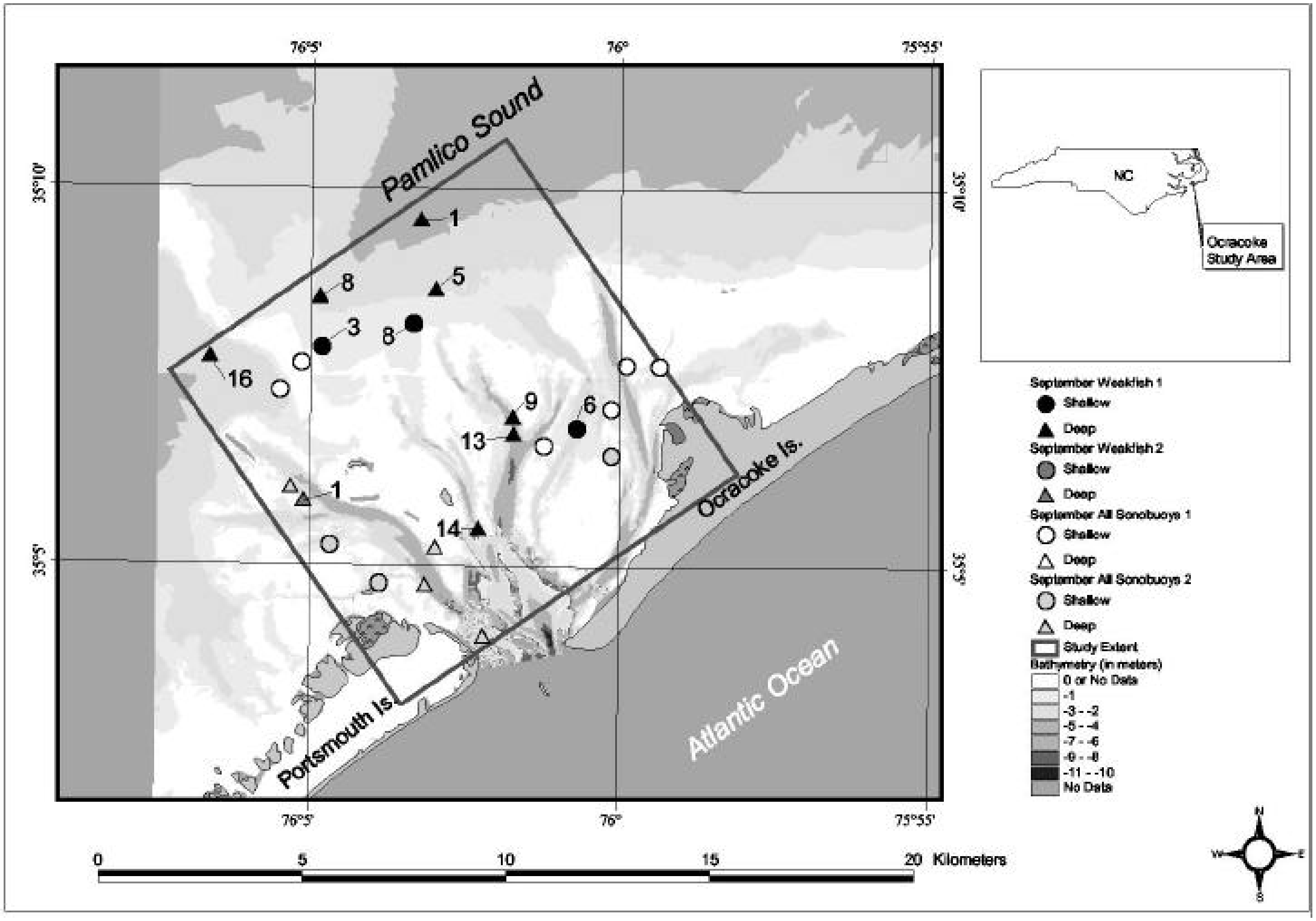

Figure 54. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in September 1998. Filled symbols represent stations at which weakfish "purring" was detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

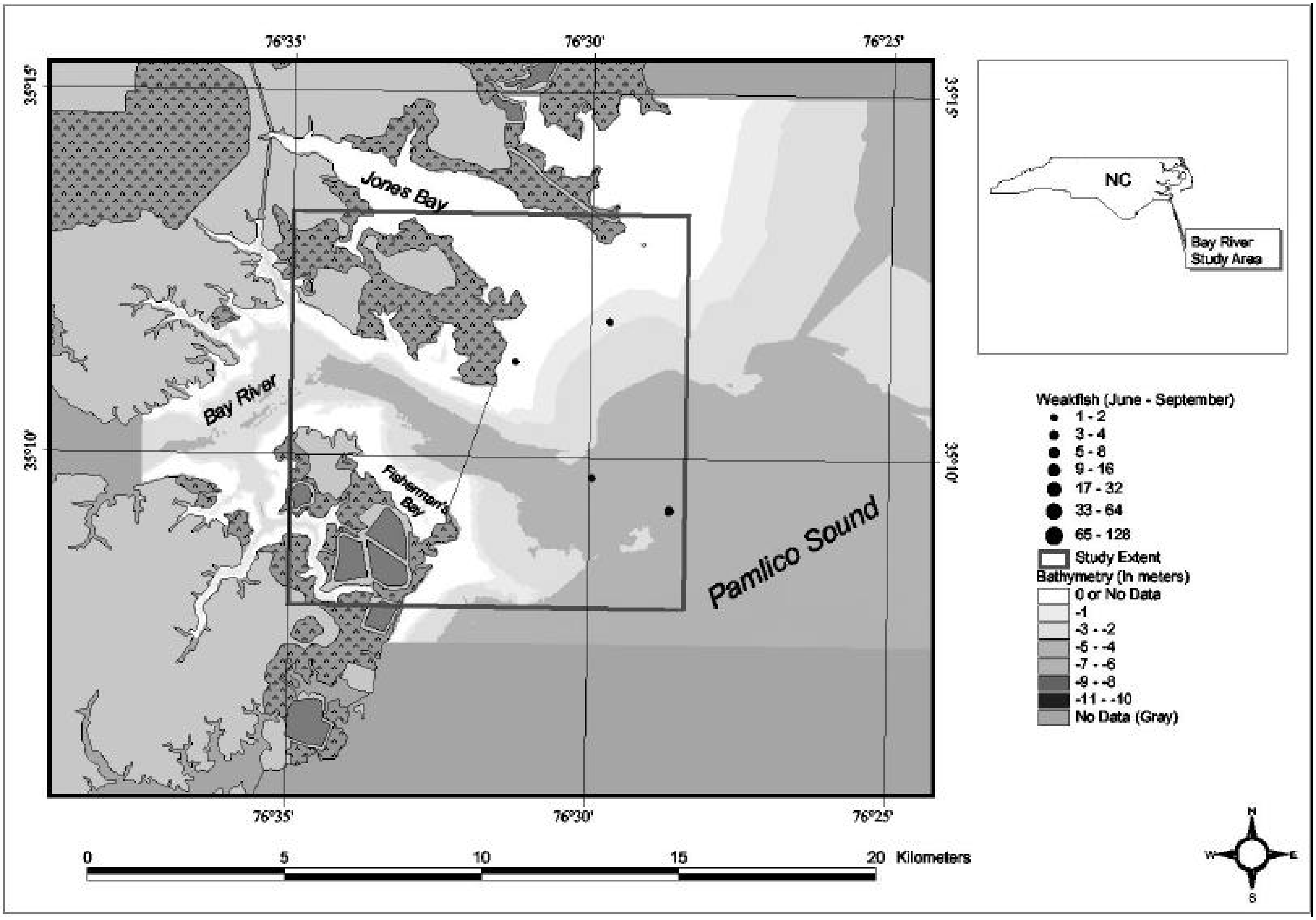

Figure 55. Weakfish drumming index sum from all sonobuoys deployed at Bay River, May - October 1998

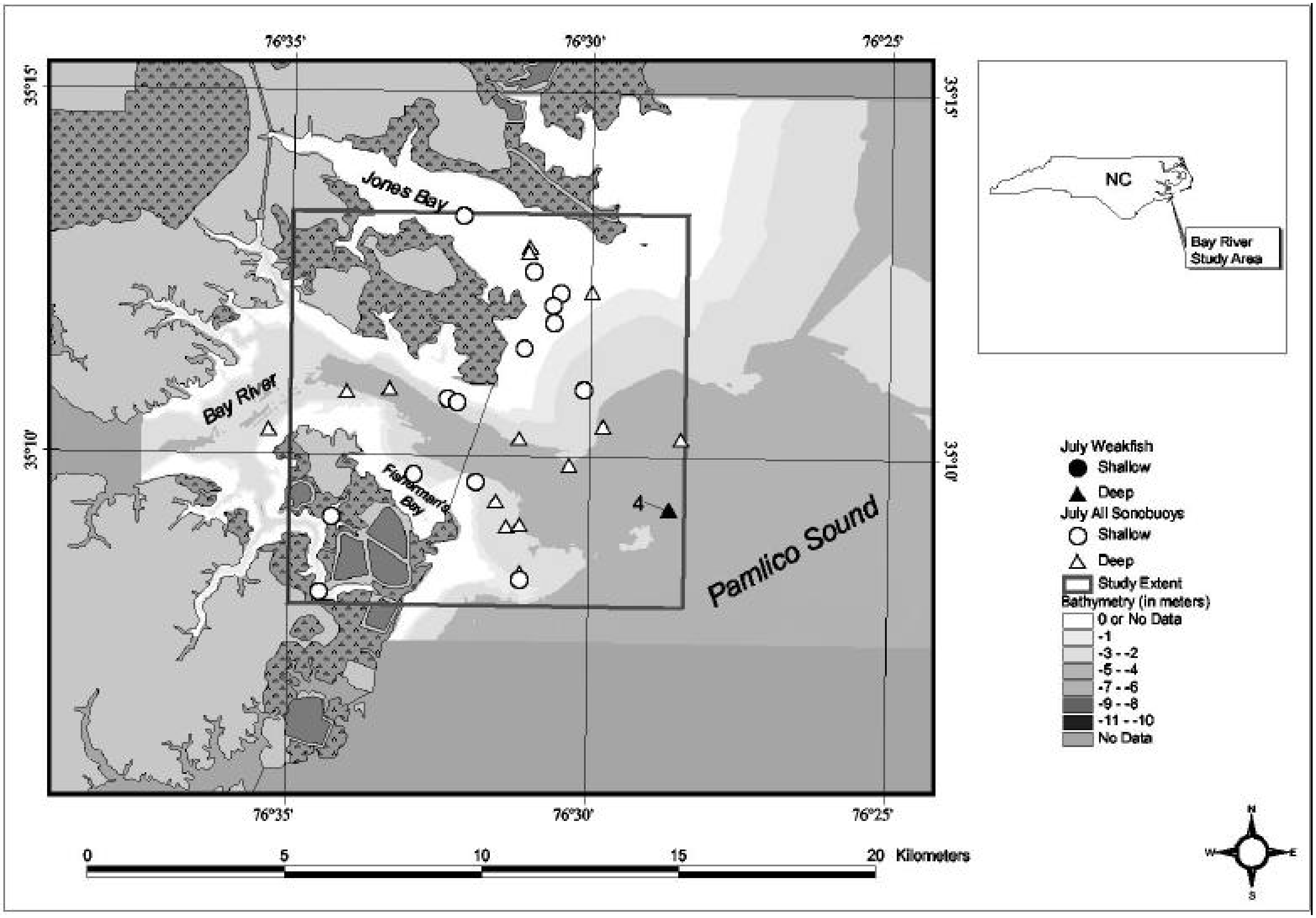

Figure 56. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in July 1998. Filled symbols represent stations in which weakfish "purring" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

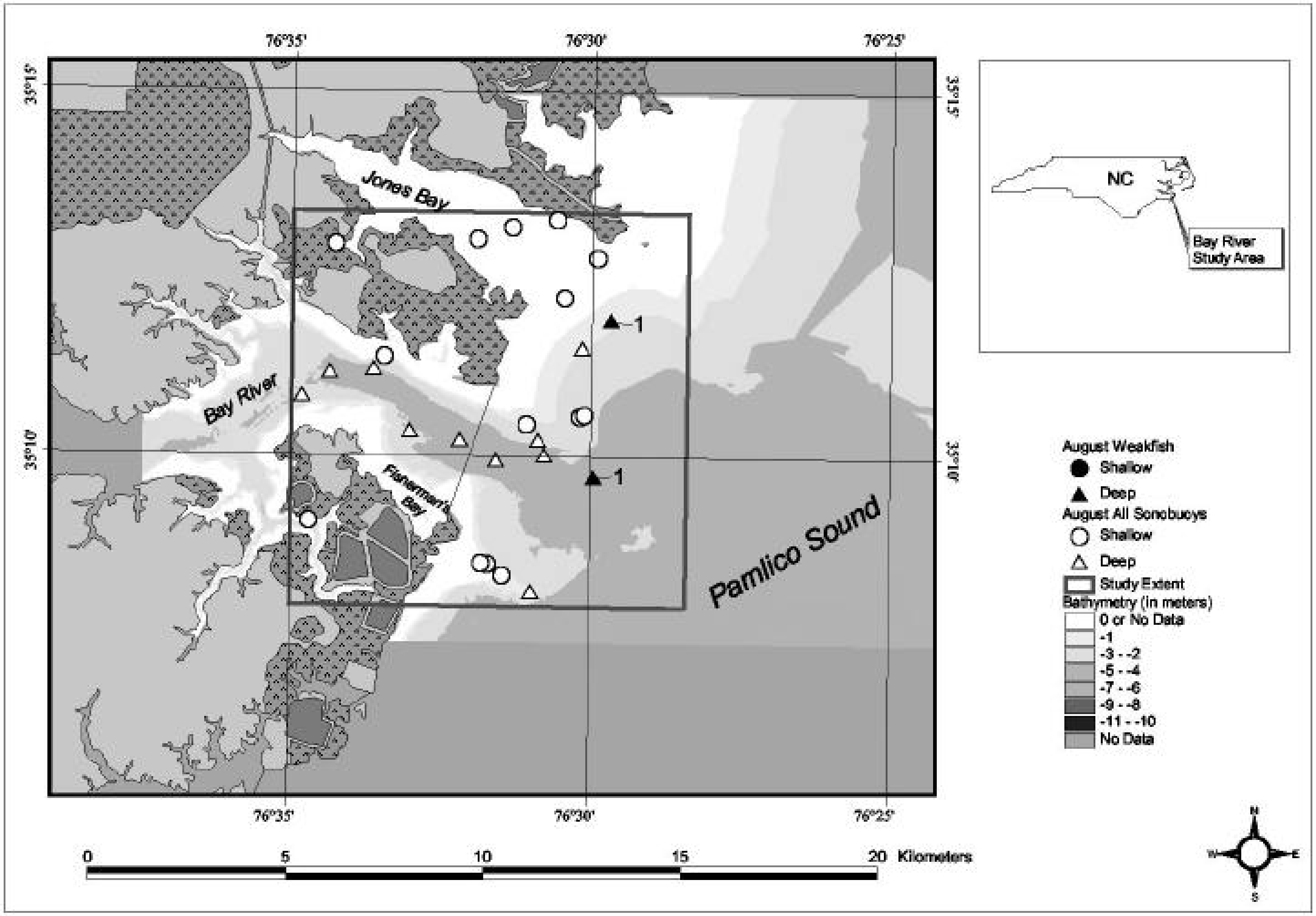

Figure 57. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in August 1998. Filled symbols represent stations in which weakfish "purring" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

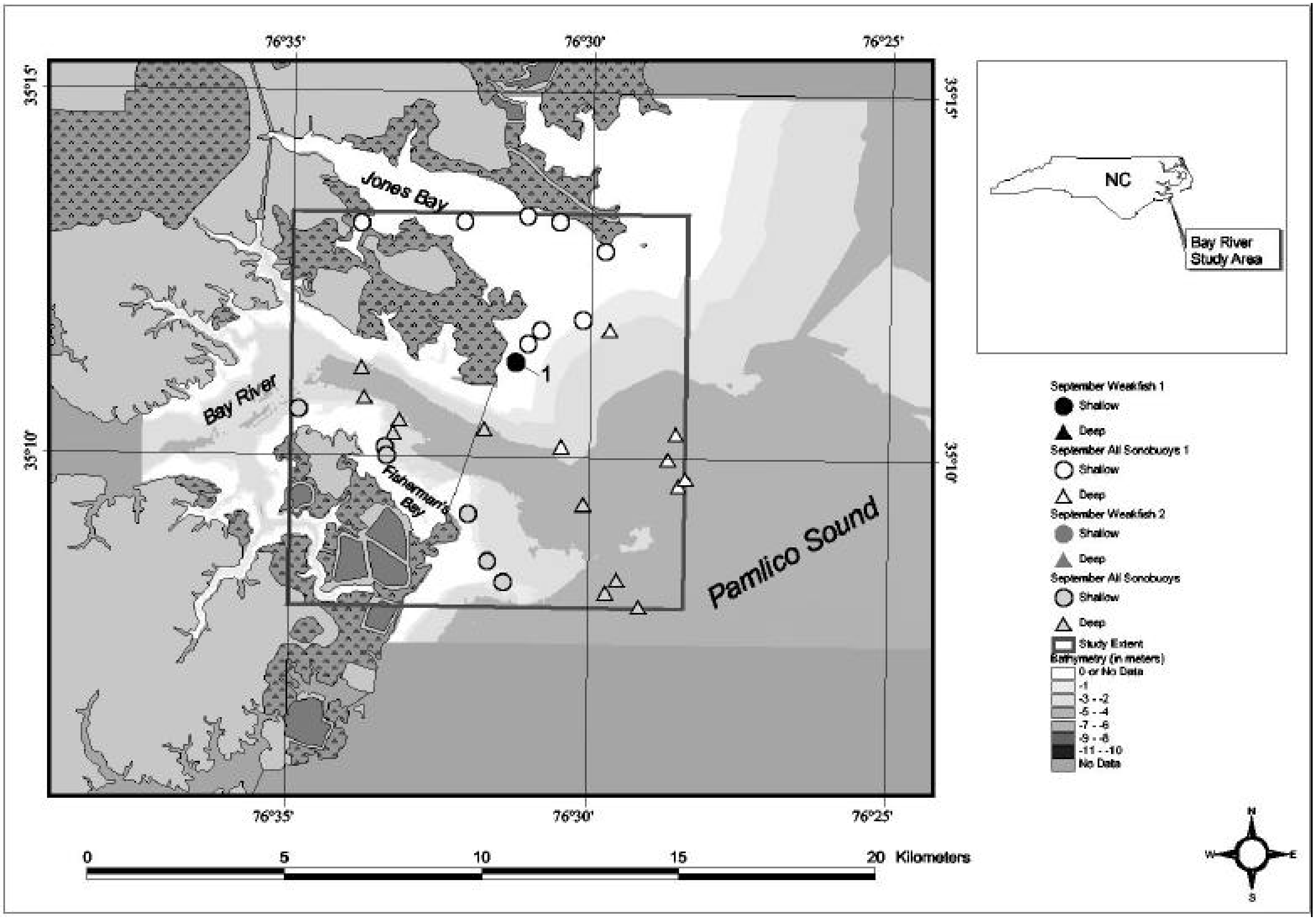

Figure 58. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in September 1998. Filled symbols represent stations in which weakfish "purring" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

Spotted Seatrout Spawning Areas

The following pages display maps of the spotted seatrout spawning areas as determined by plots of the drumming index at each sonobuoy location in the Bay River and Ocracoke study areas May through October 1998.

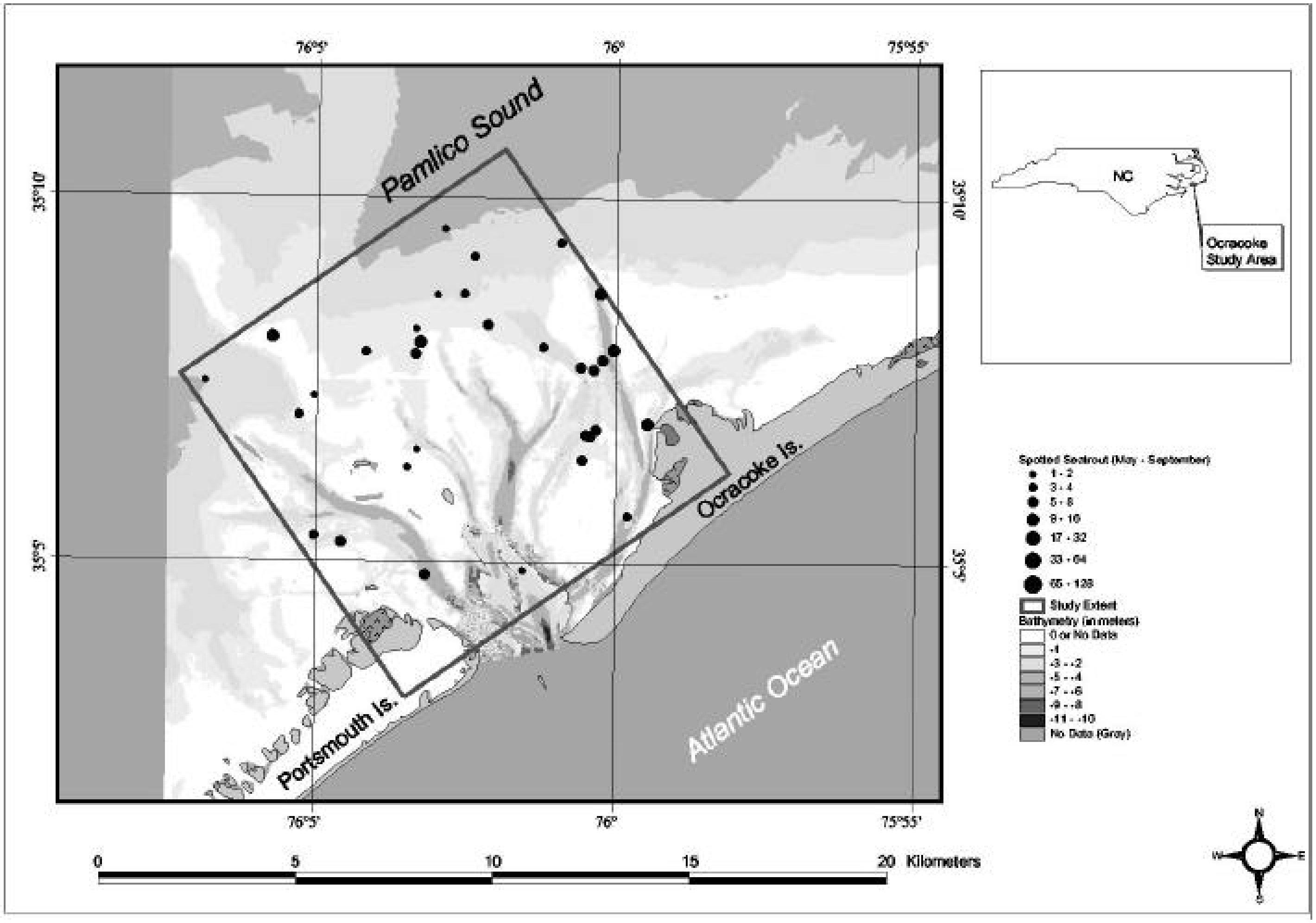

Figure 59. Spotted seatrout drumming index sum from all sonobuoys deployed at Ocracoke, May - October 1998

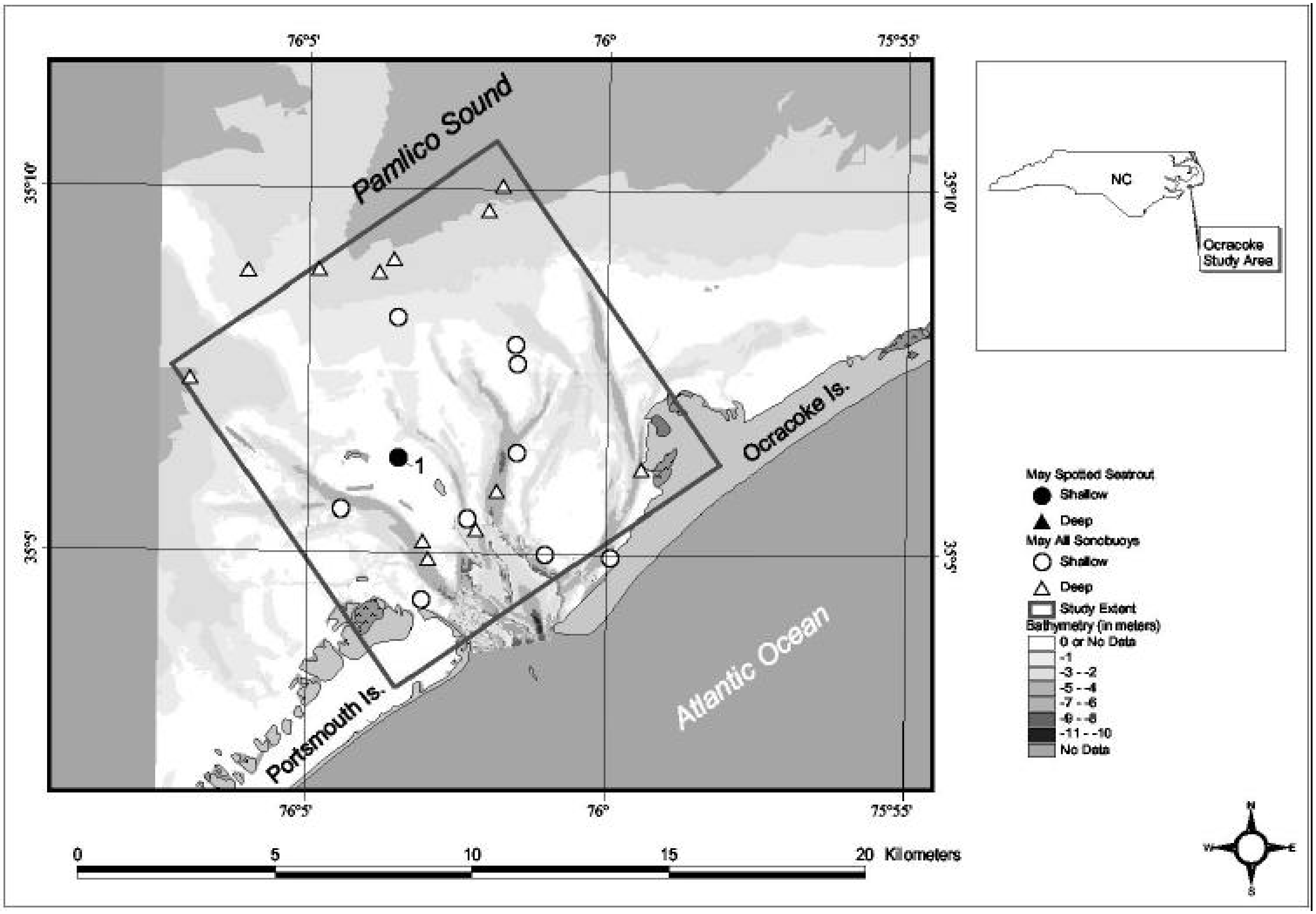

Figure 60. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in May 1998. Filled symbols represent stations at which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

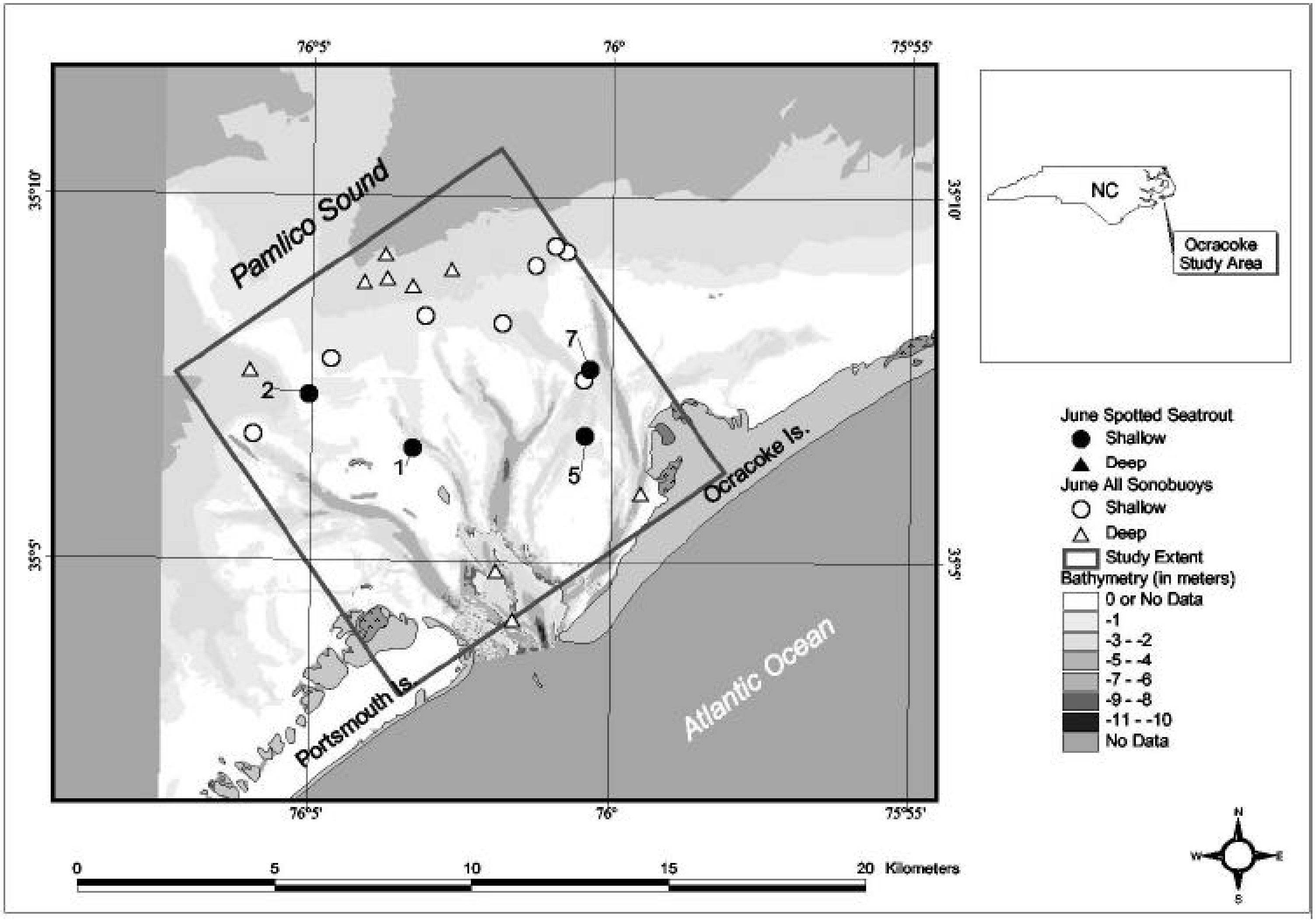

Figure 61. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in June1998. Filled symbols represent stations at which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

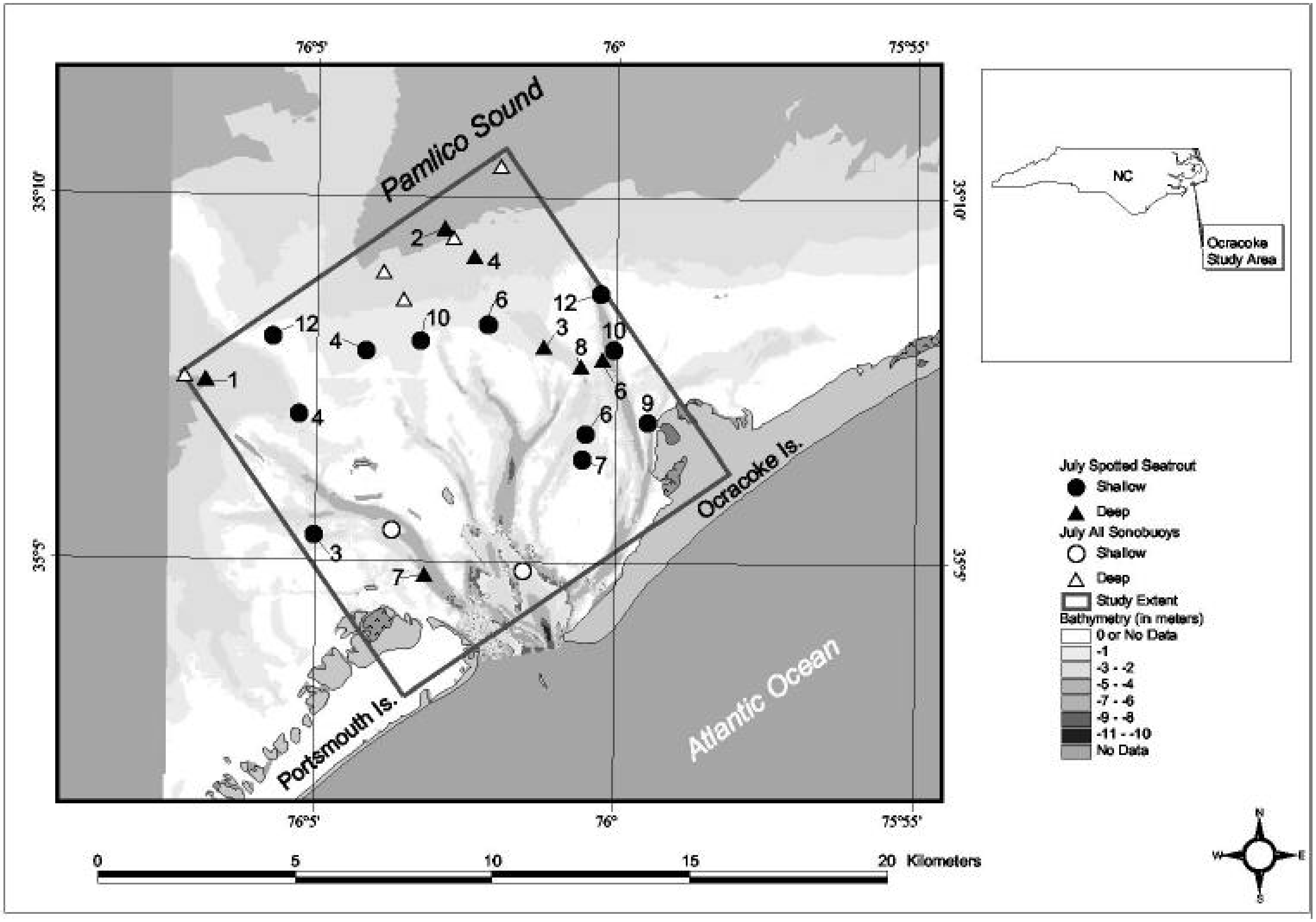

Figure 62. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in July 1998. Filled symbols represent stations at which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

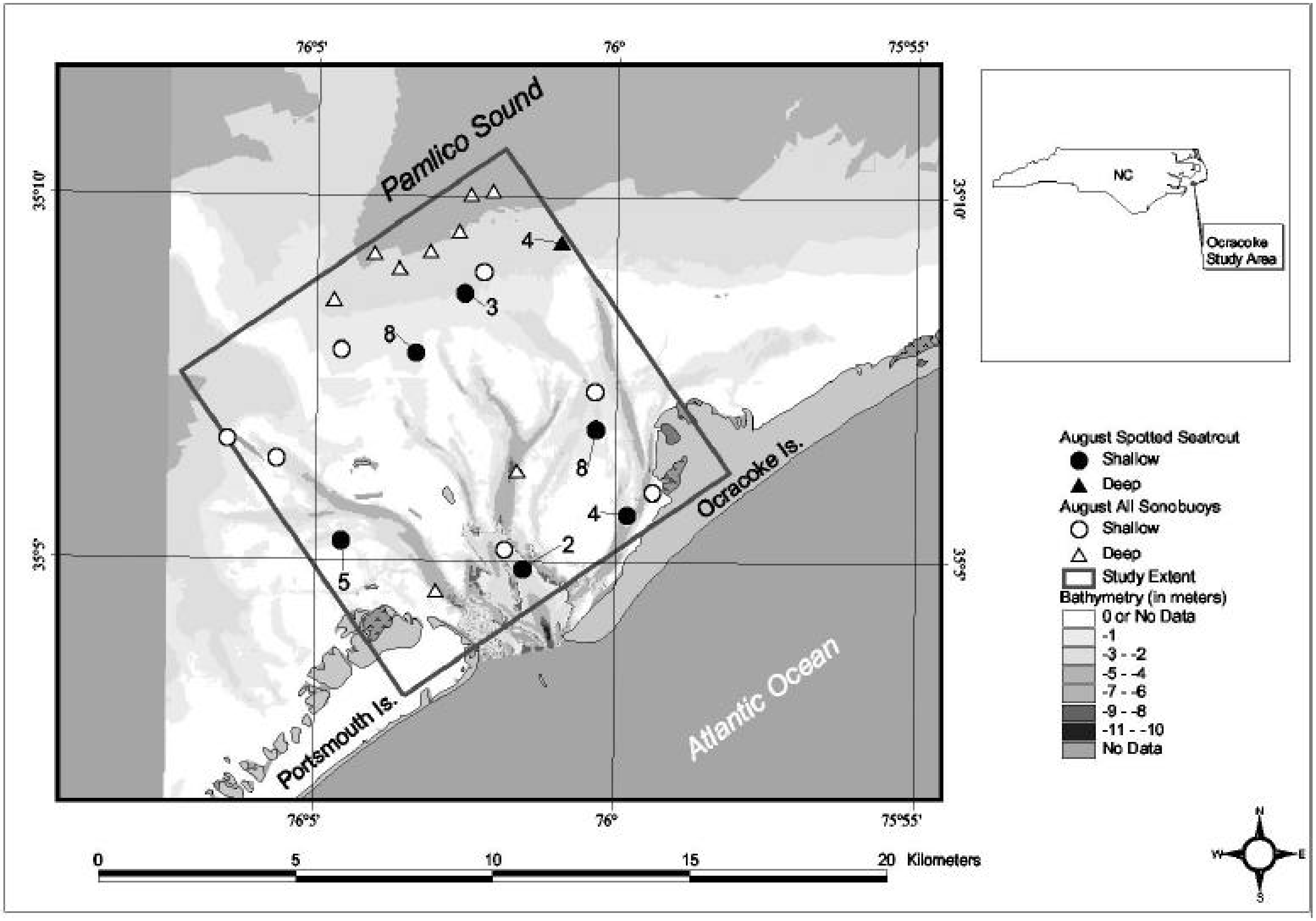

Figure 63. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in August 1998. Filled symbols represent stations at which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

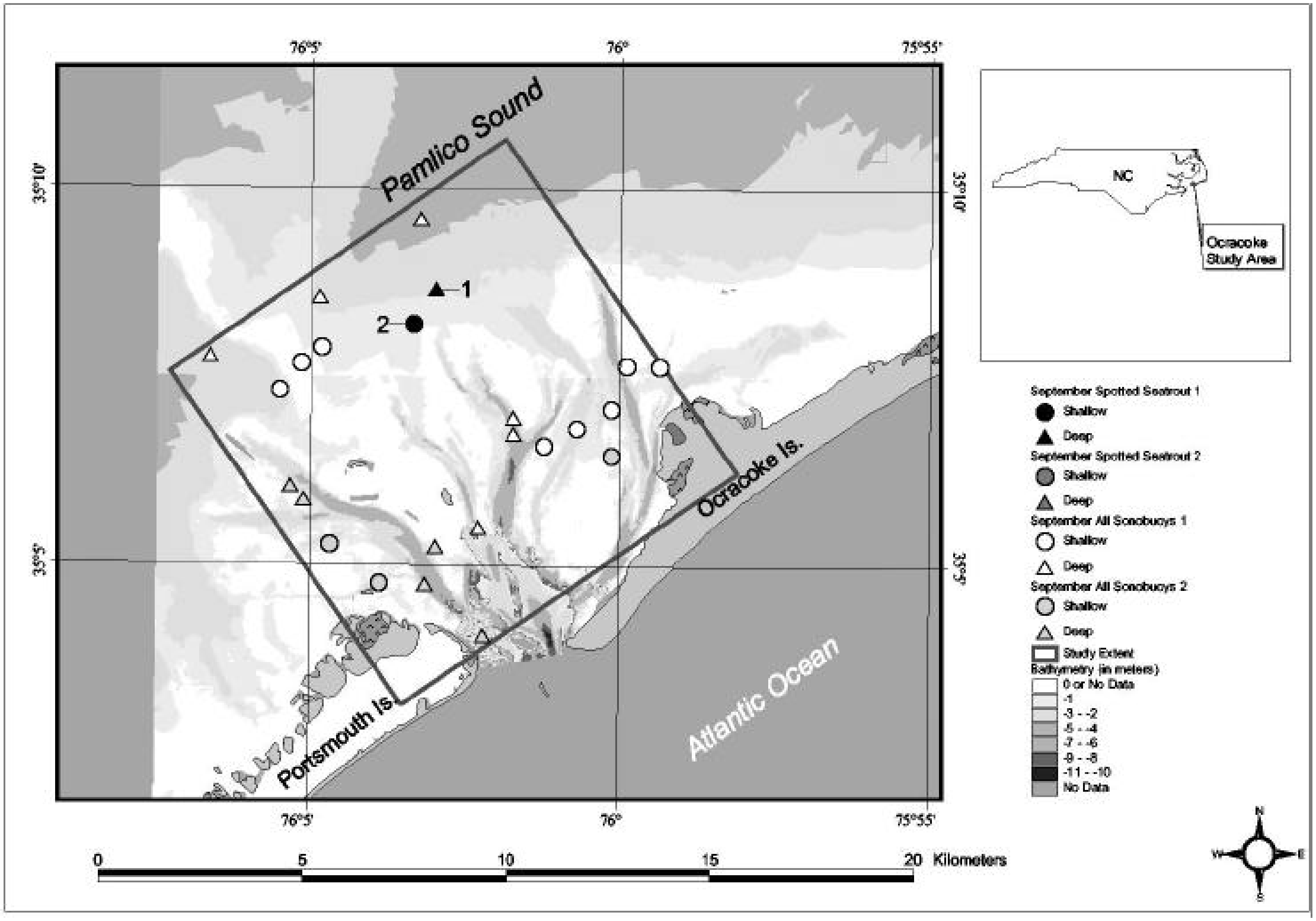

Figure 64. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in September1998. Filled symbols represent stations at which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

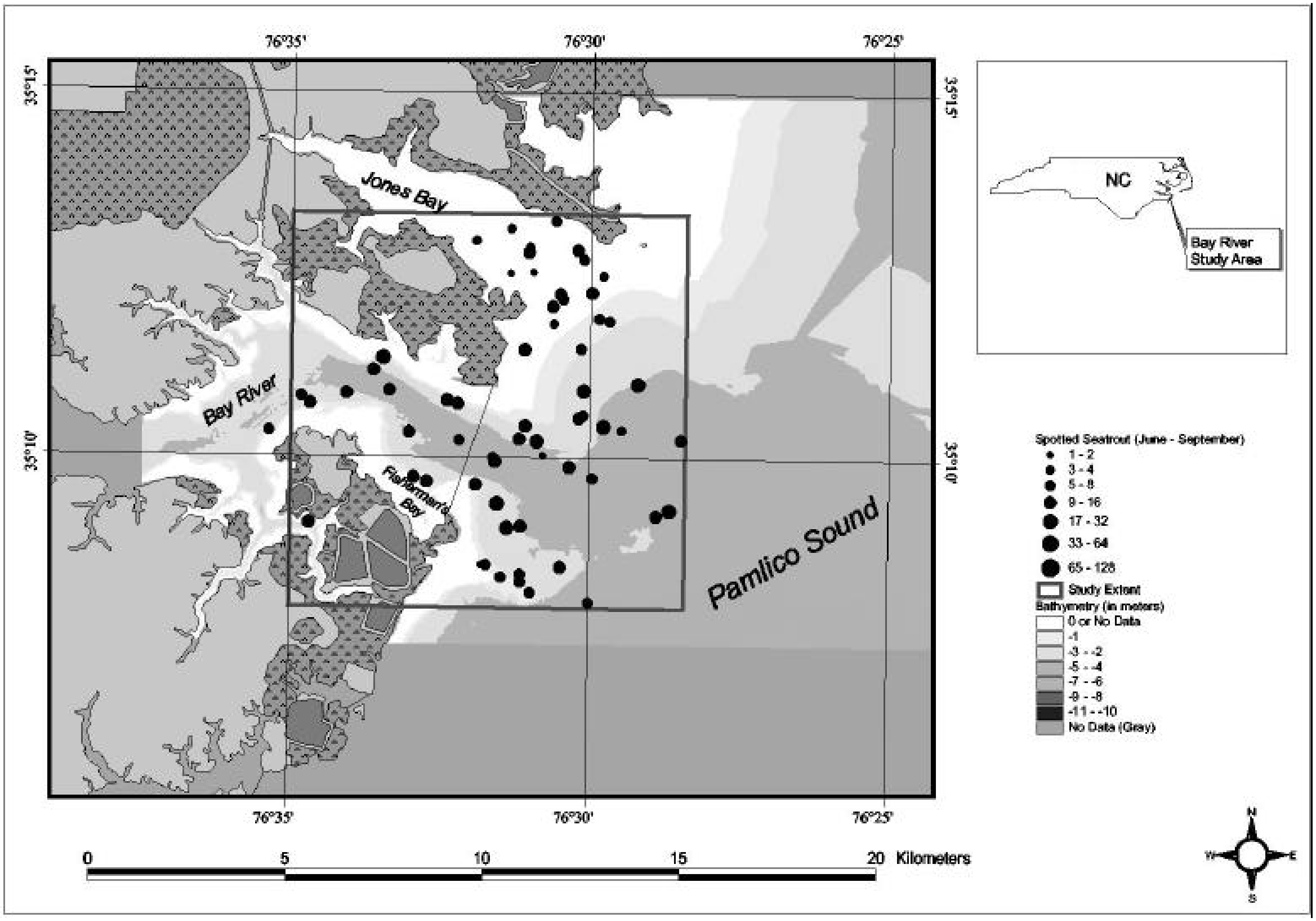

Figure 65. Spotted seatrout drumming index sum from all sonobuoys deployed at Bay River, May - October 1998.

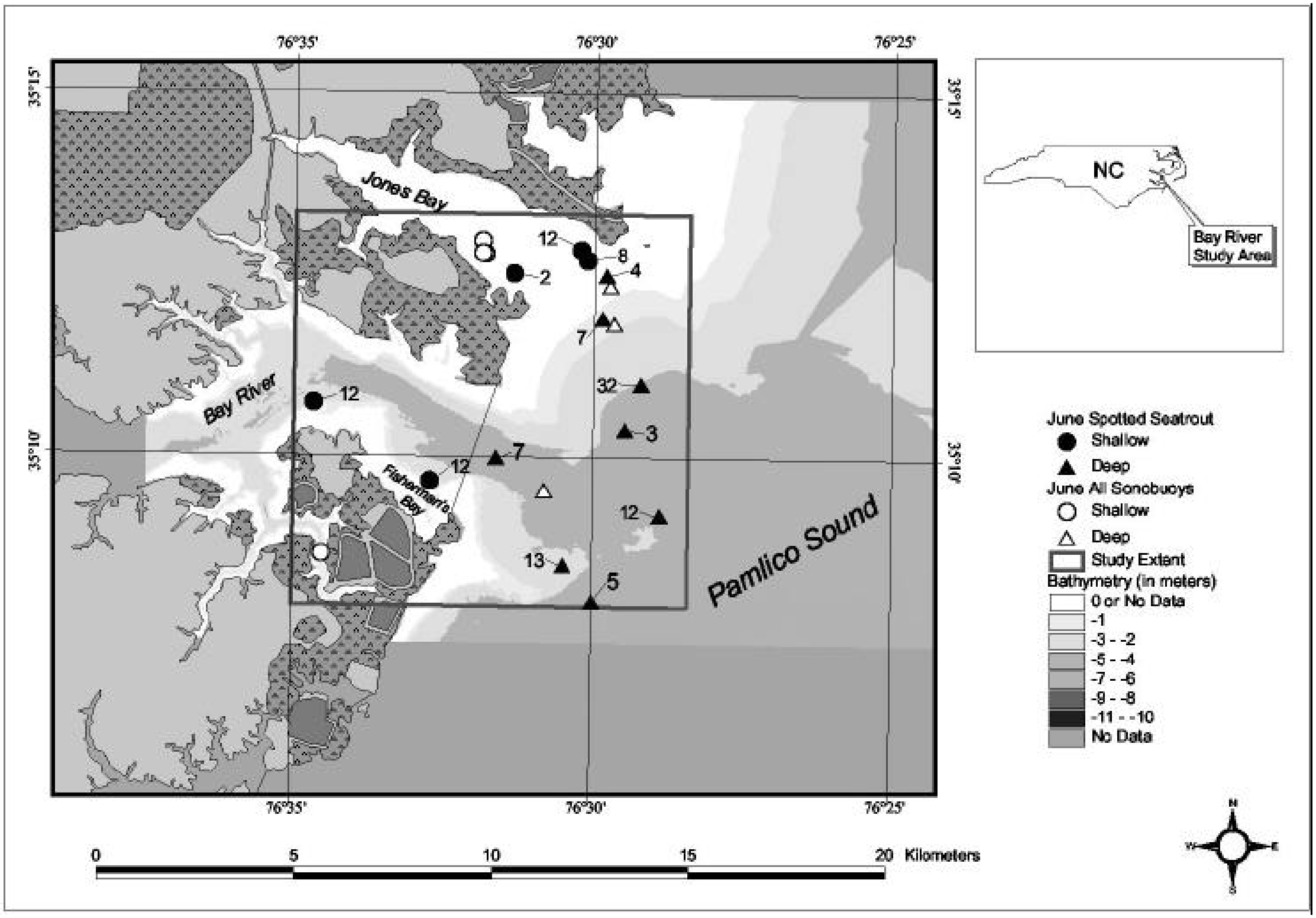

Figure 66. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in June 1998. Filled symbols represent stations in which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

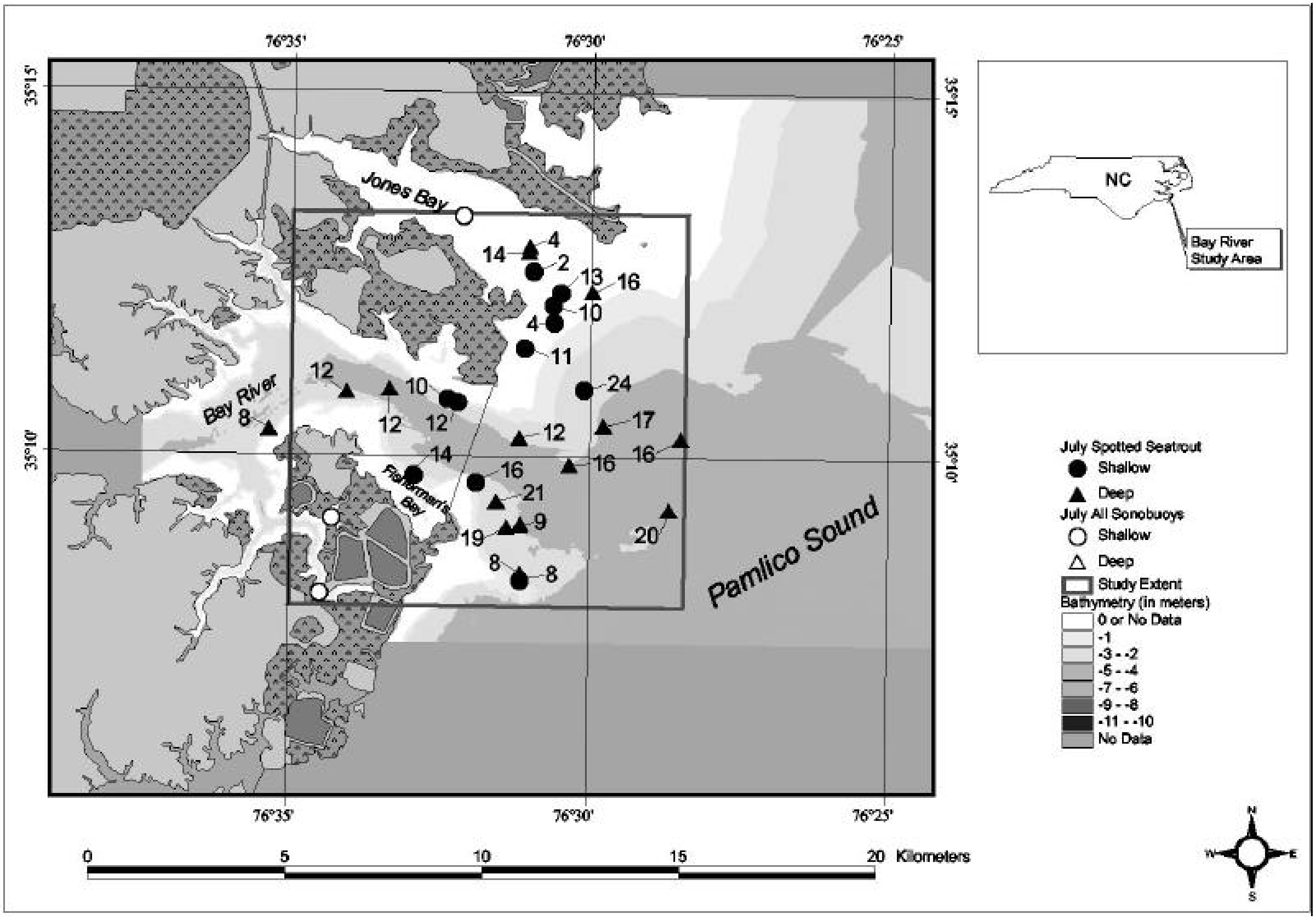

Figure 67. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in July 1998. Filled symbols represent stations in which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

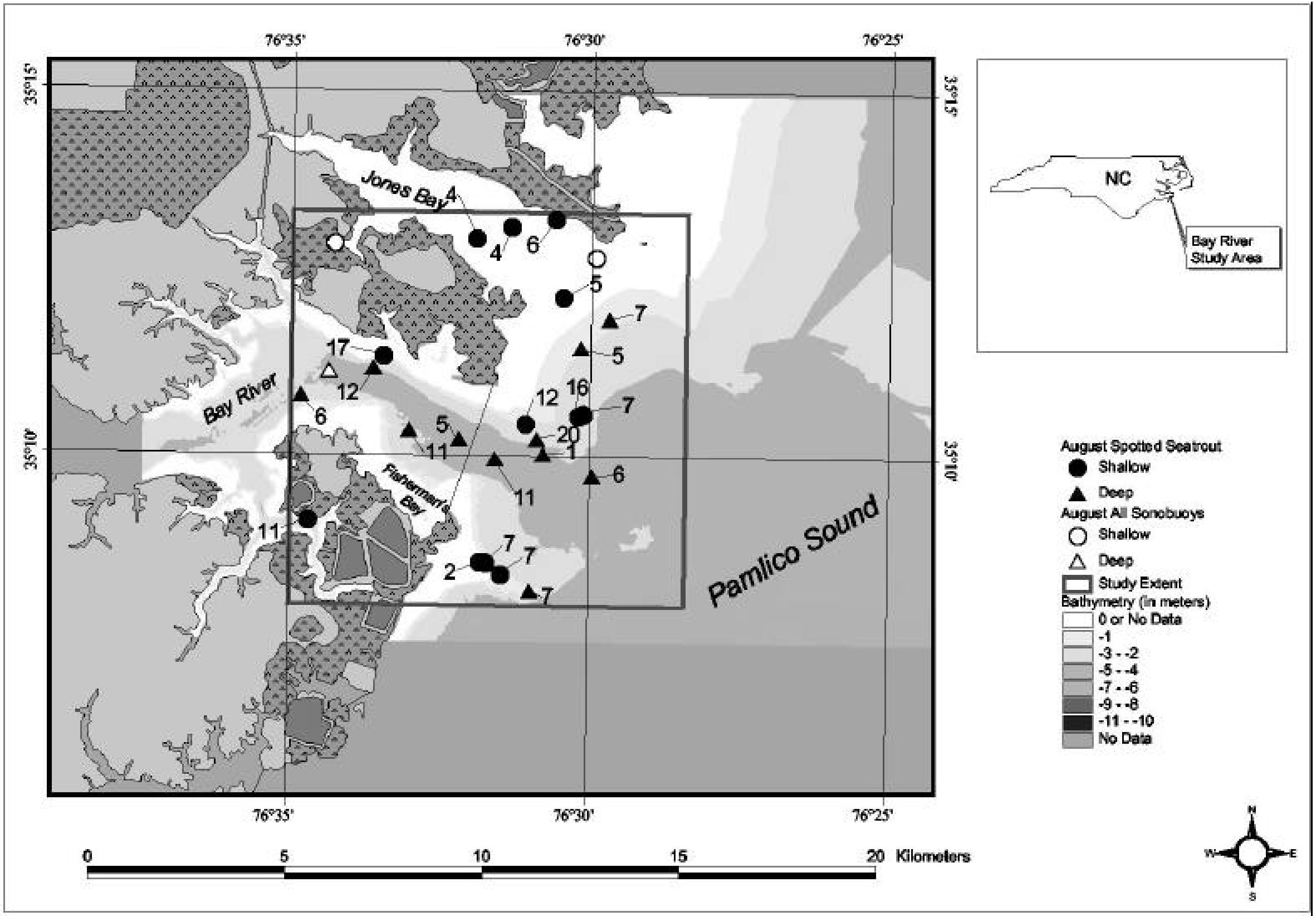

Figure 68. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in August 1998. Filled symbols represent stations in which spotted seatrout "heartbeat," "burp" or "staccato" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

Red drum Spawning Areas

The following pages display maps of the red drum spawning areas as determined by plots of the drumming index at each sonobuoy location in the Bay River and Ocracoke study areas May through October 1998

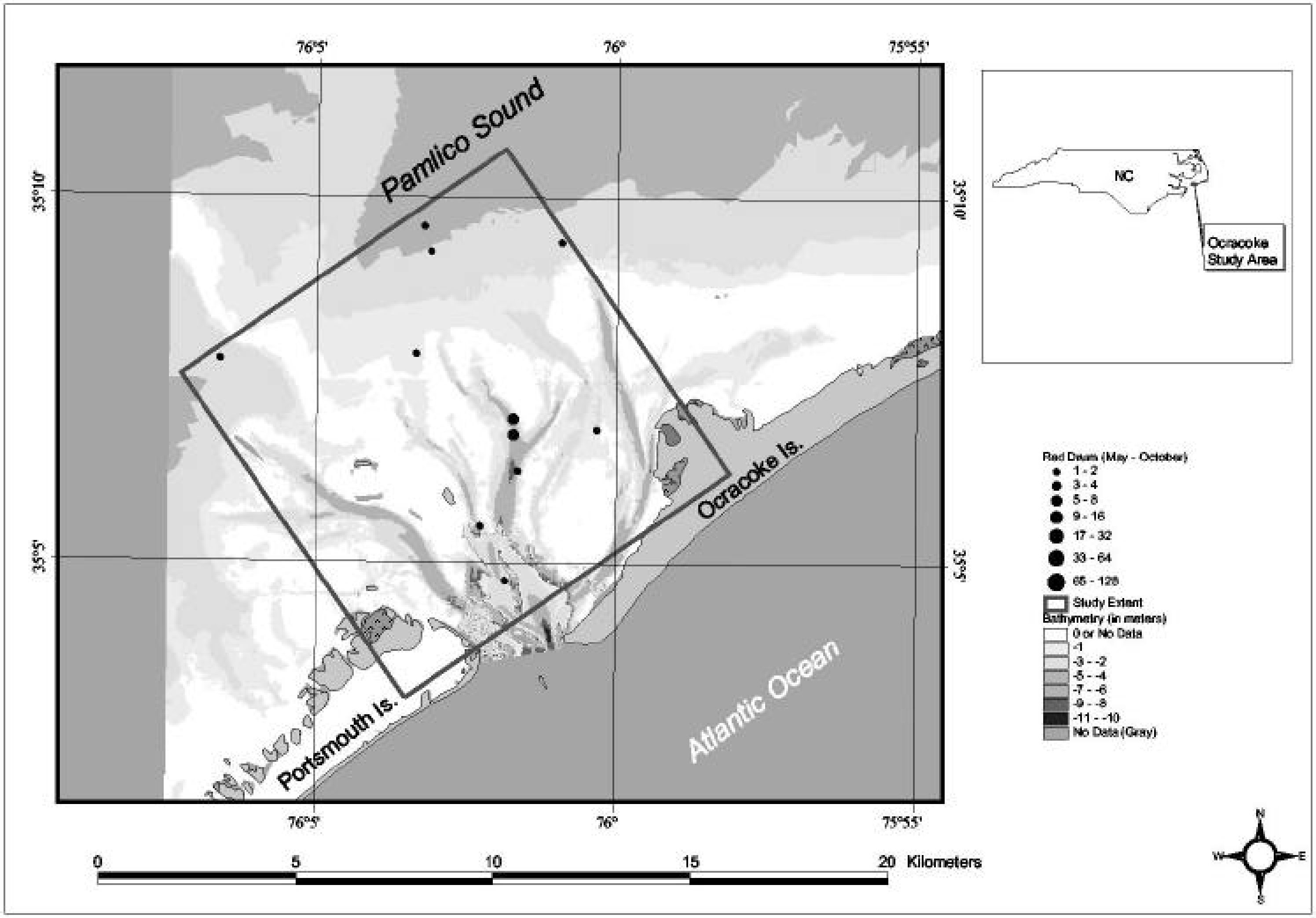

Figure 69. Red drum drumming index sum from all sonobuoys deployed at Ocracoke, May - October 1998.

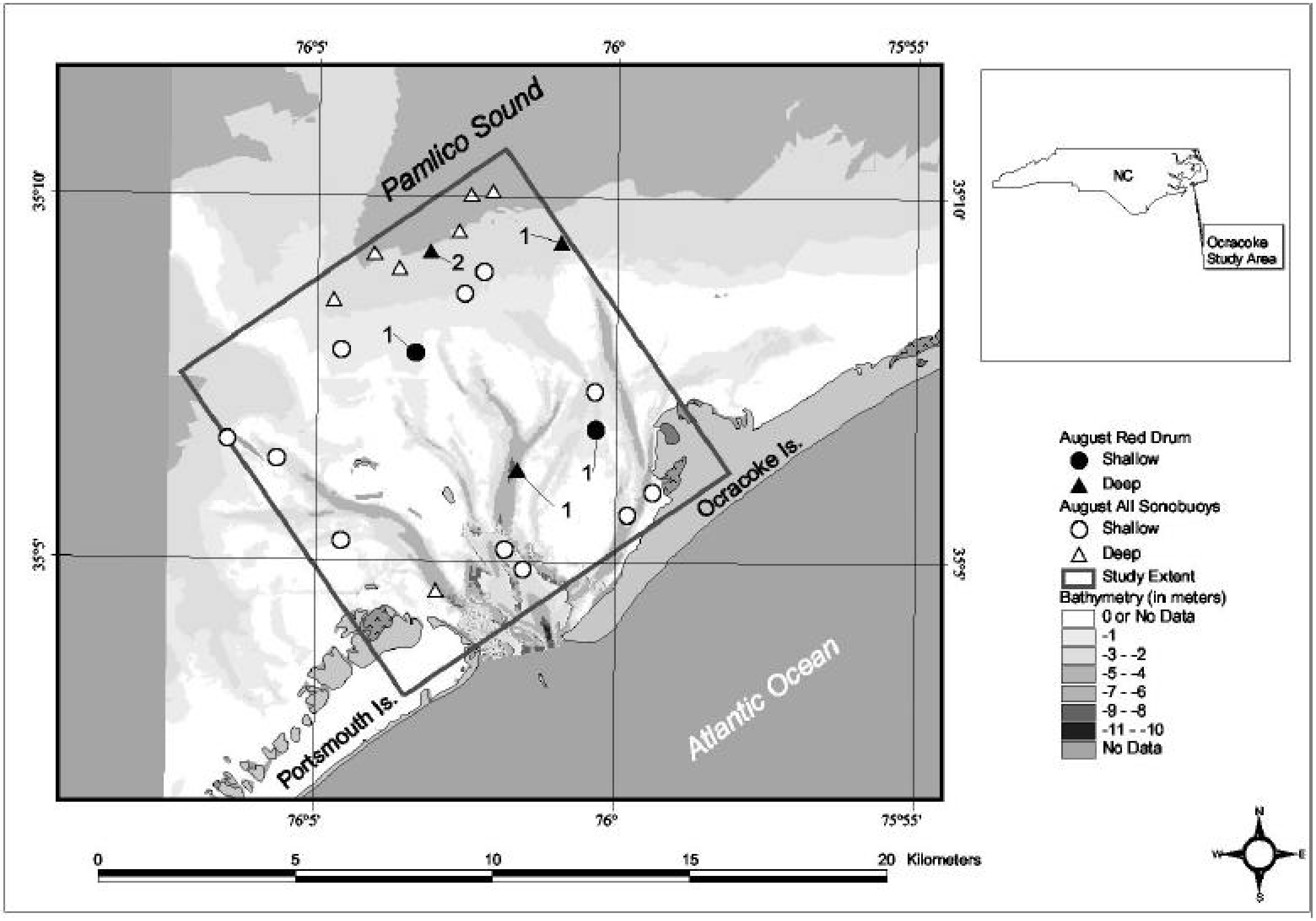

Figure 70. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in August 1998. Filled symbols represent stations at which red drum "knocking" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

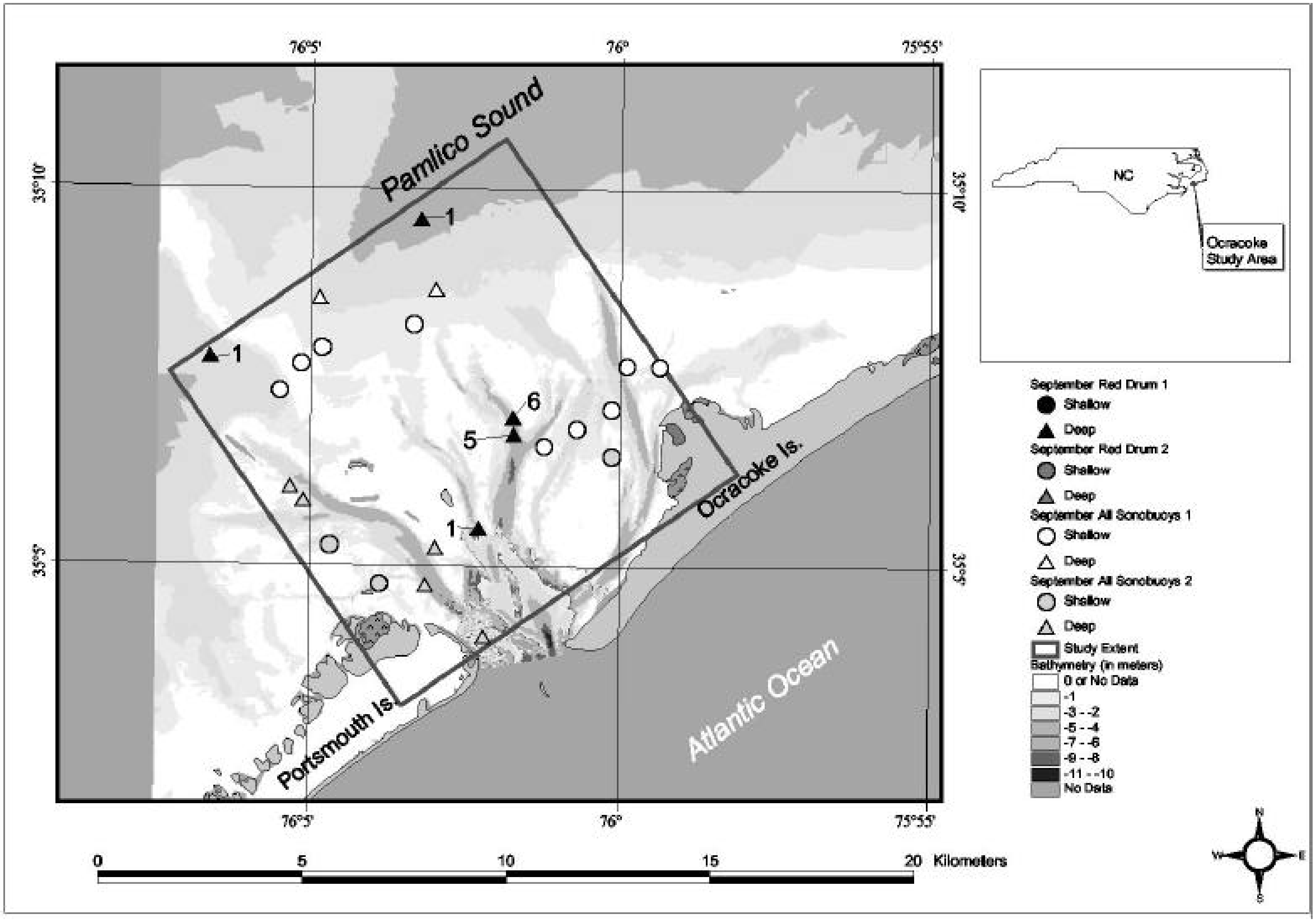

Figure 71. A map of the Ocracoke study area showing shallow (< 10') and deep (> 10') sonobuoy locations in September 1998. Filled symbols represent stations at which red drum "knocking" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

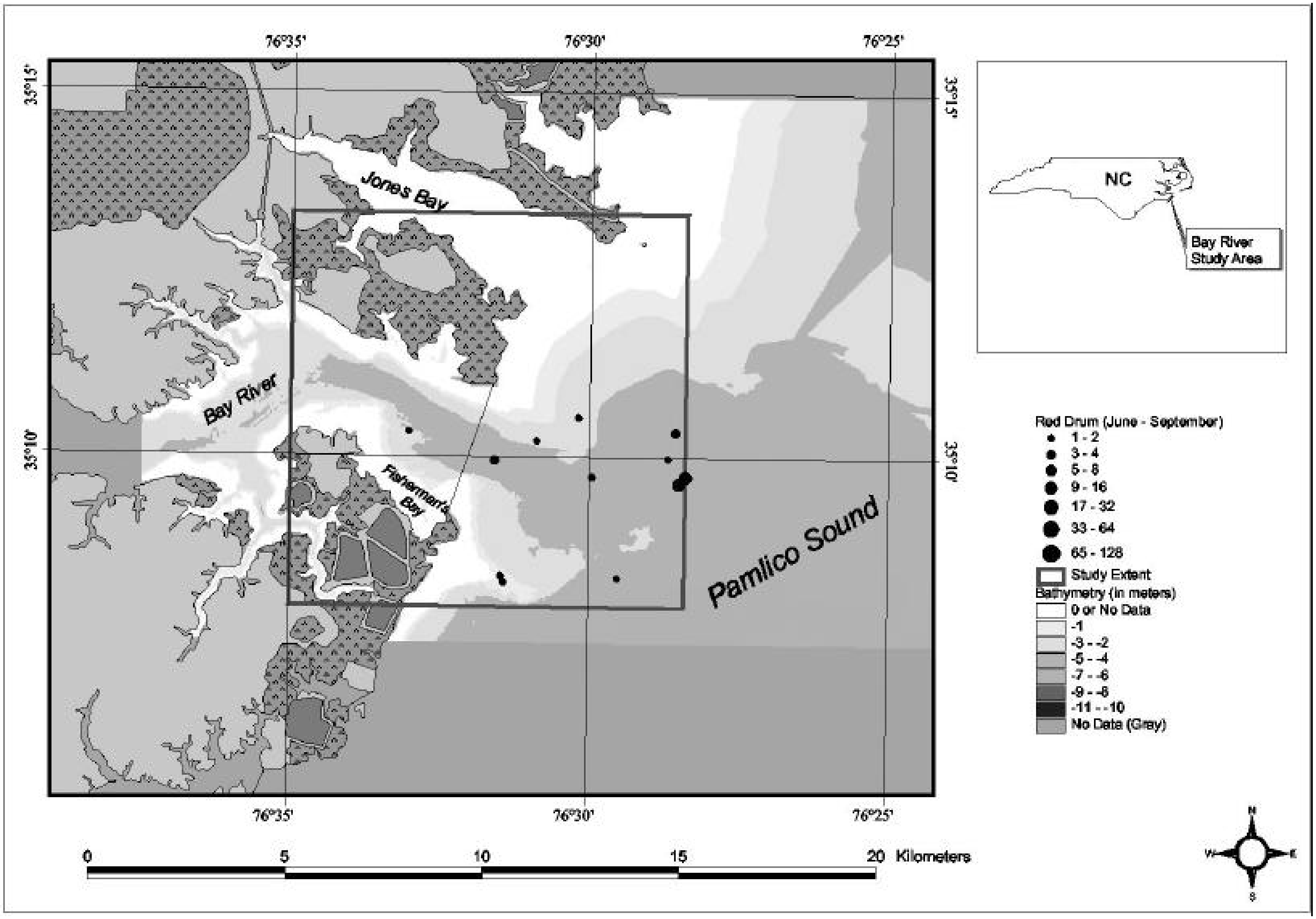

Figure 72. Red drum drumming index sum from all sonobuoys deployed at Bay River, June - October 1998. (No red drum were detected in October 1998 at this site.)

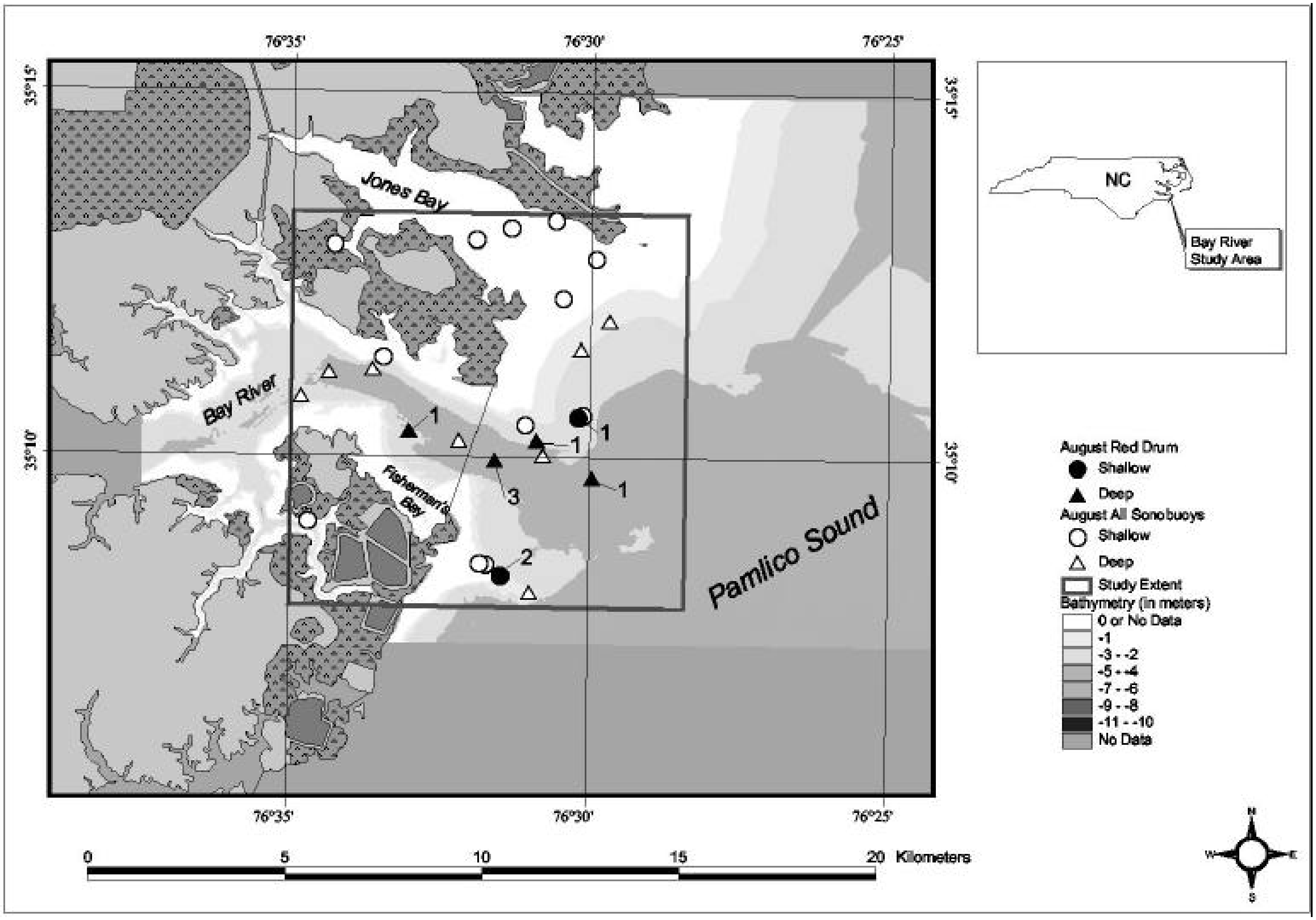

Figure 73. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in August 1998. Filled symbols represent stations in which red drum "knocking" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

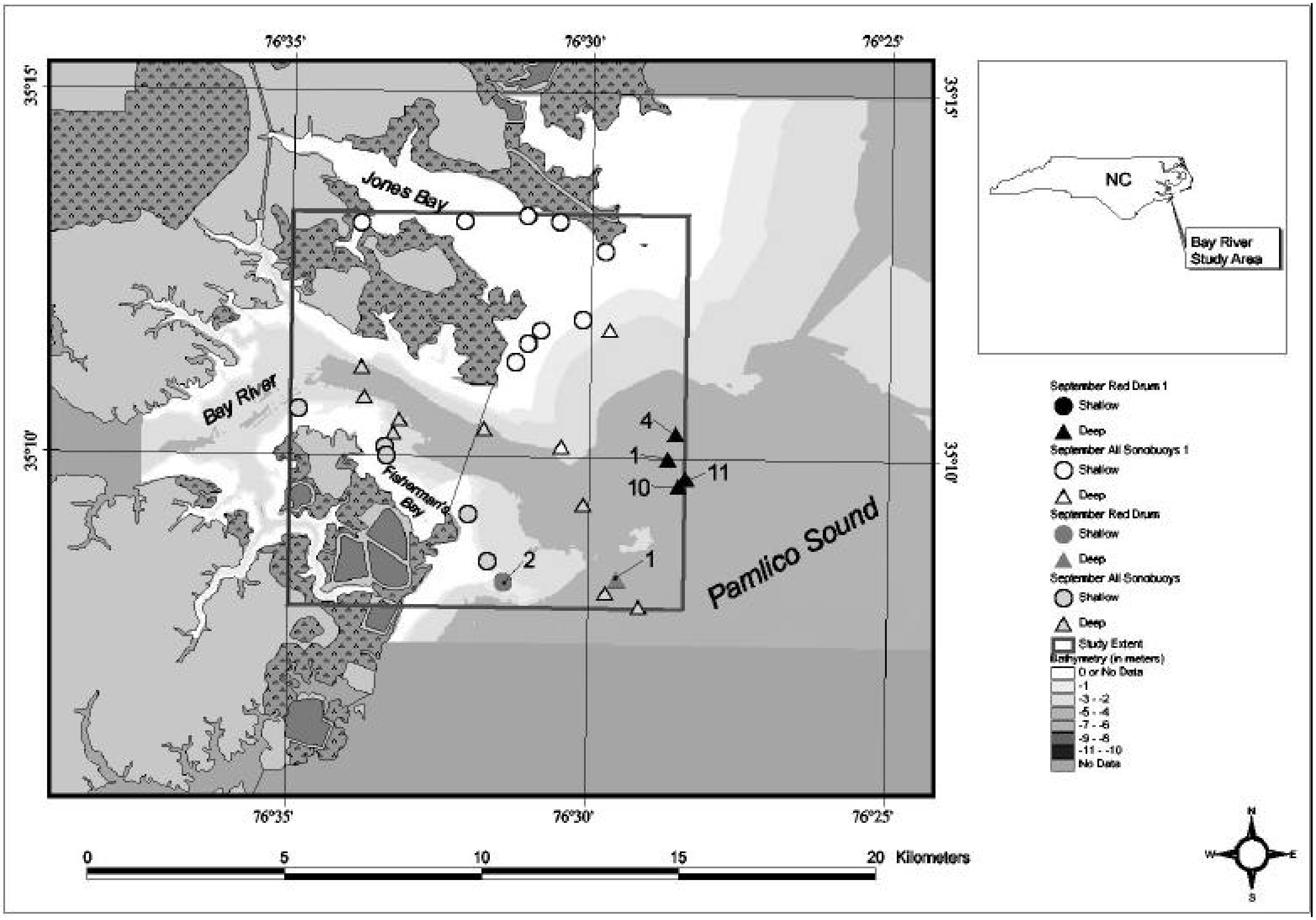

Figure 74. A map of the Bay River study area showing shallow (< 10') and deep (> 10') sonobuoy locations in September 1998. Filled symbols represent stations at which red drum "knocking" sounds were detected on a sonobuoy tape. The number next to each symbol represents the drumming index sum for the sonobuoy recording at that location (see text).

Conclusions

We have recorded and spectrographically analyzed the sounds produced by individual male weakfish, *Cynoscion regalis,* spotted seatrout, *C. nebulous*, red drum, *Sciaenops ocellatus*, and silver perch, *Bairdiella chrysoura*. The sounds were as loud as 127 dB (re 1 μPa) for individual weakfish, 136 dB for individual silver perch, and 147 dB for groups of these fishes. In May and June, it was apparent that some of the recordings contained the "purring" sounds of many individual male weakfish along with “clucking” sounds of many individual silver perch drumming simultaneously. At those times, the sound pressure levels were near the maximum recorded. Thus, both weakfish and silver perch males may “purr” and “cluck” in groups, but we do not have enough information about spatial distribution or abundance of these fishes to adequately model the propagation of the sound produced by these aggregations. We expect that the group drumming of weakfish and silver perch would be louder than an individual fish "purring" or "clucking", and thus would explain the maximal sound pressure levels that we recorded at those sites. The spectrographic analysis presented here allows good discrimination between weakfish and silver perch. We have mapped both species spawning areas based on the sound production alone. Although the areas overlap, the silver perch "clucking" was heard on both sides of the sound, but weakfish "purring" was recorded only at the inlets. Thus, the sciaenid-type eggs that we collected appear to be more closely associated with weakfish "purring", although we cannot rule out the possibility of silver perch eggs contributing to the sciaenid-type egg abundance.

The spotted seatrout and red drum sound production tended to not occur at the same places and times as weakfish and silver perch, indicating that spawning habitats were spatially and temporally partitioned by these sciaenid species. Whereas weakfish and silver perch sound production peaked in May and June each year, spotted seatrout sound production peaked in July, and red drum sound production peaked in September of each year. Weakfish were only heard near the inlets in high-salinity waters; all other species were heard on both sides of the sound. At some locations (i.e., Ocracoke Inlet stations), all three target species could be heard at different times during the study period, but normally not at the same time. Spotted seatrout and weakfish were heard only rarely in the same place at the same time. They appeared to partition the habitat where they co-occurred (Ocracoke Inlet) by occupying different depth ranges: weakfish were in waters > 10 feet, while spotted seatrout were in waters < 10 feet.

Significantly, there is a correlation between overall sound pressure levels of the two most common sciaenid fish sounds (weakfish “purring” and silver perch “clucking”) and sciaenid-type egg densities in the surface waters at the hydrophone stations. This correlation was most likely due to either one or both of the following factors: 1) differences in the number of weakfish and silver perch in the spawning aggregations at some stations, which would influence both the recorded sound pressure levels and the sciaenid egg density measured at any site; or 2) variations in the distance between our hydrophone and the spawning aggregation, which would cause low sound pressure levels due to sound attenuation and a corresponding plume of eggs that was dispersed in the water column, thus appearing as a low density in our samples. At stations where no weakfish "purring" was detected, we can assume that they were absent from those areas, or perhaps that weakfish males were present, but not drumming, because environmental factors (e.g., photoperiod or temperature) were poor for spawning. During some nights at some locations, we recorded “purring” sounds but did not collect developing eggs, which contributed to the imperfect correlation between sound pressure level and egg density. Most of these instances occurred early in the evening just prior to or at sunset. Connaughton and Taylor (1996) reported that the "purring" or drumming sound made by male weakfish under laboratory conditions began before spawning, ceased during the actual spawning activity, then began again immediately after spawning. In our samples, the detection of weakfish “purring” and the absence of eggs may indicate that male weakfish were present and signaling their readiness to spawn, but that spawning had not yet occurred (perhaps because females were not yet present or ready to spawn at that time). Other alternative explanations are that the weakfish could be heard at over a large area (8 km^2), but the pelagic eggs were present in a smaller area and we missed them with the plankton net. In either case, our plankton net did not intercept a plume of eggs released during spawning at these stations. We favor the idea that weakfish make their presence known just prior to spawning, but do not necessarily spawn when drumming. Thus, stations where weakfish produced "purring" early in the evening may be best referred to as potential spawning sites that indicate where eggs will produced at some later time. We may conclude that the strong correlation between weakfish "purring" and sciaenid-type eggs suggests that weakfish were spawning near the inlets of Pamlico Sound in May of 1997. Because we obtained similar qualitative results for both 1997 and 1998, this is good evidence that the inlet areas are being used as spawning areas for this species in May each year.

Although the eggs we collected appear externally similar to descriptions of eggs produced by weakfish, spotted seatrout and red drum, we cannot conclusively identify the sciaenid-type eggs collected in this study as belonging to the target species eggs based on morphological characteristics alone. Because early-stage eggs of sciaenids are very close in appearance, a molecular genetics approach has been used to distinguish them (Daniel and Graves 1994). Although the molecular approach is precise with regard to species identification, it is labor-intensive and it is nearly impossible to perform on the numerous eggs that are typically collected in a plankton sample. Our data suggest that there is a good correlation between sound production and egg production; thus, passive acoustics can be used as a more rapid (but less precise) method for identifying species-specific sciaenid spawning areas. The sound production can be easily discriminated among species using a spectrographic approach as detailed in this report (see also Appendix III).

We cannot rule out several alternative interpretations of our results. Weakfish, spotted seatrout, and red drum may spawn in areas not adequately sampled in this study (center of the Pamlico Sound, other areas along the western and eastern side of the sound offshore in the Atlantic Ocean, etc.), but we would not have detected them because of their great distance from our listening stations. Areas such as Adams Creek, Garbacon Shoal, Legged Lump, Swan Island, and the mouth of the Neuse River should be sampled using passive acoustics and planktonic egg collections in the future, because they have all been suggested as areas where ripe red drum females have been observed and may spawn (personal communication with M. Wolff, B. Burns, NC DMF). Likewise, all inlets in North Carolina are potentially important spawning habitats for weakfish, so future surveys need to be done to include Oregon Inlet, Drum Inlet, Barden Inlet, Beaufort Inlet, etc. We simply did not have enough resources to visit all these areas, but the use of the sonobuoy described in this report could improve the areal extent of sampling in the future.

Passive hydroacoustic surveys will greatly reduce the effort required in planning marine reserves for weakfish, because spawning areas of fishes can be easily delimited using hydrophones. It appears that due to the declining status of red drum along the Atlantic Coast (Vaughan 1996), and the relative rarity of red drum in our passive hydroacoustic sampling reported in this study, spawning reserves should be established for red drum. Specific areas that should be considered for closure as red drum spawning reserves in North Carolina, based on the data contained in this report, include the areas near the mouth of the Bay River (Figure 72), and Ocracoke Inlet (Figure 69). For weakfish, areas near Ocracoke (Figure 49) and Hatteras Inlets should be considered for spawning reserves. Finally, good areas for spotted seatrout spawning reserves would appear to be areas on both sides of the sound, including the Bay River (Figure 65) and Ocracoke Inlet (Figure 59). It should be emphasized that spawning reserves may only need to be implemented to prevent fishing on the spawning stock as an emergency measure during peak of spawning seasons. We also emphasize that because all spawning areas are not fully known at the current time, priority areas for closure cannot be determined. As further passive hydroacoustic surveys are conducted, a complete map of the major spawning areas in North Carolina may be developed. Only then should spawning reserves be established, unless emergency management measures are required. For the region studied here, the peak of spawning for weakfish (May and June), spotted seatrout (July) and red drum (September) have been clearly identified. The peak of spawning in other areas can be determined by continued use of passive acoustic methods (i.e., monitoring with a moored sonobuoy). Spawning reserves may not be warranted for catch-and-release fishing, unless high rates of hooking mortality, based on studies similar to those being conducted now on red drum (personal communication, Peter Rand, North Carolina State University), indicate that additional protection of these spawning fishes is needed.

Although passive acoustic sampling method used in this report cannot totally replace the careful estimation of fish egg production by traditional means, it is a reliable, rapid, and non-disruptive method of determining the location of spawning grounds of soniferous fishes in the family Sciaenidae. The approach may be applicable to other commercial species as well (cod, *Gadus morhua*, and penaeid shrimp are two examples of commercially valuable soniferous animals). We recommend that the fishery management agencies (such as NC DMF) continue to use this passive hydroacoustic approach to identify EFH-HAPC regions for the fishery management plans as required by the Magnuson-Stevens Sustainable Fishery Act and the South Atlantic Fishery Management Council Habitat Plan (SAFMC, 1998). In addition, consideration should be given to the use of passive hydroacoustic surveys to estimate a yearly index of spawning stock biomass, which would be useful in the future as a correlate to traditional stock assessment data. An extension of some of the passive acoustic techniques we used here can be made, with the proper model validation, to develop an accurate unbiased fishery-independent estimator of adult populations for soniferous species.

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Appendix I – Data on Compact Disc, Instructions for Use

IMPORTANT

Read this and the Contents of the READMEFIRST.TXT file on the CD to view any updates.

THIS COMPACT DISC (CD) CONTAINS BOTH DATA AND AUDIO FILES.

Therefore, you should have the following 2 different components to view on your CD:

1. THE DATA PORTION, which contains, an Hyper-Text-Markup Language (HTML) coded Catalogue of Fish Sounds, Spectrographs, and Spawning Maps (START.htm)

To access the Catalogue, open your Internet browser (Netscape or Microsoft Explorer), and open the file labeled START.htm, in the folder titled 'Final Report'. Another way to do this is to double-click the letter of your CD drive, then the folder labeled 'Final Report', and then the file labeled START.htm. Maps of critical spawning areas of weakfish, spotted seatrout, and red drum in Pamlico Sound are also linked to this file. These files can be viewed in any program that supports *.jpg files.

2. THE AUDIO PORTION, which contains recordings of fish sounds that can be listened to using a stereo with a CD player or directly on your computer, if it has a sound card and CD drive.

MAKE SURE THAT YOU ADVANCE TO AUDIO TRACK # 2

Track #1 contains the computer data, which will sound like noise if you play it through an audio CD player.

Appendix II – Gill Net Collections 1997

Gill Net Collections 1997

Gill Net 1 = 3"/6" stretch mesh net used before 6 Aug
Gill Net 2 = 3"/6.5" multi-panel net used on and after 6 Aug 97
Gill Net 3 = 12 " mesh, large mesh net , or drum net

NA= not applicable
NR= not recorded

Station	Latitude	Longitude	Depth (ft)	Gear	Set Date	Time	Re-covery Date	Re-covery Time	Soak Time (h:min)	Species Caught	Fish ID No.	SL (mm)	Body Mass (g)	Sex	Gonad mass (g)	GSI (%)
Fisherman's Bay	35*10'03.85"	76*32'53.28"	13	Gill net 1	5/13/97	18:50	5/13/97	22:27	3:37	no target sp.	NA	NA	NA	NA	NA	
Fisherman's Bay	35*09'36.01"	76*32'42.12"	5	Gill net 1	5/13/97	18:59	5/13/97	22:09	3:10	no target sp.	NA	NA	NA	NA	NA	
Rose Bay	35*22'38.7"	76*25'09.6"	5	Gill net 1	5/15/97	19:19	5/15/97	22:30	3:11	CYNNEB	97-1	308	NR	NR	NR	
RB Net 2	35*27'24.8"	76*24'12.2"	5	Gill net 2	5/15/97	20:55	5/15/97	21:53	0:58	no target sp.	NA	NA	NA	NA	NA	
Wallace Channel	35*04'14.6"	76*02'54.8"	10	Gill net 1	5/16/97	19:39	5/17/97	7:17	11:38	no target sp.	NA	NA	NA	NA	NA	
Teach's Hole	35*04'56.5"	75*59'57.9"	7	Gill net 1	5/16/97	21:00	5/17/97	6:45	9:45	CYNNEB	NA	NA	NA	NA	NA	
Lehigh Dredge	35*09'18.7"	76*00'48.1"	9	Gill net 1	5/18/97	18:03	5/18/97	21:15	3:12	no target sp.	NA	NA	NA	NA	NA	
Royal Shoal	35*08'17.2"	76*05'59.2"	7	Gill net 1	5/18/97	19:58	5/19/97	8:30	12:32	CYNREG	9705RS01	365	NR	male	NR	
Royal Shoal	35*08'17.2"	76*05'59.2"	7	Gill net 1	5/18/97	19:58	5/19/97	8:30	12:32	CYNREG	9705RS02	335	NR	female	NR	
Royal Shoal	35*08'17.2"	76*05'59.2"	7	Gill net 1	5/18/97	19:58	5/19/97	8:30	12:32	CYNREG	9705RS03	285	NR	female	NR	
Royal Shoal	35*08'17.2"	76*05'59.2"	7	Gill net 1	5/18/97	19:58	5/19/97	8:30	12:32	CYNREG	9705RS04	188	80	female	NR	
Royal Shoal	35*08'17.2"	76*05'59.2"	7	Gill net 1	5/18/97	19:58	5/19/97	8:30	12:32	CYNREG	9705RS05	178	80	male	NR	
Teach's Hole	35*06'11.9"	75*59'30.3"	10	Gill net 2	5/19/97	18:50	5/20/97	7:10	12:20	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'14.2"	75*59'47.2"	8	Gill net 1	5/19/97	18:42	5/20/97	6:30	11:48	no target sp.	NA	NA	NA	NA	NA	
Teach's Net 3	35*05'14.1"	75*59'46.3"	7	Gill net 1	5/21/97	19:29	5/22/97	7:50	12:21	CYNREG	NR	NR	NR	NR	NR	
Teach's Net 3	35*05'14.1"	75*59'46.3"	7	Gill net 1	5/21/97	19:29	5/22/97	7:50	12:21	CYNREG	NR	NR	NR	NR	NR	
Marker 11 Net	35*08'45.8"	76*00'30.3"	7	Gill net 1	5/21/97	19:13	5/22/97	7:36	12:23	no target sp.	NA	NA	NA	NA	NA	
Hatteras	35*11'49"	76*46'47"	15	Gill net 1	5/22/97	19:30	5/23/97	7:30	12:00	CYNREG	9705HH01	365	755	MALE	NR	
Hatteras	35*11'49"	76*46'47"	15	Gill net 1	5/22/97	19:30	5/23/97	7:30	12:00	CYNREG	9705HH02	345	602	FEMALE	NR	
Hatteras	35*11'49"	76*46'47"	15	Gill net 1	5/22/97	19:30	5/23/97	7:30	12:00	CYNREG	9705HH03	345	620	FEMALE	NR	
Hatteras	35*11'49"	76*46'47"	15	Gill net 1	5/22/97	19:30	5/23/97	7:30	12:00	CYNREG	9705HH04	330	481	MALE	NR	
Marker 13	35*12'14.3"	75*43'51.7"	8	Gill net 1	5/22/97	20:39	5/22/97	21:45	1:06	no target sp.	NA	NA	NA	NA	NA	
Royal Shoal Gap	35*08'14.96"	76*06'00.66"	7	Gill net 1	6/16/97	19:39	6/17/97	8:30	12:51	no target sp.	NA	NA	NA	NA	NA	
Lehigh Dredge	35*09'18.97"	76*00'45.79"	10	Gill net 1	6/16/97	18:00	6/17/97	8:00	14:00	CYNREG	970616MF1	200	0.22	female	NR	
Hatteras Island	35*11'38.47"	75*44'53.85"	12	Gill net 1	6/17/97	18:47	6/18/97	8:15	13:28	CYNREG	970617NH01	320	460	female	NR	
Hatteras Hole	35*12'00.74"	75*46'50.44"	16	Gill net 1	6/17/97	19:00	6/18/97	8:45	13:45	no target sp.	NA	NA	NA	NA	NA	
Wallace channel	NR	NR	NR	Gill net 1	6/18/97	19:45	NR	NR	NR	CYNREG	NA	NA	NA	NA	NA	
Teach's Hole	35*06'10.01"	75*59'24.74"	NR	Gill net 1	6/18/97	18:59	6/19/97	7:00	12:01	no target sp.	NA	NA	NA	NA	NA	
Rose Bay	NR	NR	NR	Gill net 1	6/19/97	19:45	6/20/97	8:30	12:45	no target sp.	NA	NA	NA	NA	NA	
Bay River	35*10'01.15"	76*32'55.94"	12	Gill net 1	6/25/97	17:00	6/26/97	8:15	15:15	no target sp.	NA	NA	NA	NA	NA	
Bay River	35*09'37.24"	76*32'40.81"	6	Gill net 1	6/25/97	17:18	6/26/97	8:30	15:12	CYNNEB	970625BR2	363	707	male	22	3.14
Bay River	35*09'37.24"	76*32'40.81"	6	Gill net 1	6/25/97	17:18	6/26/97	8:30	15:12	CYNNEB	970625BR1	290	420	female	23	5.35

Wallace Channel	35*04'18.14"	76*02'59.75"	12	Gill net 1	7/14/97	18:25	7/15/97	8:15	13:50	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'03.28"	75*59'53.28"	12	Gill net 1	7/14/97	20:49	7/15/97	7:45	10:56	CYNREG	970714M29-1	371	627	female	8.7	1.3
Hatteras Hole	35*12'07.590"	75*46'53.360"	14	Gill net 1	7/15/97	18:15	7/16/97	8:35	14:20	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'38.107"	75*46'53.360"	NR	Gill net 1	7/15/97	20:15	7/16/97	8:10	11:55	no target sp.	NA	NA	NA	NA	NA	
Lehigh dredge	35*09'20.52"	76*00'46.52"	8	Gill net 1	7/16/97	18:22	7/17/97	6:25	12:03	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Cr.	35*27'15.70"	76*24'18.41"	5	Gill net 1	7/17/97	18:27	7/18/97	7:46	13:19	no target sp.	NA	NA	NA	NA	NA	
Deep Bay Cove	35*22'21.76"	76*24'39.96"	4	Gill net 1	7/17/97	19:15	7/18/97	8:25	13:10	no target sp.	NA	NA	NA	NA	NA	
Jones Bay West	35*13'43.59"	76*32'22.63"	7	Gill net 1	7/24/97	19:14	NR	NR	NR	no target sp.	NA	NA	NA	NA	NA	
Jones Bay East	35*13'11.38"	76*30'39.23"	10	Gill net 1	7/24/97	19:40	NR	NR	NR	no target sp.	NA	NA	NA	NA	NA	
Teach's Hole	35*06'02.503"	75*59'25.970"	9	Gill net 1	7/28/97	20:00	7/29/97	9:10	13:10	no target sp.	NA	NA	NA	NA	NA	
Wallace Channel	35*05'24.830"	76*01'10.939"	15	Gill net 1	7/28/97	18:30	7/29/97	8:24	13:54	no target sp.	NA	NA	NA	NA	NA	
Howard's Reef	35*07'44.953"	75*58'41.830"	7	Gill net 1	7/29/97	19:55	NR	NR	NR	no target sp.	NA	NA	NA	NA	NA	
Lehigh Dredge	35*09'13.084"	76*00'51.462"	7	Gill net 1	7/29/97	21:01	7/30/97	15:45	18:44	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Cr.	35*22'31.158"	76*25'18.564"	4	Gill net 1	7/31/97	18:21	8/1/97	8:20	13:59	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Mouth	35*22'32.189"	76*25'16.752"	5	Gill net 1	7/31/97	19:06	8/1/97	9:00	13:54	no target sp.	NA	NA	NA	NA	NA	
Fisherman's Bay	35*09'35.180"	76*32'44.807"	4	Gill net 2	8/6/97	21:00	8/7/97	10:50	13:50	no target sp.	NA	NA	NA	NA	NA	
Jones Bay West	35*13'43.350"	76*32'19.920"	7	Gill net 2	8/6/97	19:03	8/7/97	8:25	13:22	no target sp.	NA	NA	NA	NA	NA	
Jones Bay East	35*13'12.750"	76*30'44.426"	7	Gill net 2	8/6/97	19:51	8/7/97	9:17	13:26	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'57.575"	75*46'47.546"	10	Gill net 2	8/11/97	19:32	8/12/97	9:30	13:58	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'36.218"	76*45'05.266"	10	Gill net 2	8/11/97	20:28	8/12/97	8:26	11:58	no target sp.	NA	NA	NA	NA	NA	
Howard's Reef	35*07'44.745"	75*58'45.016"	NR	Gill net 2	8/12/97	18:06	8/13/97	7:17	13:11	no target sp.	NA	NA	NA	NA	NA	
Lehigh Dredge	35*09'11.416"	76*00'56.85"	9	Gill net 2	8/12/97	19:37	8/13/97	9:00	13:23	CYNREG	1	283	329	female	7.3	2.2
Royal Shoal	35*08'18.57"	76*05'54.953"	9	Gill net 2	8/12/97	20:54	8/13/97	10:00	13:06	no target sp.	NA	NA	NA	NA	NA	
Wallace Channel	35*04'16.309"	76*02'57.029"	20	Gill net 2	8/13/97	18:45	8/14/97	6:30	11:45	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*04'59.591"	75*59'54.459"	5	Gill net 2	8/13/97	20:20	8/14/97	7:07	10:47	no target sp.	NA	NA	NA	NA	NA	
Teach's Hole	35*06'3.835"	75*59'24.471"	5	Gill net 2	8/13/97	21:11	8/14/97	7:45	10:34	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Creek	35*27'21.200"	76*24'13.254"	3	Gill net 2	8/14/97	18:59	8/15/97	8:28	13:29	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Mouth	35*42'58.334"	76*25'22.049"	12	Gill net 2	8/14/97	20:02	NR	NR	NR	CYNNEB	NA	NA	NA	NA	NA	
Rose bay Mouth	35*22'27.217"	76*25'17.333"	5	Gill net 2	8/11/97	20:14	NR	NR	NR	no target sp.	NA	NA	NA	NA	NA	
Jones Bay West	35*13'40.067"	76*32'17.071"	10	Gill net 2	8/19/97	19:19	8/20/97	9:00	13:41	no target sp.	NA	NA	NA	NA	NA	
Jones Bay East	35*13'12.407"	76*30'45.689"	8	Gill net 2	8/19/97	19:53	8/20/97	10:00	14:07	no target sp.	NA	NA	NA	NA	NA	
Fisherman's Bay	35*09'36.645"	76*32'52.332"	5	Gill net 2	8/19/97	22:15	8/20/97	11:55	13:40	CYNREG	NA	240	NA	NA	NA	
Hatteras Hole	35*11'48.250"	75*46'44.160"	14	Gill net 2	8/25/97	18:41	8/26/97	8:40	13:59	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'35.918"	75*45'02.771"	10	Gill net 2	8/25/97	19:58	8/26/97	7:35	11:37	CYNREG	HN970825CR1	330	490	male	2.5	0.5
Teach's Hole	35*06'04.563"	75*59'27.021"	8	Gill net 2	8/26/97	17:29	8/27/97	7:15	13:46	no target sp.	NA	NA	NA	NA	NA	
Wallace Channel	35*04'15.214"	75*02'56.005"	4	Gill net 2	8/26/97	18:17	8/27/97	8:00	13:43	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'06.266"	75*59'51.026"	10	Gill net 2	8/27/97	17:40	8/28/97	7:20	13:40	no target sp.	NA	NA	NA	NA	NA	

Howard's Reef	35*07'45.621"	75*58'42.075"	8	Gill net 2	8/27/97	19:36	8/28/97	7:45	12:09	CYNREG	970827HR1	195	120	female	1	0.8
Rose Bay Creek	35*27'17.387"	76*24'21.671"	4	Gill net 2	8/28/97	18:25	8/29/97	8:05	13:40	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Mouth	35*22'32.476"	76*25'25.412"	5	Gill net 2	8/28/97	19:33	8/29/97	9:25	13:52	CYNREG	NA	190	NA	NA	NA	
Jones Bay East	35*13'11.230"	76*30'46.712"	8	Gill net 2	8/29/97	18:10	8/30/97	10:00	15:50	no target sp.	NA	NA	NA	NA	NA	
Brant Island Shoal	35*10'41.847"	76*23'39.555"	10	Gill net 2	8/29/97	19:30	8/30/97	8:22	12:52	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'52.570"	75*46'52.406"	14	Gill net 2	9/8/97	18:45	9/9/97	8:41	13:56	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'36.608"	75*45'02.468"	13	Gill net 2	9/8/97	19:40	9/9/97	7:30	11:50	CYNREG	NA	240	NA	NA	NA	
Lehigh Dredge	35*09'12.259"	76*00'50.922"	10	Gill net 2	9/9/97	18:00	9/10/97	7:38	13:38	no target sp.	NA	NA	NA	NA	NA	
Royal Shoal	35*08'43.348"	76*04'27.343"	10	Gill net 2	9/9/97	18:48	9/10/97	8:35	13:47	no target sp.	NA	NA	NA	NA	NA	
Royal Shoal	35*08'46.437"	76*04'26.134"	13	Gill net 3	9/9/97	19:21	9/10/97	9:08	13:47	no target sp.	NA	NA	NA	NA	NA	
Teach's Hole	35*06'01.212"	75*59'27.951"	10	Gill net 2	9/10/97	17:27	9/11/97	7:45	14:18	CYNREG	970910TH1	300	395	female	7	1.7
Teach's Hole	35*05'57.107"	75*59'30.163"	13	Gill net 3	9/10/97	19:45	9/11/97	7:45	12:00	no target sp.	NA	NA	NA	NA	NA	
Wallace Channel	35*04'20.202"	76*03'02.923"	8	Gill net 2	9/10/97	18:13	9/11/97	8:30	14:17	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Creek	35*27'18.916"	76*24'20.620"	4	Gill net 2	9/11/97	17:49	9/12/97	8:10	14:21	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Mouth	35*22'31.031"	76*25'15.114"	5	Gill net 2	9/11/97	18:43	9/12/97	8:39	13:56	CYNREG	970911RBM1	255	260	female	2.5	0.9
Rose Bay Mouth	35*22'31.031"	76*25'15.114"	5	Gill net 2	9/11/97	18:43	9/12/97	8:39	13:56	CYNREG	970911RBM2	275	285	female	2.5	0.8
Rose Bay Mouth	35*22'30.106"	76*25'18.254"	5	Gill net 3	9/11/97	18:49	9/12/97	9:15	14:26	no target sp.	NA	NA	NA	NA	NA	
Jones Bay East	35*13'11.073"	76*30'48.607"	8	Gill net 2	9/17/97	18:05	9/18/97	9:00	14:55	CYNREG	970917JBE1	270	NA	female	2	
Brant Island Shoal	35*11'04.740"	76*22'53.000"	9	Gill net 2	9/17/97	19:20	9/18/97	10:27	15:07	SCIOCE	970917BS1	###	####	male	339	1.9
Brant Island Shoal	35*11'00.174"	76*22'49.457"	9	Gill net 3	9/17/97	19:25	9/18/97	10:10	14:45	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'48.679"	75*46'50.901"	10	Gill net 2	9/22/97	18:07	9/23/97	8:08	14:01	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'56.297"	75*46'55.103"	12	Gill net 3	9/22/97	18:14	9/23/97	8:00	13:46	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'36.513"	75*45'00.708"	11	Gill net 2	9/22/97	19:24	9/23/97	7:11	11:47	no target sp.	NA	NA	NA	NA	NA	
Lehigh Dredge	35*09'00.642"	76*01'03.470"	6	Gill net 2	9/23/97	17:52	9/24/97	7:40	13:48	no target sp.	NA	NA	NA	NA	NA	
Lehigh Dredge	35*09'02.800"	76*01'01.055"	6	Gill net 3	9/23/97	18:00	9/24/97	7:45	13:45	no target sp.	NA	NA	NA	NA	NA	
Royal Shoal	35*08'42.467"	76*04'27.372"	13	Gill net 2	9/23/97	18:30	9/24/97	8:45	14:15	no target sp.	NA	NA	NA	NA	NA	
Teach's Hole	35*06'59.957"	75*59'25.945"	6	Gill net 2	9/24/97	17:19	9/25/97	7:40	14:21	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'04.958"	75*59'51.301"	7	Gill net 2	9/24/97	17:49	9/25/97	8:10	14:21	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'02.480"	75*59'52.950"	9	Gill net 2	9/24/97	17:52	9/25/97	8:20	14:28	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Creek	35*27'17.345"	76*24'20.672"	5	Gill net 2	9/25/97	17:52	9/26/97	8:34	14:42	CYNREG	970925RBC1	264	295	female	2	0.6
Rose Bay Creek	35*27'17.345"	76*24'20.672"	5	Gill net 2	9/25/97	17:52	9/26/97	8:34	14:42	CYNREG	970925RBC2	315	525	female	3.2	0.6
Rose Bay Mouth	35*22'22.895"	76*25'19.921"	5	Gill net 2	9/25/97	18:38	9/26/97	9:35	14:57	CYNREG		240	NA			
Rose Bay Mouth	35*22'22.895"	76*25'19.921"	5	Gill net 2	9/25/97	18:38	9/26/97	9:35	14:57	CYNREG	970925RBM1			female	2	
Rose Bay Mouth	35*22'25.175"	76*25'18.217"	5	Gill net 3	9/25/97	18:40	9/26/97	9:41	15:01	no target sp.	NA	NA	NA	NA	NA	
Bay River Mouth	35*10'13.977"	76*30'23.848"	12	Gill net 2	9/26/97	17:57	9/27/97	9:00	15:03	no target sp.	NA	NA	NA	NA	NA	
Bay River Mouth	35*10'14.595"	76*30'27.100"	12	Gill net 3	9/26/97	18:02	9/27/97	8:50	14:48	no target sp.	NA	NA	NA	NA	NA	
Brant Island Shoal	35*10'59.817"	76*22'48.585"	9	Gill net 2	9/26/97	18:59	10/3/97	14:00	163:01:00	CYNREG	970926BS1	220	175	female	1	0.5

Brant Island Shoal	35*10'59.817"	76*22'48.585"	9	Gill net 2	9/26/97	18:59	10/3/97	14:00	163:01:00	CYNREG	970926BS2	270	330	female	NA	
Brant Island Shoal	35*10'59.817"	76*22'48.585"	9	Gill net 2	9/26/97	18:59	10/3/97	14:00	163:01:00	CYNREG	970926BS3	290	NA	female	NA	
Lehigh Dredge	35*09'06.492"	76*01'03.125"	7	Gill net 2	10/5/97	17:41	10/6/97	7:40	13:59	CYNREG	971006LD1	380	810	female	8	0.99
Royal Shoal	35*08'40.980"	76*04'27.372"	12	Gill net 2	10/6/97	18:21	10/7/97	8:20	13:59	CYNREG	971006RS1	239	191	immature	0.9	0.47
Royal Shoal	35*08'42.068"	76*04'31.997"	12	Gill net 3	10/6/97	18:26	10/7/97	8:20	13:54	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*12'00.409"	75*46'57.718"	12	Gill net 2	10/7/97	17:25	10/8/97	8:15	14:50	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'56.524"	75*46'55.654"	12	Gill net 3	10/7/97	17:29	10/8/97	8:10	14:41	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'35.687"	75*45'05.461"	7	Gill net 2	10/7/97	19:04	10/8/97	7:20	12:16	CYNREG	971007NH1	348	666	female	4.5	0.68
Marker 29	35*05'01.996"	75*59'53.445"	7	Gill net 2	10/8/97	17:42	10/9/97	7:47	14:05	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'59.185"	75*59'55.370"	7	Gill net 3	10/8/97	17:45	10/9/97	7:58	14:13	no target sp.	NA	NA	NA	NA	NA	
Wallace Channel	35*04'21.357"	76*03'04.312"	8	Gill net 2	10/8/97	18:14	10/9/97	8:36	14:22	Cynoscion sp.	NA	NA	NA	NA	NA	
Bay River Mouth	35*10'15.226"	76*30'22.596"	11	Gill net 2	10/14/97	17:49	10/15/97	8:26	14:37	no target sp.	NA	NA	NA	NA	NA	
Bay River Mouth	35*10'15.885"	76*30'20.186"	10	Gill net 3	10/14/97	17:57	10/15/97	8:10	14:13	no target sp.	NA	NA	NA	NA	NA	
Fisherman's Bay	35*08'28.880"	76*31'31.555"	7	Gill net 2	10/14/97	18:42	10/15/97	8:55	14:13	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'56.074"	75*46'55.949"	10	Gill net 2	10/20/97	17:23	10/21/97	8:20	14:57	no target sp.	NA	NA	NA	NA	NA	
Hatteras Hole	35*11'51.958"	75*46'53.498"	10	Gill net 3	10/20/97	17:29	10/21/97	8:25	14:56	no target sp.	NA	NA	NA	NA	NA	
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH01	415		immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH02	385	955	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH03	360		immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH04	355	780	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH05	360	885	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH06	310	555	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH07	360		immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH08	340	645	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH09	345	760	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH10	355	690	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	SCIOCE	971020NH11	330	590	immature		
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	CYNNEB	971020NH12	435	1300	female	8.8	0.67
North Hatteras	35*11'36.309"	75*45'03.057"	7	Gill net 2	10/20/97	18:52	10/21/97	7:15	12:23	CYNREG	971020NH13	360	600	female	5	0.83
Wallace Channel	35*04'23.263"	76*03'07.794"	7	Gill net 2	10/21/97	17:55	10/22/97	8:12	14:17	CYNREG	971021WC1	360	770	female	6	0.78
Wallace Channel	35*04'22.822"	76*03'05.959"	7	Gill net 3	10/21/97	18:00	10/22/97	8:17	14:17	no target sp.	NA	NA	NA	NA	NA	
Teach's Hole	35*05"53.319"	75*59'28.534"	7	Gill net 2	10/21/97	17:13	10/22/97	9:20	16:07	no target sp.	NA	NA	NA	NA	NA	
Marker 29	35*05'03.939"	75*59'52.274"	7	Gill net 2	10/22/97	17:12	10/23/97	8:15	15:03	no target sp.	NA	NA	NA	NA	NA	
Howard's Reef	35*07'40.136"	75*58'51.512"	10	Gill net 2	10/22/97	17:51	10/23/97	7:44	13:53	no target sp.	NA	NA	NA	NA	NA	
Howard's Reef	35*07'39.505"	75*58'51.161"	10	Gill net 3	10/22/97	17:57	10/23/97	7:49	13:52	no target sp.	NA	NA	NA	NA	NA	
Rose Bay Creek	35*27'19.120"	76*24'19.120"	6	Gill net 2	10/23/97	17:16	10/24/97	8:30	15:14	CYNNEB	971023RBC1	360	780	female	7.5	0.96
Rose Bay Mouth	35*22'39.460"	76*25'09.993"	8	Gill net 2	10/23/97	18:20	10/24/97	9:15	14:55	CYNREG	971023RBM1	210	140	female	1	0.71

Rose Bay Mouth	35*22'40.514"	76*25'09.800"	8	Gill net 3	10/23/97	18:23	10/24/97	9:10	14:47	no target sp.	NA	NA	NA	NA	NA	
Jones Bay East	35*13'11.500"	76*30'47.494"	7	Gill net 2	10/29/97	14:57	10/30/97	8:18	17:21	CYNREG	971029JBE1	345	560	female	5	0.8
Jones Bay East	35*13'11.500"	76*30'47.494"	7	Gill net 2	10/29/97	14:57	10/30/97	8:18	17:21	CYNREG	971029JBE2	320	460	female	3.5	0.7
Bay River Mouth	35*10'17.825"	76*30'22.712"	6	Gill net 2	10/29/97	16:38	10/30/97	9:35	16:57	no target sp.	NA	NA	NA	NA	NA	
Bay River Mouth	35*10'18.219"	76*30'24.447"	6	Gill net 3	10/29/97	16:42	10/30/97	9:47	17:05	no target sp.	NA	NA	NA	NA	NA	

Appendix III - A Primer on Acoustical Analysis of Fish Sounds

Recording Fish Sounds

The fish sounds analyzed in this report were recorded in two ways. Digital recordings of fish sounds were made using Sony TCD-D8 Digital Audio Tape (DAT) recorders, and analog recordings of fish sounds were made using sonobuoys (see the sonobuoy description) with analog cassette recorders in them.

Recording Equipment

Digital recordings were made using an InterOcean Model 902 Acoustic Listening and Calibration System, (frequency range: 20 Hz to 10,000 Hz; sensitivity: 100 dB re 1 μPa RMS pressure), which consisted of an InterOcean Model T-902 hydrophone (omnidirectional with sensitivity –195 dB Nominal re 1 V/μPa) connected to an amplifier (gain adjustable from 15 dB to 95 dB in 10 dB increments plus vernier adjustment) with a rectifier-type AC meter (peak deflection within 3 dB of continuous signal for 100 ms pulse) calibrated in dB connected to the amplifier output. The hydrophone was placed at 1-2 m depth below the water surface. The sound pressure levels, both during background sound measurements during the day and during periods of fish sound production at night, were measured over the entire frequency range. The acoustical data were recorded with a portable battery-operated digital audio tape (DAT) cassette recorder (Sony TCD-D8 recorder, frequency range: 20 Hz-22,000 Hz ± 1 dB).

Aliasing

Many problems can occur when working with digitally sampled data. One of these involves the sampling rate used by the digital sampling device (DAT recorder). If the data are not sampled at a high enough frequency, the sampled waveforms obtained may be misleading. The following example will help illustrate this potential problem.

Frequency spectra of experimentally measured signals are computed by sampling the signal at discrete points and performing an FFT using a computer or a frequency analyzer. The sampled signal is an approximation to the actual signal and it can have unwanted artifacts due to *aliasing* and the *windowing* function used to obtain the data.

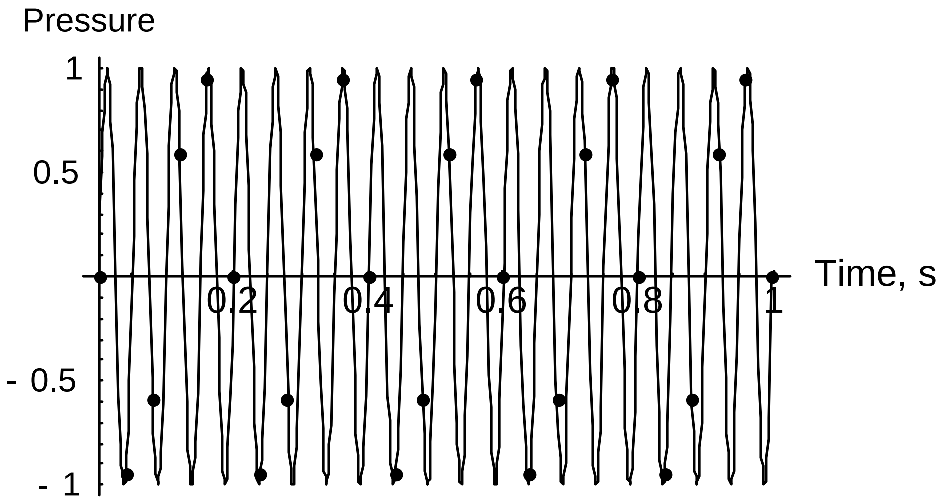


Figure 75. Discrete sampling of a signal. The dots are the sample points.

Aliasing is a result of discretely sampling a signal at a rate that is too low to give the necessary resolution. Figure 75 is an example of a 20 Hz signal that is sampled at a rate of 25 Hz (25 samples per second). Figure 76 shows the sampled signal that appears to be a 5 Hz signal, and a discrete Fourier transform taken at this sampling rate would give a peak at 5 Hz. This is an example of aliasing. The Nyquist rate is the minimum sampling rate that will not alias the frequency information. The Nyquist rate is twice the highest frequency in the signal. Thus, if a fish is drumming at 100 Hz, a minimum digital sampling rate would be 200 Hz (the Nyquist rate) in order to detect the 100 Hz wave without aliasing.

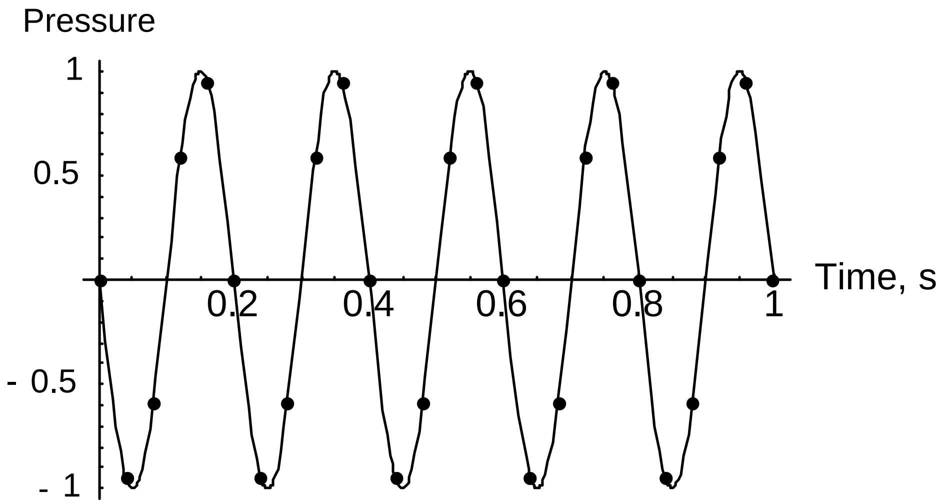


Figure 76: A signal constructed from the sampled data.

We recorded the fish sounds presented in this report on a Digital Audio Tape recorder (Sony TCD-D8) sampling at 48 kHz, far above the Nyquist frequency for fish sounds. To reduce storage space and computation speed, the recorded sounds were re-sampled at 24 kHz using a National Instruments NB2100 A/D board with anti-aliasing filters. We did all spectral analysis with the 24 kHz-sampled signal with no further re-sampling yielding information for frequencies 12 kHz and below.

Spectral Analysis of Fish Sounds

Spectral analysis of a signal provides information about the frequencies present in the signal. This information is important for identifying the species present in a recording. We analyzed the sounds presented in this report using *Labview* 5.0 (National Instruments Corp., 6504 Bridge Point Pkwy., Austin, TX 78730). *Labview* is a programming environment for data acquisition and analysis in which the user can create custom algorithms called virtual instruments (VI's). Digitally recorded fish sounds were first stored on a computer, and spectral analysis consisted of computing power spectra and spectrographs using VI's of our own creation.

The algorithm for producing a spectrograph is as follows.

1. Read the desired number of samples N from the digitized sound file. (We used N = 1024 points for most sounds.)
2. Multiply the samples by the appropriate window function. (We used a Hanning window.)
3. Use an FFT algorithm to compute a Fourier transform of the samples.
4. Compute a power spectrum using the FFT output.
5. Move through the sound file by the slide factor s_f and repeat the procedure. (We used the smallest power-of-2 slide factor allowed by the memory constraints of our computer. s_f = 1024 for samples longer than 15 s; s_f = 512 for samples longer than 7.5 s; s_f = 256 for samples longer than 3.75 s; etc.)

We also produced average power spectra over interesting intervals in the sampled sounds.

The Fourier Transform

The Fourier transform is a mathematical operation that separates a time waveform (*i.e.*, a recorded fish sound) into its frequency spectrum, a representation of the frequencies present in the waveform. It is a useful tool for signal analysis and identification because complex waveforms are easier to characterize by their frequency spectra. In the following section, a simple example will be given to show how a complex signal (Figure 77), similar to that produced by sciaenid fishes, may be decomposed into its frequency spectra using the Fourier transform.

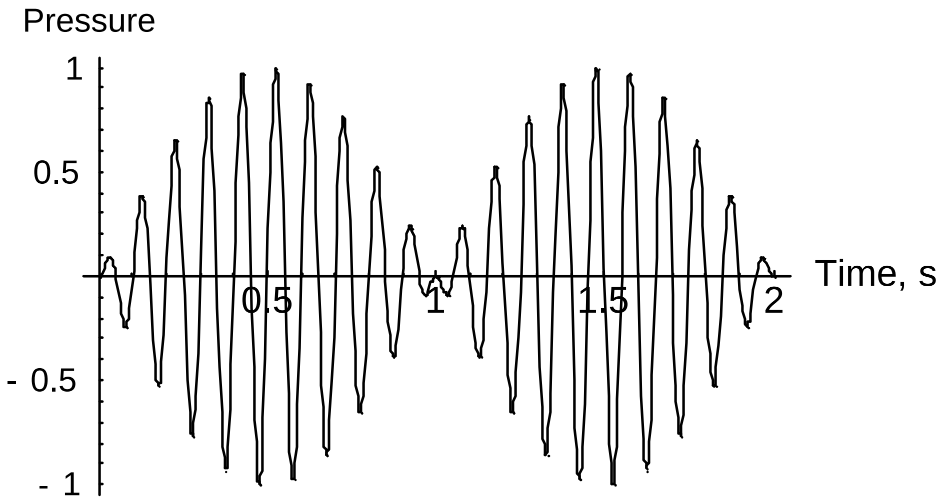


Figure 77. A 10 Hz sine wave with a 0.5 Hz envelope. This is a hypothetical time signal similar to that produced by sciaenid fishes.

Complex signals consist of many different frequency components, which are separated from the time signal with a Fourier transform (FT).

$$\text{FT\{time signal\}} = \text{frequency spectrum} \tag{1}$$

Mathematically, the Fourier transform is defined as

$$S(f) = \int_{-\infty}^{\infty} s(f) e^{-i2pft} dt\,, \tag{2}$$

where *t* is time; *f* is frequency; *s*(*t*) is the time waveform; and *S*(*f*) is the frequency spectrum. The frequency spectrum contains all of the information that was in the time signal, and the original time signal can be reconstructed using an inverse Fourier transform (IFT).

$$\text{IFT\{frequency spectrum\}} = \text{time signal} \tag{3}$$

Mathematically, the inverse Fourier transform is

$$s(t) = \int_{-\infty}^{\infty} S(f) e^{i2pft} df \tag{4}$$

One advantage of Fourier analysis is the ability to identify envelope functions. An envelope function is a slowly varying function that modulates the primary function. For example, Figure 4 is a complex wave created by convolving a 10 Hz sine wave with a 0.5 Hz envelope function. A Fourier transform of the signal gives a frequency spectrum with peaks at -10.5, -9.5, +9.5, and +10.5 Hz, indicating a 10 Hz tone with a 0.5 Hz envelope. This transform is shown in Figure 78.

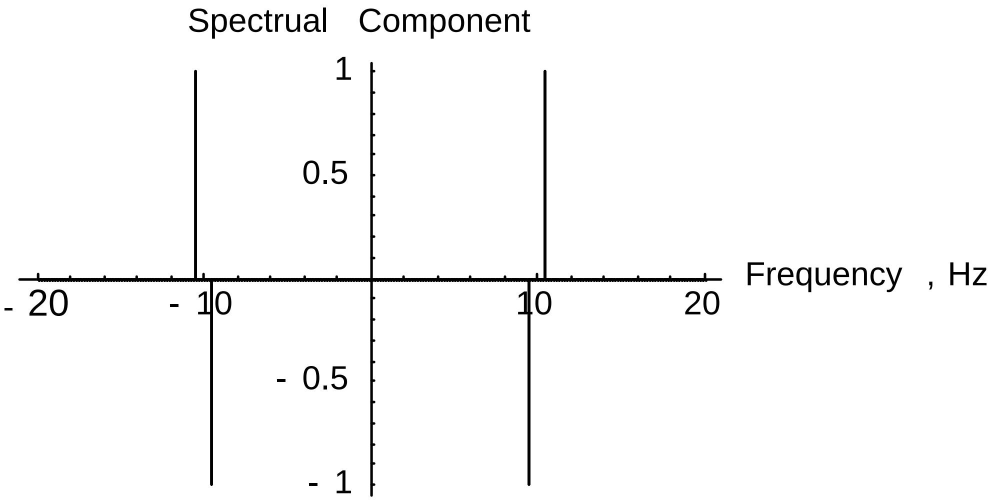


Figure 78. Fourier transform of the signal shown in Figure 77.

Window Functions

When the sample is taken over a finite time period, an artificial envelope pattern is introduced to the frequency spectrum. This envelope results from turning the signal on and off and it causes the frequency components in the spectrum to smear into nearby frequencies as they did for the envelope shown in Figure 77 and Figure 78. By using an appropriate filter function the signal can be turned on and off in such a way that its envelope does not introduce leakage that obscures the frequency spectrum. The appropriate window causes the sample frequency spectrum to accurately represent the signal. Some commonly used window functions include Cesaro, Hanning, Hamming, Gauss, Parzen, and Welch (Walker, 1991). Each of the windows has different envelope characteristics that it introduces to the spectrum. All of the spectra in this report were computed using a Hanning window.

Fast Fourier Transforms (FFT's)

Fourier transforms of numerical data are computed using the Fast Fourier Transform (FFT) algorithm (Walker, 1991). This technique uses bit manipulation to transform a sampled time signal to a frequency spectrum. The FFT algorithm uses fewer steps to perform the transform than the conventional discrete Fourier transform, and the results of the FFT are identical to those of the discrete Fourier transform. Most commercially available signal processing packages use FFT’s to obtain the frequency spectrum of the data.

The FFT algorithm requires that the number of samples of the signal be a power of 2 (i.e., 2, 4, 8, 16, …) and returns the same number of samples of the frequency spectrum. Since the frequency spectrum contains both magnitude and phase information for each frequency component, the number of different frequencies is half of the total number of samples. The frequency resolution, or increment between frequency samples, Δf is given by

$$\Delta f = f_0/N, \tag{5}$$

where f_0 is the sampling rate and N is the number of samples in the FFT. For example, an FFT of 1024 (or 2^{10}) points sampled at 24,000 Hz returns a spectrum containing the magnitude and phase of 512 different frequencies separated by an increment Δf of 23.4375 Hz.

Power Spectra

A power spectrum is used when only the magnitude of the spectrum is necessary. The power spectrum folds the positive and negative frequency components of the Fourier transform together into a function of positive frequencies in which all values are non-negative real numbers representing the power spectral density which is the sound power of a 1 Hz-wide band centered at a given frequency in the signal. Figure 79 shows a power spectrum of the signal from Figure 77. Notice how it compares with the Fourier transform shown in Figure 78.

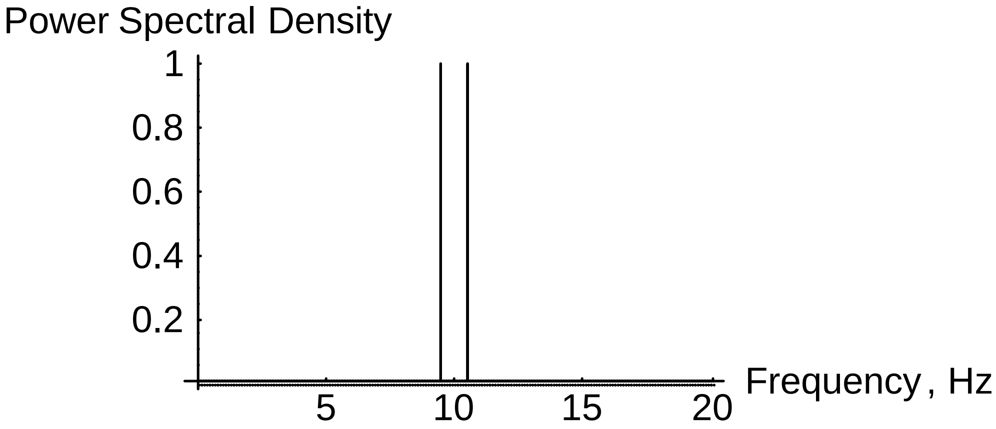


Figure 79. Power Spectrum of the signal shown in Figure 77.

An average power spectrum is the average of each frequency component of several consecutive power spectra in a sample. If the power spectra are given in logarithmic units (i.e. decibels) each frequency component must be converted to linear units before computing the mean. (See the section on decibels below.)

Spectrographs

A spectrograph (often called a spectrogram or sonogram) shows the time variation of the frequency content of the signal with a three dimensional plot of consecutive windowed power spectra. In most spectrographs the horizontal (*x*) axis is time, the vertical (*y*) axis frequency, and the color (*z*) axis power spectral density. Thus, a vertical slice of a spectrograph is a power spectrum.

Spectrographs calculated from sampled data are usually computed using FFT's and are subject to the same frequency resolution as the FFT. The time resolution in a spectrograph is determined by the time required to calculate a power spectrum,

$$\Delta t = N / f_0 \,, \tag{6}$$

where N is the number of points in the FFT, and f_0 is the sampling frequency. Since the time and frequency resolutions are reciprocals, increasing the time resolution decreases the frequency resolution and *vice versa*.

One technique for artificially enhancing the time resolution in a spectrograph is to introduce an overlap in the segments used to compute consecutive power spectra. This overlap Ω is determined by the slide factor s_f or the number of samples between the starts of the segments used for consecutive power spectra by

$$\Omega = 1 - s_f / N \,. \tag{7}$$

For example a spectrograph computed with a 1024-point FFT with a slide factor of 256 points would have an overlap of 3/4 because each power spectrum contains 3/4 of the points from the previous power spectrum. Using an overlap in a spectrograph will not decrease the time window required for each power spectrum, but it will increase the number of power spectra in the spectrograph and the similarity of each power to the previous power spectrum thereby causing the spectrograph to appear less "grainy" in the time direction.

Sound Levels

The terms sound level, sound pressure level, and sound intensity level are measured in decibels, and each have specific definitions, but they are often confused and used in the wrong context. Because each of the above parameters is slightly different, but all are reported in the unitless ratio decibel, when reporting sound level data, it must be specified what type of level is being reported in decibels (sound pressure levels or sound intensity levels). In this section, definitions are given for each parameter.

Decibel

A decibel is a convenient unit for expressing the ratio between two signals on a logarithmic scale. The denominator of the ratio is always some kind of reference signal. The power of a signal is the amount of energy per unit time, measured in Watts (W) in the SI system of units. A power ratio is given in decibels, which is the ratio between two signals with powers W_1 and W_2 and is defined by the equation,

$$\text{number of decibels} = 10\log_{10} W_1/W_2 \,. \tag{8}$$

Since the quantities W_1 and W_2 have the same units, the decibel is a unitless ratio. Equation (8) is also valid for expressing the ratio of two intensities (See below). Intensity is defined as the power per unit area (W/m^2) (Beranek, 1988, pp. 18-19).

The power or intensity of a signal is proportional to the square of easily measured quantities such as voltage, current, sound pressure, and sound particle velocity. When ratios of these quantities are expressed in decibels, the ratio is squared so that it varies in the same way as a ratio of signal powers or intensities. A ratio in decibels of these types of quantity is,

$$\text{number of decibels} = 10\log_{10} a_1^{\,2}/a_2^{\,2} = 20\log_{10} a_1/a_2 \,, \tag{9}$$

where a_1 and a_2 are quantities such as voltage, current, sound pressure, or particle velocity, such that the signal power is proportional to the square of the quantity. a_1 and a_2 must be measured in the same units. For sound pressures, the units are Pascals (Pa) (Beranek, 1988, pp. 18-19)

Sound Pressure Level

In acoustics, the term “sound pressure level” refers to the ratio in decibels of a sound pressure measured by a meter with a flat frequency response to a standard reference pressure. This is given by

$$SPL = 20\log_{10} p/p_0 \,, \tag{10}$$

where p is the root mean square (RMS) pressure and p_0 is the standard reference pressure for air or water. When giving sound pressure levels, it is important to specify the standard reference pressure used for the measurement. (Beranek, 1988, pp. 18-19)

RMS pressure is the square root of the mean squared sound pressure, essentially the standard deviation of the sound pressure. Typically, RMS pressures are evaluated on an exponentially weighted time window. The time constant determines the importance of past pressures to the result. A "slow" detector has a time constant of 1000 ms and averages the signal over a long time. A "fast" detector has a time window of 125 ms and only recent values are important (Beranek, 1988, p. 810). The time constant for the meter on the InterOcean Model 902 Acoustic Listening System, which was used to determine the levels of the recorded sounds, is 100 ms.

Traditionally a standard reference pressure of 20 µPa is used for sounds measured in air and 1 µPa is used for sounds measured in water (Pierce, 1989, pp. 60-61). Therefore, a sound measured under water will have a sound pressure level 26 dB higher than a sound of the same pressure level measured in air. For example, a sound pressure level of 145 dB re 1 µPa is the same RMS pressure and a sound pressure level of 119 dB re 20 µPa.

Intensity Level

The term “intensity level” refers to a ratio of the sound intensity to a standard reference intensity, given by

$$SIL = 10\log_{10} I/I_0\,, \tag{11}$$

where I_0 is the standard reference intensity, traditionally 10^{-12} W/m^2. The sound intensity level is not commonly used because sound intensity is a vector quantity with a specific definition (see American National Standards Institute, Standards for Acoustical Terminology: ANSI S1.1-1960 (R1976)). While the sound intensity is well defined and easy to calculate theoretically, it is a difficult quantity to measure, and there is no simple relationship between pressure and intensity for all sound fields. (Pierce, 1989, p. 65)

Spectrum Level

The term "spectrum level" refers to the Fourier transform of the sound pressure level. It is the contribution to the sound pressure level from a single-frequency component of the signal. The spectrum level at a frequency is defined as the effective sound pressure level of a 1-Hz wide band centered at the frequency. The spectrum level is given by

$$SL(f) = 20\log_{10} p(f)/p_0\,, \tag{12}$$

where $p(f)$ is the Fourier-transformed sound pressure at frequency f, and p_0 in either air or water is the standard reference sound pressure. (Beranek, 1988, p. 19)

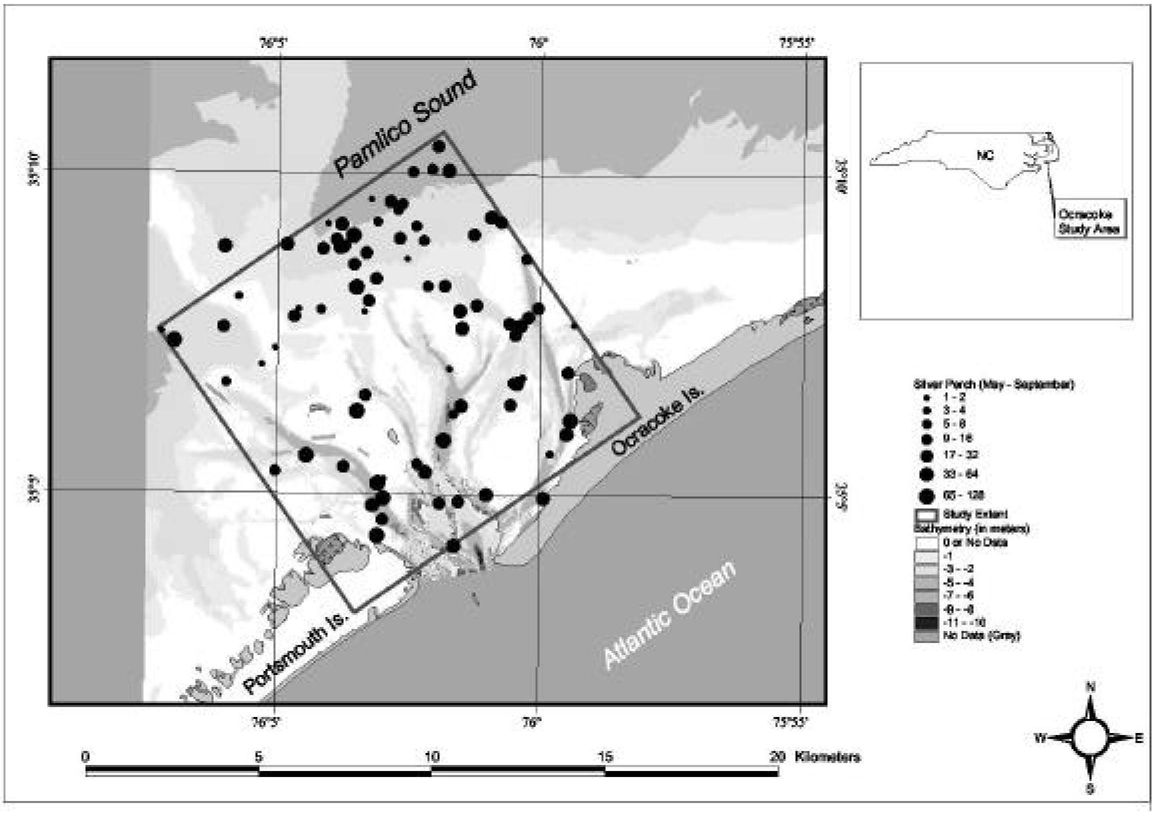
Power Spectral Density

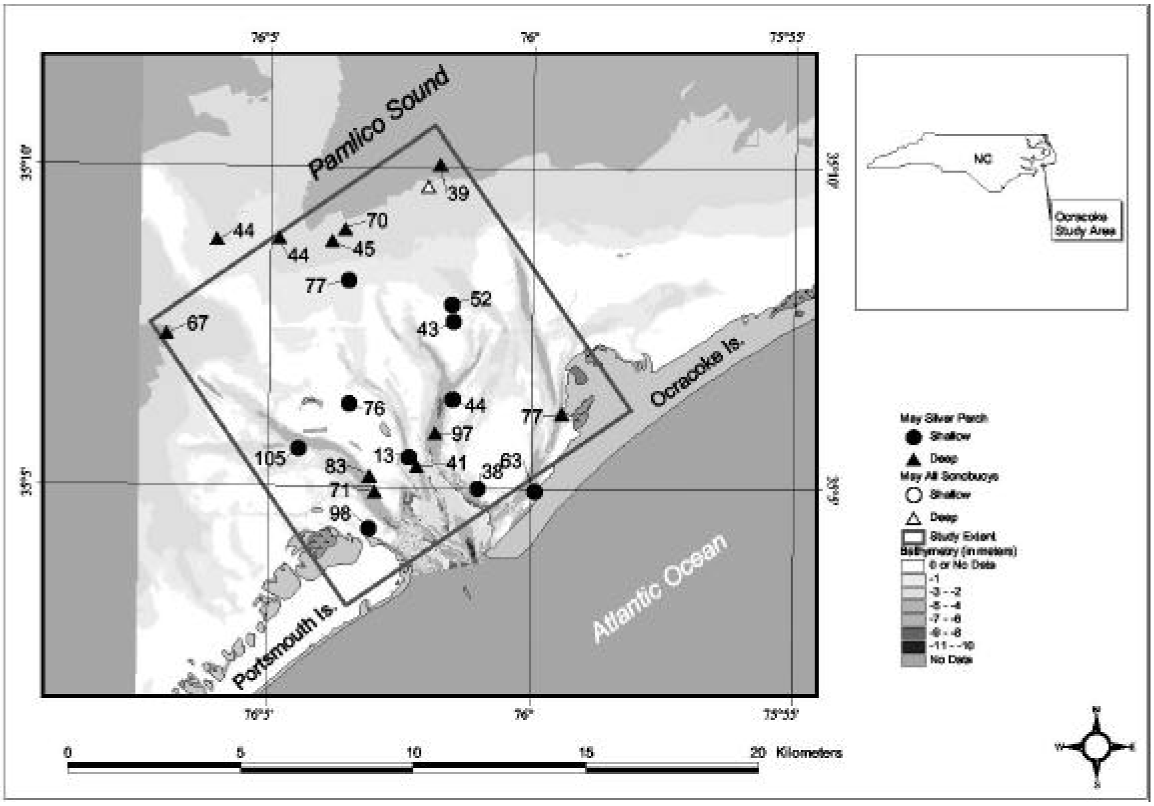
Power spectral density refers to the power spectrum of the sound pressure level. It is the spectrum level calculated from a power spectrum function. When averaging power spectra, it is important to compute the average of each frequency component in linear units. The average power spectrum may then be converted back to decibels.

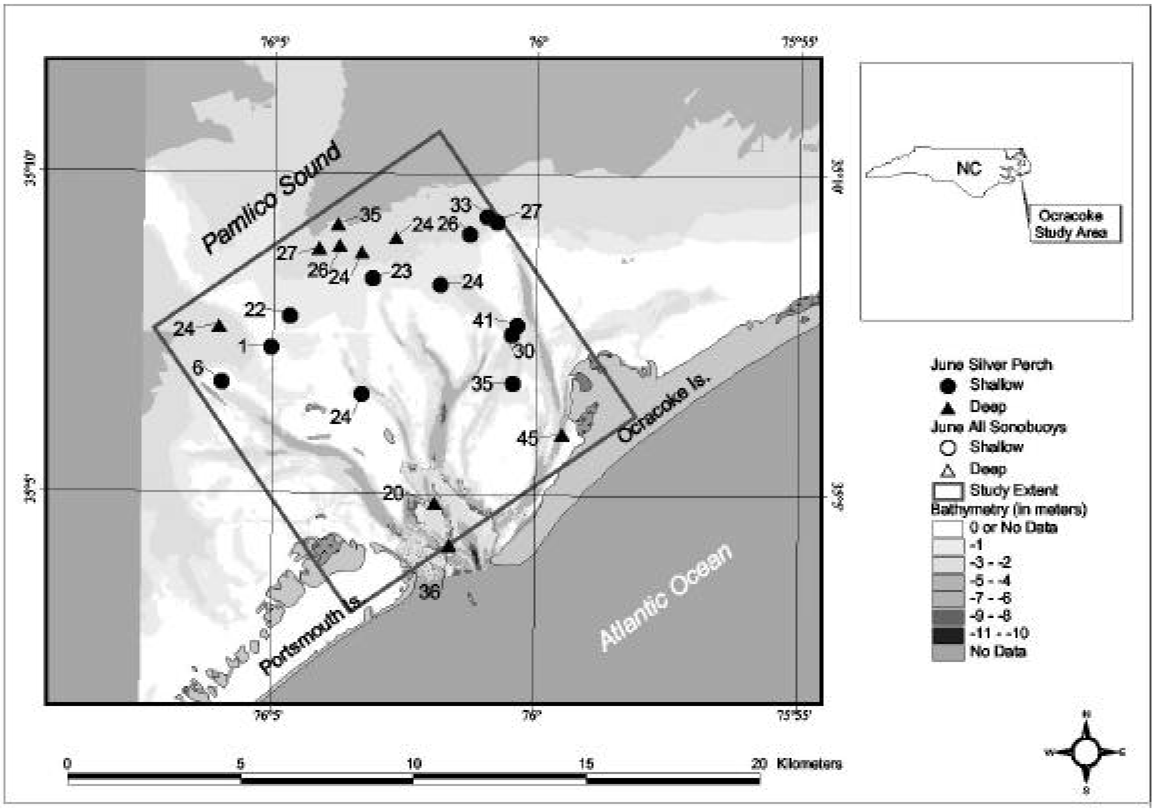
Relative Levels

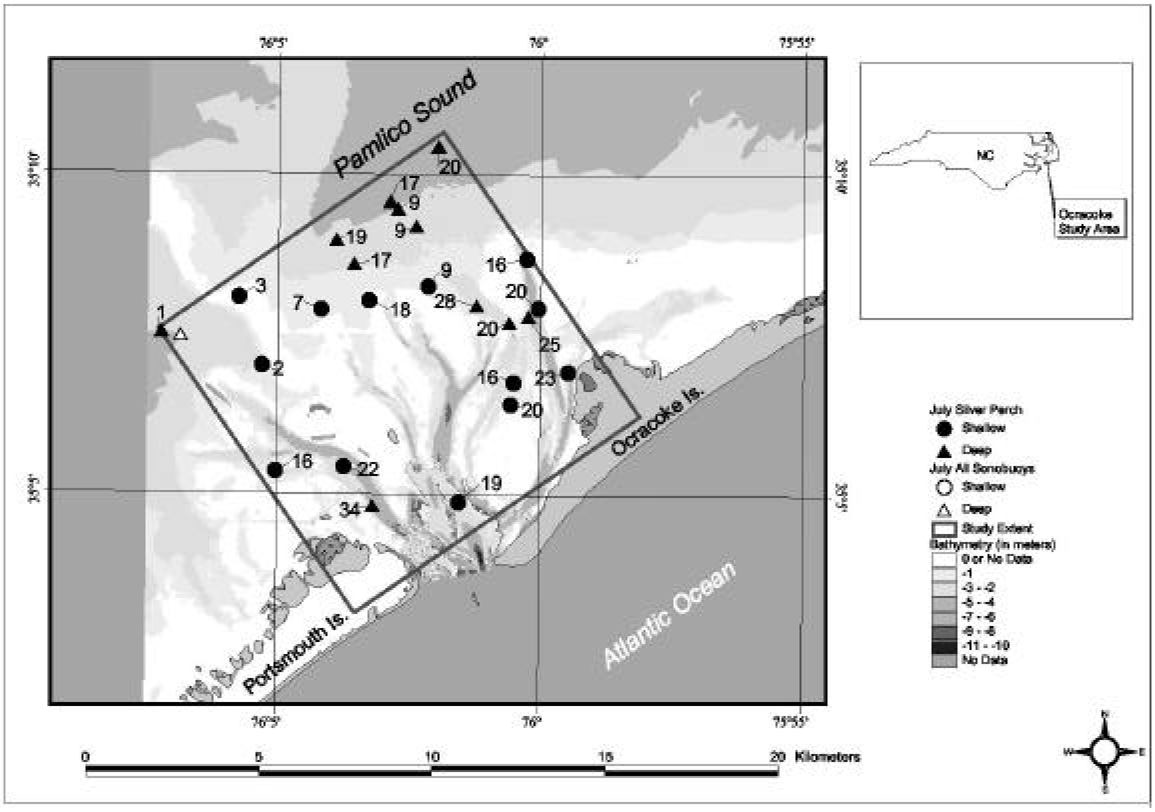
The terms "relative sound pressure level" and "relative spectrum level" refer to levels that are measured with respect to a common, arbitrary reference pressure. Often the relative level is used when reporting the spectra sound producing organisms. When reporting these levels it is important to indicate that they are relative levels.

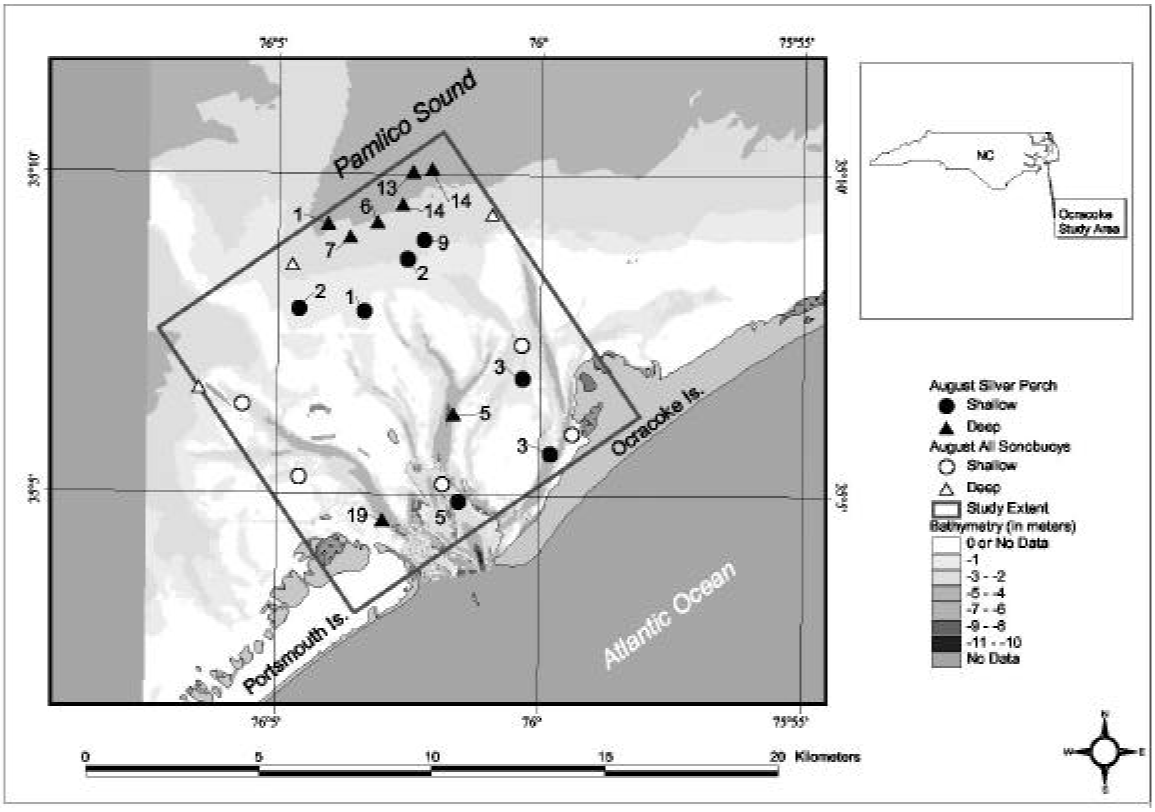
Appendix IV - Silver perch, *Bairdiella chrysoura*, drumming maps in 1997 and 1998 in Pamlico Sound, NC.


76°5'
76°
75°55'
35°10'
35°5'
Pamlico Sound
Ocracoke Is.
Portsmouth Is.
Atlantic Ocean
NC
Ocracoke Study Area
Silver Perch (May - September)
1 - 2
3 - 4
5 - 8
9 - 16
17 - 32
33 - 64
65 - 128
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data (Grey)
0
5
10
15
20 Kilometers
N
W
E
S

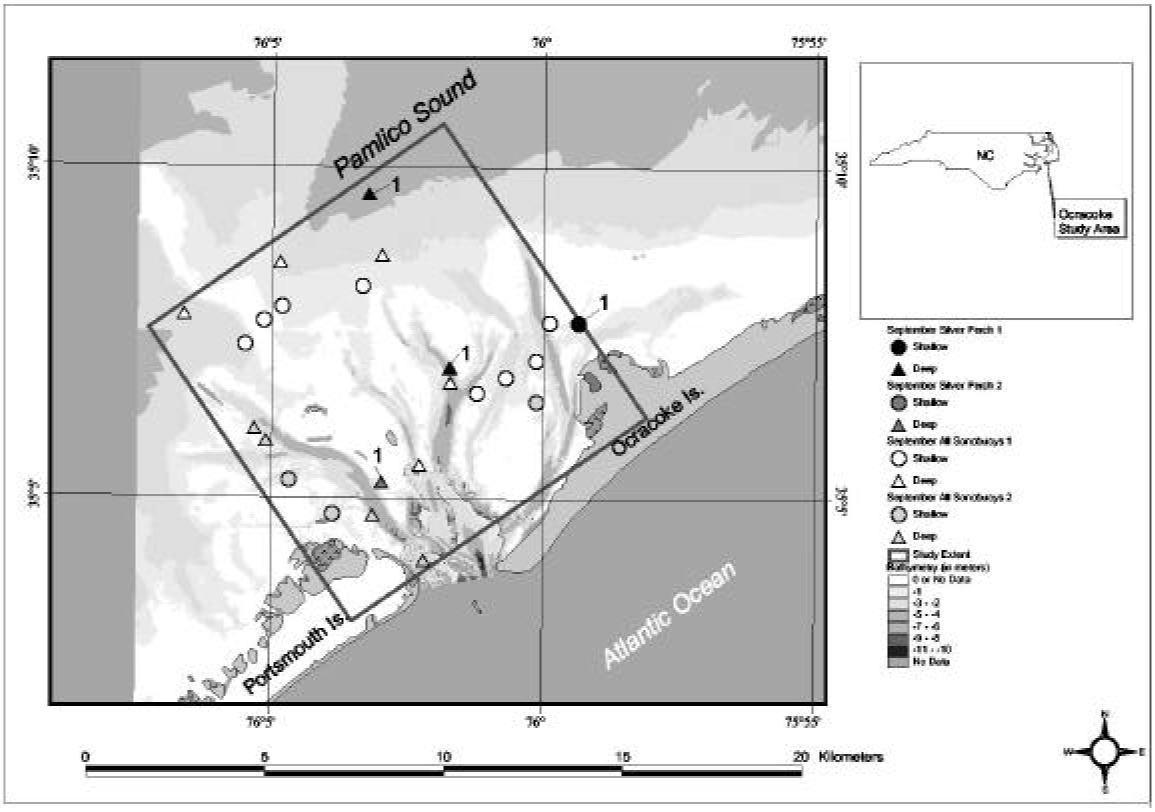

76°5'
76°
75°55'
35°10'
35°5'
Pamlico Sound
Ocracoke Is.
Portsmouth Is.
Atlantic Ocean
39
44
44
70
45
77
52
43
67
76
44
77
97
105
13
41
83
63
38
71
98
NC
Ocracoke Study Area
May Silver Perch
Shallow
Deep
May All Sonobuoys
Shallow
Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
0
5
10
15
20 Kilometers
N
W
E
S


76°5'
76°
75°55'
35°10'
35°5'
Pamlico Sound
35
24
26
33
27
27
26
24
23
24
22
24
1
41
30
6
35
24
45
20
36
Ocracoke Is.
Portsmouth Is.
Atlantic Ocean
NC
Ocracoke Study Area
June Silver Perch
● Shallow
▲ Deep
June All Sonobuoys
○ Shallow
△ Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
0
5
10
15
20
Kilometers
N
W
E
S

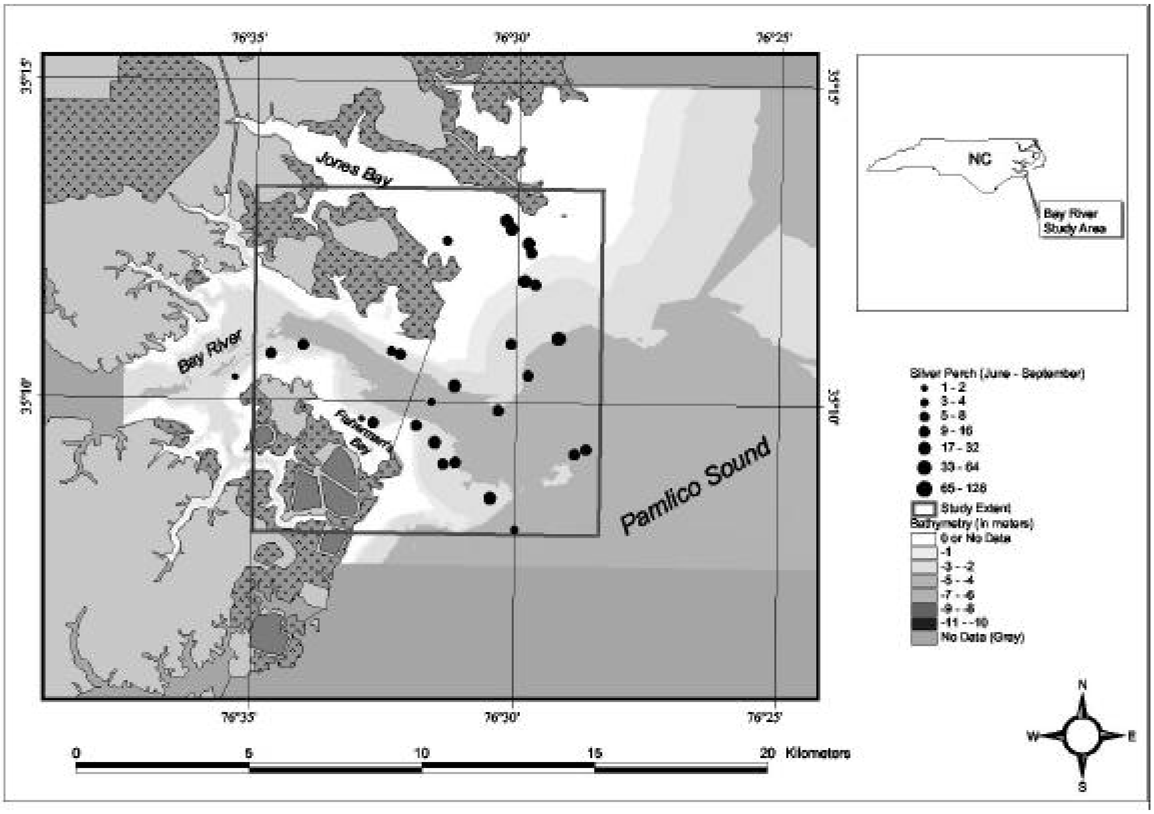

76°5'
76°
75°55'
35°10'
35°5'
Pamlico Sound
20
17
9
9
19
17
16
3
9
1
7
18
28
20
20
25
2
16
23
20
16
22
19
34
Ocracoke Is.
Portsmouth Is.
Atlantic Ocean
NC
Ocracoke Study Area
July Silver Perch
● Shallow
▲ Deep
July All Sonobuoys
○ Shallow
△ Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
0
5
10
15
20
Kilometers
N
W
E
S

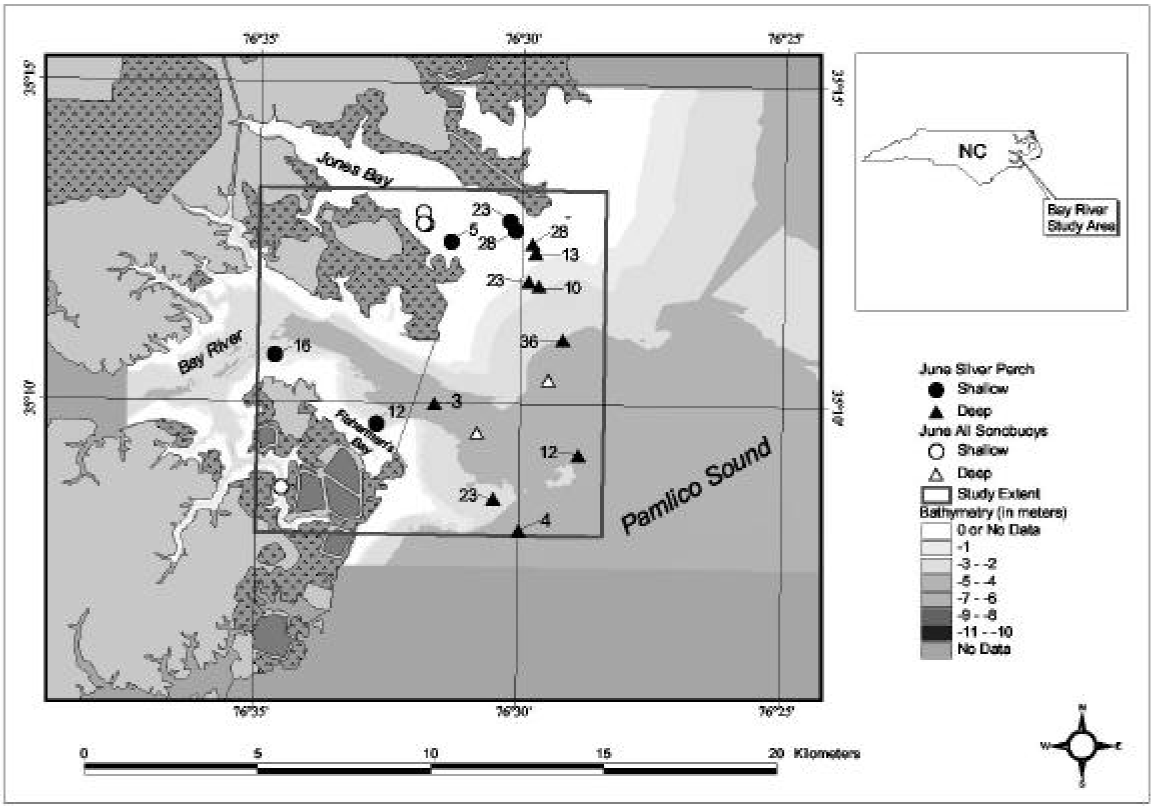


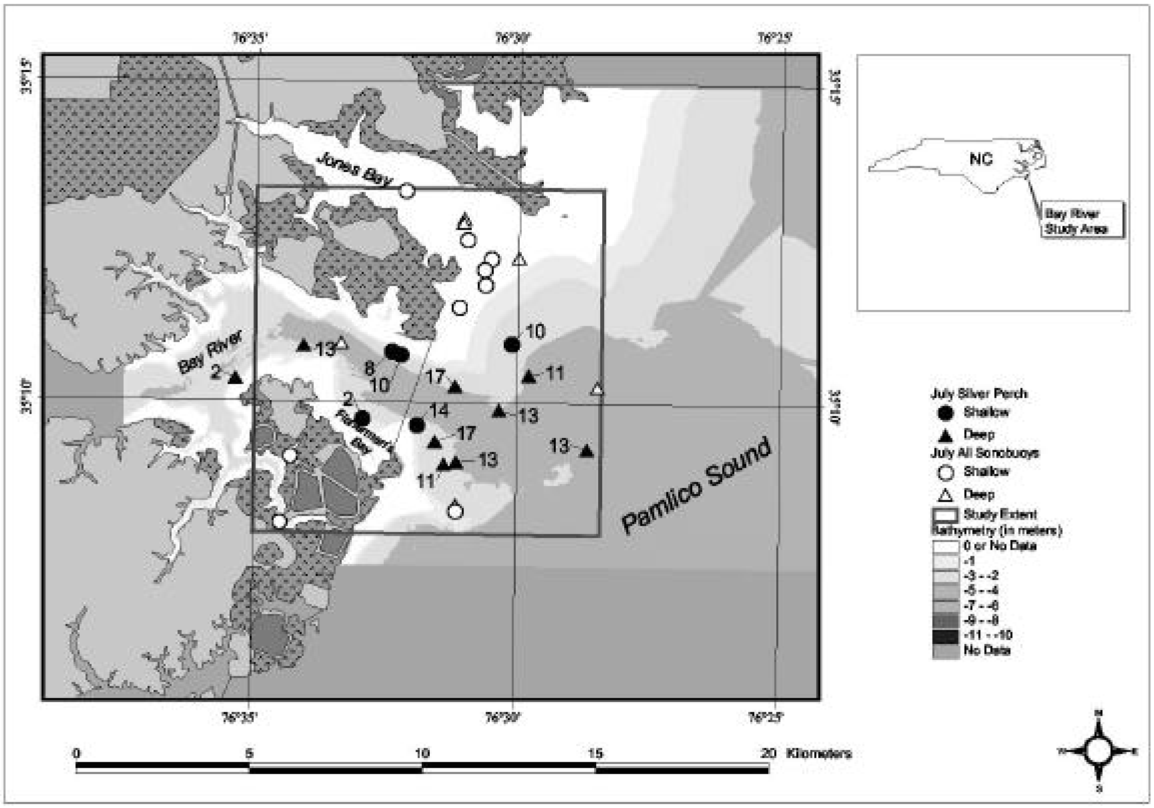
76°5'
76°
75°55'
35°10'
35°5'
Pamlico Sound
Ocracoke Is.
Portsmouth Is.
Atlantic Ocean
NC
Ocracoke Study Area
13
14
14
1
6
7
9
2
2
1
3
5
3
19
5
August Silver Perch
Shallow
Deep
August All Sonobuoys
Shallow
Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
0
5
10
15
20 Kilometers
N
S
E
W



76°5'
76°
75°55'
35°10'
35°5'
Pamlico Sound
Ocracoke Is.
Portsmouth Is.
Atlantic Ocean
NC
Ocracoke Study Area
1
1
1
1
September Silver Perch 1
Shallow
Deep
September Silver Perch 2
Shallow
Deep
September All Sonobuoys 1
Shallow
Deep
September All Sonobuoys 2
Shallow
Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
0
5
10
15
20 Kilometers
N
S
E
W


76°35'
76°30'
76°25'
35°15'
35°10'
Jones Bay
Bay River
Fisherman's Bay
Pamlico Sound
NC
Bay River Study Area
Silver Perch (June - September)
1 - 2
3 - 4
5 - 8
9 - 16
17 - 32
33 - 64
65 - 128
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data (Grey)
N
W
E
S
0
5
10
15
20 Kilometers


76°35'
76°30'
76°25'
35°15'
35°10'
Jones Bay
Bay River
Fisherman's Bay
Pamlico Sound
23
5
28
28
13
23
10
36
16
12
3
12
23
4
NC
Bay River Study Area
June Silver Perch
Shallow
Deep
June All Sonobuoys
Shallow
Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
N
W
E
S
0
5
10
15
20 Kilometers


76°35'
76°30'
76°25'
35°15'
35°10'
Jones Bay
Bay River
Fisherman's Bay
Pamlico Sound
2
13
8
10
10
17
11
2
14
17
13
13
13
11
NC
Bay River Study Area
July Silver Perch
● Shallow
▲ Deep
July All Sonobuoys
○ Shallow
△ Deep
Study Extent
Bathymetry (in meters)
0 or No Data
-1
-3 - -2
-5 - -4
-7 - -6
-9 - -8
-11 - -10
No Data
0
5
10
15
20
Kilometers
N
S
E
W