Assessing trends in the density of Atlantic croaker (*Micropogonias undulatus*): a comparison of passive acoustic and trawl methods

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Abstract—Using data collected simultaneously from a trawl and a hydrophone, we found that temporal and spatial trends in densities of juvenile Atlantic croaker (*Micropogonias undulatus*) in the Neuse River estuary in North Carolina can be identified by monitoring their sound production. Multivariate analysis of covariance (MANCOVA) revealed that catch per unit of effort (CPUE) of Atlantic croaker had a significant relationship with the dependent variables of sound level and peak frequency of Atlantic croaker calls. Tests of between-subject correspondence failed to detect relationships between CPUE and either of the call parameters, but statistical power was low. Williamson's index of spatial overlap indicated that call detection rate (expressed by a 0–3 calling index) was correlated in time and space with Atlantic croaker CPUE. The correspondence between acoustic parameters and trawl catch rates varied by month and by habitat. In general, the calling index had a higher degree of overlap with this species' density than did the received sound level of their calls. Classification and regression tree analysis identified calling index as the strongest correlate of CPUE. Passive acoustics has the potential to be an inexpensive means of identifying spatial and temporal trends in abundance for soniferous fish species.

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Many fish species communicate acoustically, producing sounds under a variety of conditions, such as when engaging in reproductive activities, defending territory, competing for food, or responding to threats (Ladich, 1997, 2004; Myrberg, 1997). Fishery scientists can take advantage of sounds produced by fishes to study certain aspects of fish biology. Passive acoustic techniques (i.e., the use of hydrophones to receive and record sounds made by fishes) have been used by fishery scientists primarily to characterize temporal and spatial patterns in spawning activity (reviewed by Gannon, 2008). For example, some investigators have coupled passive acoustic surveys with collection of adult fish or newly fertilized oocytes to help confirm the species identifications of the callers and to correlate certain behaviors (i.e., spawning) with specific sound types (e.g., Saucier and Baltz, 1993). However, the objective of many fishery assessments is to obtain some measure of fish abundance. Passive acoustics may provide data that would allow indices of abundance to be calculated. But to our knowledge, passive acoustic methods have not yet been used in this fashion and no studies have formally evaluated their potential for assessing relative abundance.

Atlantic croaker (Sciaenidae: *Micropogonias undulatus*) are an abundant, estuary-dependent sciaenid found in the coastal waters of the mid-Atlantic and southern United States. Off North Carolina, Atlantic croaker spawn in the ocean from September to February, and juveniles are found in mesohaline estuaries throughout summer (see Eby, 2001). Sciaenids, including Atlantic croaker, are well known for their noise-making abilities. Members of the family produce sound by the movement of bilaterally paired muscles that surround the swim bladder, which cause the swim bladder to vibrate (reviewed by Ramcharitar et al., 2006). In Atlantic croaker, both males and females possess well-developed sonic muscles. Their sonic muscles develop when they are approximately 45 mm standard length (long before sexual maturation commences), and their sonic muscles do not undergo atrophy after spawning to the extent seen in other sciaenids (Hill et al., 1987; Vance et al., 2002). Atlantic croaker produce three types of acoustic calls: reproductive calls, disturbance calls, and "knock calls" (Connaughton et al., 2003; Fine et al., 2004; Gannon, 2007). Knock calls consist of 1–6 brief pulses (88–106 msec), or "knocks," with a mode of 2 pulses and repetition rate of 16.1 pulses per second (Gannon, 2007). Knock calls are broadband with dominant frequencies varying inversely with fish size, ranging from approximately 600 to 1,600 Hz (Gannon, 2007). Juvenile and mature Atlantic croaker are known to produce knock calls in the estuarine waters of North Carolina from June to November (Gannon, 2007). Knock calling occurs throughout the day and night, but production of knock calls by Atlantic croaker stocked in a research pond peaked at night at an average rate of 1.0 calls per fish per minute (Gannon, 2007). Their extensive calls make Atlantic croaker ideal for a study by passive acoustic means.

We conducted trawl surveys concurrently with passive acoustic surveys for young-of-the-year Atlantic croaker in the Neuse River estuary in North Carolina to assess the utility of passive acoustics to quantify temporal and spatial trends in the density, habitat selection, and associations with environmental variables of this species. We used multivariate analysis of covariance (MANCOVA) to investigate the relationships among call parameters, fish density, and environmental variables; Williamson's index to measure the degree of spatial overlap between Atlantic croaker catches and the occurrence of their calls; and classification and regression tree analysis (CART) to identify the best set of acoustic and environmental variables for predicting Atlantic croaker distribution.

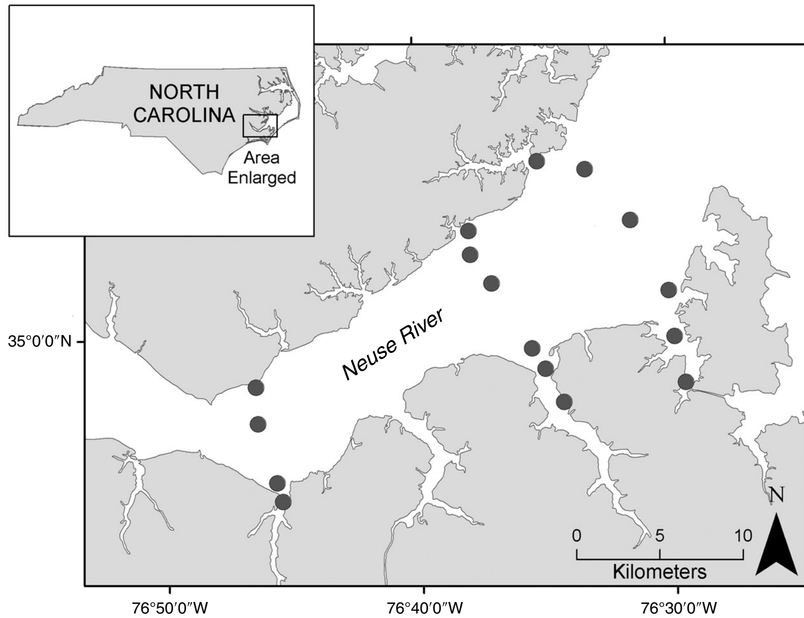

Figure 1

Neuse River study area showing stations (●) where concurrent otter trawl and passive acoustic sampling for Atlantic croaker (*Micropogonias undulatus*) took place from June to October of 2000.

Materials and methods

Field sampling

From June through October of 2000, we performed paired trawl and passive acoustic surveys from a 7-m outboard-powered vessel. Estimates of Atlantic croaker density derived from the trawl data were used as a benchmark against which acoustically derived estimates could be compared. The study area was a 300-km^2 region of the Neuse River estuary, centered at 35.0°N, 76.6°W. The survey design consisted of 16 sampling stations arranged along three transects (Fig. 1). In an effort to sample the full range of depths and habitat types within the study area, we distributed the transects along the length of the estuary, and each transect completely crossed the river.

Because sound propagation is affected by environmental conditions, each sampling station was characterized with regard to its habitat type. The three habitat types considered were 1) the tributary creeks and bays ("creeks"); 2) the main stem of the Neuse River >3.5 m in depth ("mid-river"); and 3) the main stem of the Neuse River <3.5 m in depth ("river edge"). These categories were used because the Neuse River estuary has relatively low habitat diversity: there is little submerged vegetation; oyster reefs are sparse (Lenihan and Peterson, 1998); and bottom substrate consists of sand, silt, and clay. Therefore, habitat diversity depends primarily on depth and sediment grain size (which is negatively correlated with depth).

Knock calling by Atlantic croaker peaks at night, but the fish produce sound throughout the day (Gannon, 2007). Pilot studies in the Neuse River estuary showed that the diversity of fish species producing calls was highest at night, making it difficult to quantify calling activity of any one species. Therefore, we sampled during daylight hours (from two hours after sunrise to two hours before sunset) to minimize any potential biases associated with diel variation in sound production or in vulnerability to our sampling net. Because few other species in the area call during the day, Atlantic croaker sounds dominated the sound field during the day.

At each sampling station, an acoustic recording was made before each trawl, thus each acoustic recording was paired with a trawl sample. The recording location was at the geographical midpoint of the trawl path so that the acoustic recording and trawling would sample fish from the same geographic area. The recording site was approached on a heading that was perpendicular to the trawl path to minimize any potential disturbance to the fish. Upon arrival at the recording site, the engine of the boat was turned off. Recording commenced approximately two minutes after the boat arrived on station and then a two-minute recording was made. Choice of recording length followed the reasoning and methods of Mok and Gilmore (1983) and Luczkovich et al. (1999). The recording system consisted of an HTI 96 hydrophone (High Tech, Inc., Gulfport, MS), a TCD-D8 digital audio tape (DAT) recorder (Sony Corp., Tokyo, Japan), and a personal computer. The hydrophone was omnidirectional and had a frequency range of 2 Hz to 30 kHz and a sensitivity of –164 dB re 1V/μPa (μPa=micropascal). The DAT recorder operated at a sampling rate of 44.1 kHz, with a frequency range of 20 Hz to 22 kHz (±1 dB). Data from the DAT recorder were downloaded to a personal computer running CoolEdit 2000 (Syntrillium Software, Inc., Scottsdale, AZ). The recording levels for the DAT recorder and computer were standardized for all recordings. The entire recording system (hydrophone, DAT recorder, and computer) was calibrated by recording a series of reference tones and using linear regression to determine how relative root mean squared (RMS) levels related to absolute RMS levels (r^2=0.99; Gannon et al., 2005).

To minimize movement of the hydrophone through the water (thus minimizing hydrodynamic noise), the hydrophone was suspended approximately halfway between the surface and seafloor from a 4-kg mushroom anchor that was suspended 50 cm below a 30-cm diameter buoy by an elastic shock cord. Immediately after making each two-minute recording, we deployed the trawl net. We used a 3.4-meter otter trawl with 3.8-cm mesh and a 3-mm mesh liner in the codend. We trawled in a straight line, parallel to the bottom contour, for four minutes at a speed of 1.3 m/sec, which resulted in trawl distances of approximately 300 m. The locations of each recording site and of the beginning and ending of each trawl were recorded with a Garmin GPS120 and a Garmin GBR21 differential beacon receiver (Garmin, George Town, Cayman Islands). The exact length of each trawl was calculated from the beginning and ending positions of the trawls.

Three hundred meters was chosen as the trawl length based on estimates of the maximum range over which we would likely be able to detect calling Atlantic croaker (~150 m). Maximum detection range depends upon the source level of the sound, the level of background noise at the same frequency, and characteristics of the transmitting medium (Pierce, 1989; Richardson et al., 1995). We lacked information on these parameters; therefore we used the theoretical range at which a signal would be diminished by 30 dB re 1 μPa because of spreading loss as an estimate of the range over which a sound could be detected (Gannon, 2003).

All fish and invertebrates captured were identified, counted, and measured (standard length). Catch per unit of effort (CPUE) was calculated as the number of Atlantic croaker >45 mm standard length that were caught per 100 meters of linear trawling distance. CPUE was calculated for Atlantic croaker >45 mm because this is the size at which they develop sonic muscles and thus become capable of producing sound (Hill et al., 1987; Gannon, 2007). At each sampling site we also recorded temperature, salinity, and dissolved oxygen concentration at 0.2 m above the bottom, using a Minisonde multiprobe and Surveyor 4 data logger (Hydrolab, Hach Environmental, Loveland, TX).

Acoustical analyses

Acoustical analyses were performed with CoolEdit 2000 and Raven 1.1 (Cornell Univ., Ithaca, NY). We discriminated Atlantic croaker sounds from other sounds in our field recordings by the methods outlined in Gannon (2007). Briefly, we compared spectrographs from our field recordings to spectrographs of recordings from Atlantic croaker made in captivity. We also compared our field recordings to published descriptions of calls of all other known soniferous species encountered in the estuary (e.g., Fish and Mowbray, 1970; Mok and Gilmore, 1983; Sprague and Luczkovich, 2001). We excluded recordings containing sounds of boats or rain, those in which the Beaufort sea state was ≥3, and those made when bottlenose dolphins (*Tursiops truncatus*) were present, because these factors may have affected rates of sound production by Atlantic croaker (see Luczkovich et al., 2000) or our ability to detect sounds.

We investigated how trawl CPUE and environmental conditions related to three acoustic parameters: 1) received sound level (in dB re 1 μPa), 2) rate of detection of Atlantic croaker calls by our hydrophone (expressed as a calling index), and 3) peak acoustic frequency of Atlantic croaker calls (the loudest frequency recorded). We hypothesized that received sound level and calling index should be influenced by the number of fish calling. Environmental factors were included in the analysis because of their potential to also affect the acoustic parameters. For example, temperature is known to affect the frequency characteristics of sciaenid calls (Connaughton et al., 2000), and the characteristics of the transmission medium can cause different frequency components of a call to differ in their propagation efficiencies (Richardson et al., 1995, p. 27–30).

The Atlantic croaker sounds that we recorded exhibited energy peaks between 600 and 1200 Hz (Gannon, 2007). Besides Atlantic croaker calls, there were few background sounds in this frequency range. But background noise was common at other frequencies. Therefore, we calculated the received sound level attributable to Atlantic croaker as the level within the 600 to 1200 Hz band, using an FFT (Fast Fourier Transform) size of 2048. The received sound level was calculated over the entire two-minute recording period from each sampling station.

Calling rate reached a saturation point at approximately 100 calls/min, above which the exact number of fish calls per minute could not be determined. Therefore, we used a calling index to quantify the occurrence of Atlantic croaker calls, following Heyer et al. (1994), Luczkovich et al. (1999), and Gannon (2007). The calling index was on a scale from zero to three (0=no calls, 1=1–10 calls/min, 2=10–100 calls/min, and 3=greater than 100 calls/min).

Statistical analyses

Before we performed statistical analyses, we transformed CPUE values by $\ln(x+1)$, which gave a better fit to a normal distribution in a Q-Q plot than did the raw data. To investigate whether acoustic variables were related to local densities of Atlantic croaker or other environmental variables, we performed a MANCOVA with the two continuous acoustic parameters (received sound level and peak frequency) as dependent variables, with habitat and month as factors, and with CPUE, dissolved oxygen, and temperature as covariates. MANCOVA was run in SPSS vers. 16 (SPSS, Inc., Chicago, IL).

We used Williamson's index of spatial overlap (Williamson, 1993) to characterize the extent to which Atlantic croaker calling was spatially correlated with Atlantic croaker density. Williamson's index is customarily used to measure spatial overlap of the distribution of two species, such as that between a predator and its prey (Williamson, 1993; Garrison, 2000; Garrison et al., 2002; Link and Garrison, 2002). Here we used it to compare two independent measures of distribution for the same species. The index (O_{ij}) is calculated as follows:

$$O_{ij} = \frac{\sum_{z=1}^{m}(N_{jz}n_{iz})m}{\sum_{z=1}^{m}(N_{jz})\bullet\sum_{z=1}^{m}(n_{iz})}, \quad (1)$$

where m = the number of samples;
z = a discrete sampling location;
N_j = the relative abundance of Atlantic croaker as determined by trawl CPUE; and
n_i = the relative abundance of Atlantic croaker as determined by passive acoustic methods (either calling index or relative received amplitude of calls).

Index values >1 indicate spatial overlap greater than would be expected by chance, and values <1 reflect less spatial overlap than expected.

We applied Williamson's index to compare 1) CPUE to calling index and 2) CPUE to received sound level. We determined the significance of O_{ij} using a randomization procedure developed by Garrison (2000). The test statistic O_{ij} was compared to a random distribution of overlap values in which each value of N_j was randomly paired with a value for n_i to calculate a randomized O_{ij}. This was done 4999 times, and the observed test statistic O_{ij} (the 5000$^{\text{th}}$ instance) was then compared to the generated distribution. Significance of the value was judged by the proportion of randomized O_{ij} values that were greater than the observed O_{ij} (a value was judged to be significant if fewer than 5% of the randomized values were greater than O_{ij}).

Classification and regression tree (CART) analysis was used to explore relationships between the dependent variable CPUE and eight independent variables (day of year, dissolved oxygen concentration, temperature, salinity, depth, calling index, received sound level, and distance to nearest creek). The CART method tests the global null hypothesis of independence between the dependent variable and each of the independent variables and identifies critical threshold values for the significant independent variables (Urban, 2002). Our study area was spatially structured with regard to habitat type (i.e., the mid-river habitat was a contiguous region at the center of the study area, the creeks were at the outer edges of the study area, and the mid-river habitat was a contiguous ring separating the mid-river from the creeks). Thus, the three habitat types were spatially correlated. To avoid multicollinearity in our analyses, we used a single variable ("distance to creek") to represent the position of each sampling station in relation to each of the three habitats (i.e., any station close to the creeks was necessarily far from the mid-river and vice-versa). ArcGIS vers. 9.2 (Environmental Systems Research Institute, Redlands, CA) was used to calculate the distance from each sampling location to the creek habitat. We ran the CART analysis using the "Party" library in the R software environment (vers. 2.6.1, R Development Core Team; Hothorn et al., 2006). P-values were calculated by using a quadratic test statistic.

Results

From June to October of 2000, we performed 14 simultaneous trawl and passive acoustic surveys, for a total of 224 paired samples. Sixty four samples were excluded from analysis because of a high sea state (Beaufort scale), rain, boat noise, or the presence of dolphins. Noise from rough sea conditions, rain, and passing boats may not have affected the calling behavior of Atlantic croaker, but these noise sources masked the croakers' calls, making it difficult to reliably measure their acoustic characteristics. Recordings made in the presence of bottlenose dolphins appeared to have long periods of silence punctuated by short, sporadic outbursts of croaker calls. Therefore we excluded these samples because there appeared to be a change in Atlantic croaker calling behavior when dolphins were present. Exclusion of these 64 samples left a total sample size of 160; 70 in the tributary creeks, 53 along the river edge, and 37 in middle of the river (rough sea conditions and boat noise were most prevalent in the mid-river habitat). All Atlantic croaker caught in the study were young of the year.

CPUEs were highest in all habitats during June (Fig. 2A) and the general trend was for declining densities throughout the five-month study, likely the result of a large pulse of recruitment in spring, followed by mortality or emigration throughout summer and fall. Atlantic croaker densities were lowest in the mid-river habitat during July and August, coinciding with the occurrence of hypoxic conditions. Atlantic croaker recolonized the mid-river habitat when dissolved oxygen conditions improved in September and October. Standard lengths of croaker increased throughout the period from June to October in all habitats but were consistently lower in the mid-river habitat than in the other habitats (Fig 2B). Calling indices, representing

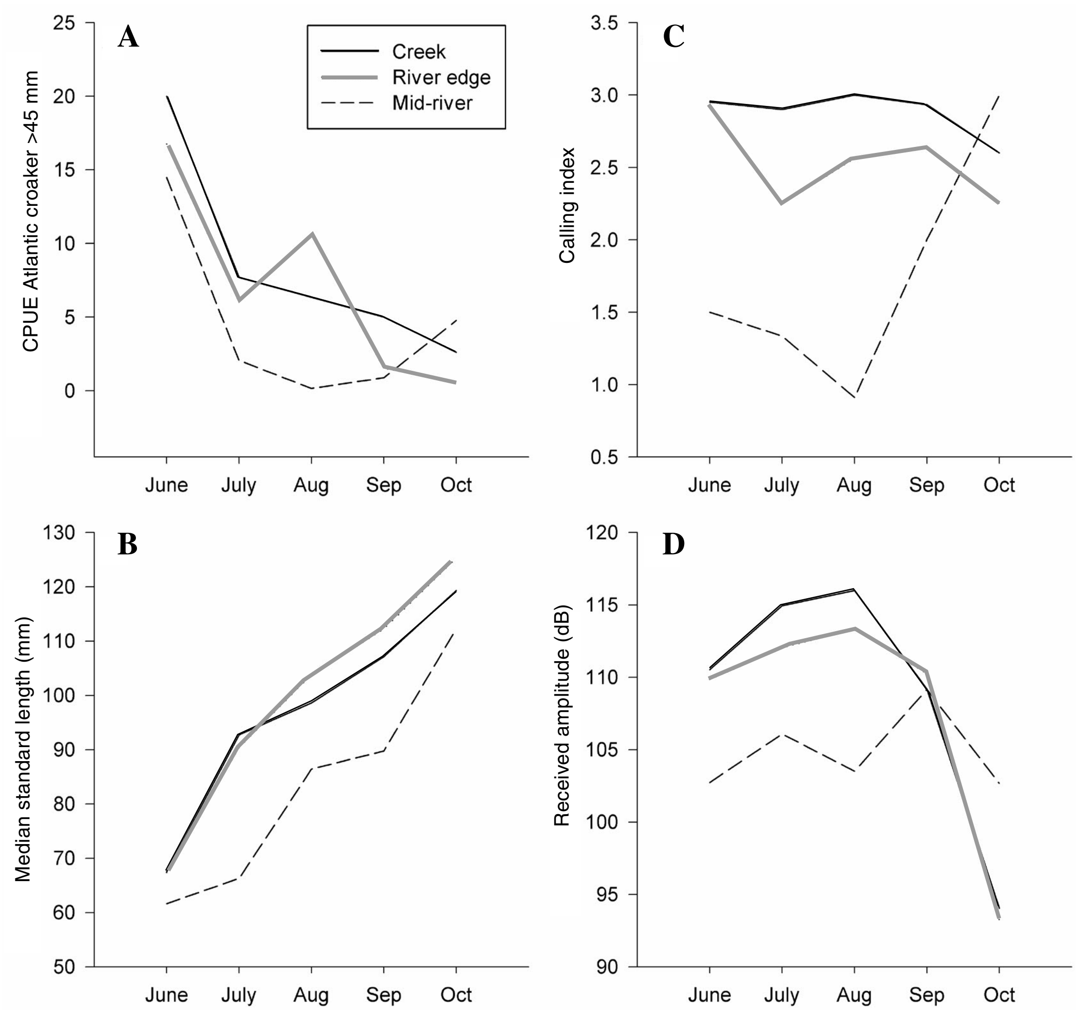

Figure 2

Monthly mean values for the creek, river edge, and mid-river habitats of the Neuse River estuary from June to October of 2000 for (**A**) trawl catch per unit of effort for Atlantic croaker greater than 45 mm standard length, (**B**) median standard length of Atlantic croaker in each trawl, (**C**) Atlantic croaker calling index, (**D**) sound level of Atlantic croaker calls received at the hydrophone, (**E**) peak acoustic frequency of Atlantic croaker calls, (**F**) temperature, (**G**) dissolved oxygen concentration, and (**H**) salinity. Dashed line at 2.3 mg/L in G represents the hypoxia avoidance threshold for Atlantic croaker (Eby and Crowder, 2002).

the rate at which Atlantic croaker knock calls were detected, were high and relatively consistent in the creek and river edge habitats throughout the study period (Fig. 2C). In contrast, the mean calling index in the mid-river fell to a minimum of 0.91 in August and then increased sharply to its maximum value of 3.0 in October. Received sound levels peaked during August in the creek and river edge habitats (116 and 113 dB, respectively), and then fell sharply (Fig. 2D). Received levels in the mid-river peaked in September (109 dB). Overall, there was a declining trend in peak acoustic frequency of the calls throughout the five-month study period (Fig 2E), after an initial increase in July in the mid-river and river edge habitats. The creek habitat was the warmest habitat in June and the coolest in October (Fig. 2F). The mid-river was the coolest habitat in June and the warmest in October. Dissolved oxygen concentration at the bottom of the water column was lowest during summer and was always lower in the mid-river habitat (the deepest portion of the study area) and highest in the river edge habitat (Fig 2G). During August, the mean dissolved oxygen concentration in the mid-river was 1.1 (standard deviation=1.6) mg/L, which was well below the 2.3 mg/L avoidance threshold that Eby and Crowder (2002) found for Atlantic croaker in the Neuse River. Salinity was highest in July and August and was higher in the mid-river habitat than in the other two habitats (Fig. 2H).

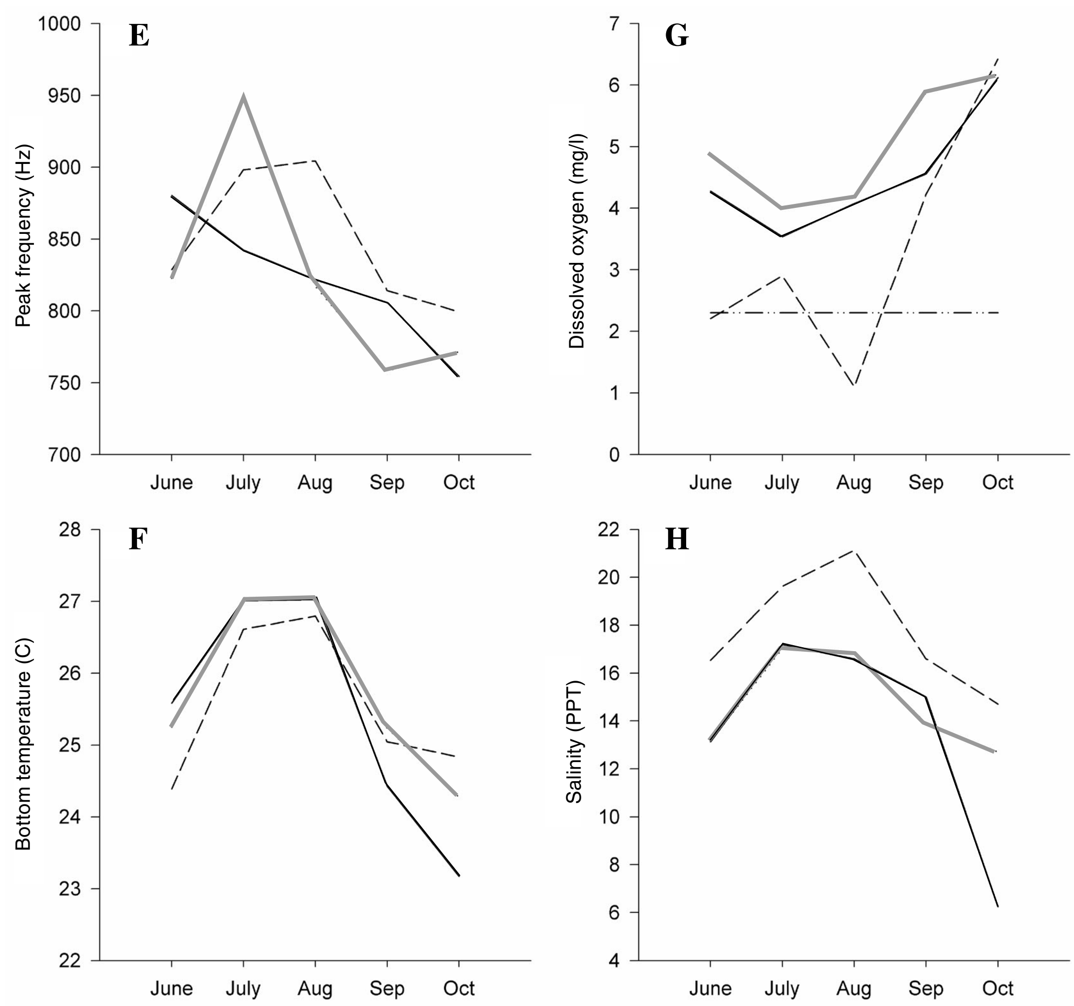

Figure 2 (continued)

CPUE ($F_{2,125}$=3.130, P=0.047), dissolved oxygen ($F_{2,125}$=4.21, P=0.017), and temperature ($F_{2,125}$=7.63, P=0.001) had significant overall effects in the MANCOVA (Table 1). Between-subject tests that provided separate results for each dependent variable showed that dissolved oxygen concentration (F_1=7.69, P=0.006), temperature (F_1=6.3, P=0.013), and habitat (F_2=4.55, P=0.012) were significantly associated with received sound level, and partial correlations indicated that each of these parameters accounted for 5–7% of the variance in sound level (Table 2). The interaction of month×habitat was associated with peak frequency of Atlantic croaker calls (F_6=2.31, P=0.038). For every 1-mg/L increase in dissolved oxygen concentration, the sound level of Atlantic croaker calls increased by 0.87 dB, and for every 1-degree increase in temperature there was an increase in sound level of 1.01 dB (Table 3). Although CPUE had significant overall effects in the MANCOVA (Table 1), the between-subject analysis did not detect significant relationships with either of the two dependent variables (Tables 2 and 3). This finding may have been due to the low statistical power associated with CPUE.

Williamson's indices demonstrated significant spatial overlap in the distributions of Atlantic croaker derived independently from acoustic and trawl data. Williamson's indices were between 0.959 and 1.488 for all combinations of month and habitat (Table 4). Calling indices corresponded better with CPUE than did received sound levels of Atlantic croaker calls. Spatial overlap between calling index and the actual density of Atlantic croaker (CPUE) was higher in July and August than in June, September, or October, and correspondence was stronger in the mid-river and river edge habitats than in the creeks.

With CART analysis we identified calling index as the predictor variable with the strongest correlation with CPUE (Fig. 3). Calling indices of 0 and 1 were associated with significantly lower densities of Atlantic croaker than were calling indices >1. Day of year and

Table 1

Multivariate analysis of covariance (MANCOVA) of the relationships among the dependent variables, received sound level and peak acoustic frequency of Atlantic croaker (*Micropogonias undulatus*) calls; the covariates dissolved oxygen concentration (DO), temperature (Temp), and trawl catch per unit of effort for Atlantic croaker (CPUE); and fixed factors of month and habitat and their interaction in North Carolina's Neuse River estuary (test statistic=Hotelling's trace statistic).

Effect	Test statistic	F	Hypothesis df	Error df	P	Partial η^2 [1]	Power
Intercept	0.812	50.75	2	125	0.000	0.45	1.00
DO	0.067	4.21	2	125	0.017	0.06	0.73
Temp	0.122	7.63	2	125	0.001	0.11	0.94
CPUE	0.05	3.13	2	125	0.047	0.05	0.59
Month	0.078	1.62	6	248	0.142	0.04	0.62
Habitat	0.072	2.25	4	248	0.065	0.04	0.65
Month × Habitat	0.155	1.60	12	248	0.092	0.07	0.83

[1] Partial η^2 is a standard measure of effect size (the proportion of variance in the dependent variable that is attributable to the independent variable, after partialling out the other independent variables). It varies between 0 and 1, and its meaning is similar to that of an R^2 in multiple regression or multiple correlation (Zar, 1999, p. 319).

Table 2

Between-subject effects from a multivariate analysis of covariance (MANCOVA) to investigate the relationships among the dependent variables received sound level (Level) and peak acoustic frequency (Frequency) of Atlantic croaker (*Micropogonias undulatus*) calls; the covariates dissolved oxygen concentration (DO), temperature (Temp), and trawl catch per unit of effort of Atlantic croaker (CPUE); and fixed factors of month and habitat, and their interaction in North Carolina's Neuse River estuary.

Source	Dependent variable	df	F	P	Partial η^2 [1]	Power
Corrected model	Level[2]	14	4.97	0.000	0.36	1.00
	Frequency[3]	14	2.74	0.001	0.23	0.99
Intercept	Level	1	56.29	0.000	0.31	1.00
	Frequency	1	12.33	0.001	0.09	0.94
DO	Level	1	7.69	0.006	0.06	0.79
	Frequency	1	3.44	0.066	0.03	0.45
Temp	Level	1	6.30	0.013	0.05	0.70
	Frequency	1	3.48	0.064	0.03	0.46
CPUE	Level	1	2.55	0.113	0.02	0.35
	Frequency	1	1.46	0.229	0.01	0.22
Month	Level	3	1.54	0.208	0.04	0.40
	Frequency	3	1.60	0.193	0.04	0.41
Habitat	Level	2	4.55	0.012	0.07	0.77
	Frequency	2	0.67	0.513	0.01	0.16
Month × Habitat	Level	6	1.08	0.377	0.05	0.41
	Frequency	6	2.31	0.038	0.10	0.78

[1] Partial η^2 is a standard measure of effect size (the proportion of variance in the dependent variable that is attributable to the independent variable, after partialling out the other independent variables). It varies between 0 and 1, and its meaning is similar to that of an R^2 in multiple regression or multiple correlation (Zar, 1999, p. 319).

[2] Type-III sum of squares: R^2=0.356 (adjusted R^2=0.284)

[3] Type-III sum of squares: R^2=0.233 (adjusted R^2=0.148)

distance to creek were also significantly correlated with Atlantic croaker density (Fig. 3). Catch rates of Atlantic croaker were significantly higher before June 11 (day 161) than were those later in the summer or fall, and catch rates were significantly higher within 525 m of the creeks than they were beyond this distance.

Discussion

The global MANCOVA test found trawl CPUE to be a significant factor (Table 1). But the MANCOVA test of between-subject correspondence failed to detect any significant relationship between CPUE and received sound

Table 3

Covariate parameter estimates from a multivariate analysis of covariance (MANCOVA) to investigate the relationships among the dependent variables received sound level (Level) and peak acoustic frequency (Frequency) of Atlantic croaker (*Micropogonias undulatus*) calls; the covariates dissolved oxygen concentration (DO), temperature (Temp), and trawl catch per unit effort of Atlantic croaker (CPUE); and fixed factors of month and habitat, and their interaction in North Carolina's Neuse River estuary.

						95% Confidence interval for B	
Dependent variable	Covariate	B	Standard error of B	t	P	Lower bound	Upper bound
Level	Intercept	79.94	10.33	7.74	<0.001	59.49	100.39
	DO	0.87	0.31	2.77	0.006	0.25	1.49
	Temp	1.01	0.40	2.51	0.013	0.21	1.80
	CPUE	0.94	0.59	1.60	0.113	–0.23	2.10
Frequency	Intercept	561.36	157.92	3.56	0.001	248.85	873.87
	DO	–8.88	4.79	–1.86	0.066	–18.36	0.59
	Temp	11.41	6.12	1.87	0.064	–0.69	23.52
	CPUE	10.83	8.96	1.21	0.229	–6.90	28.57

level for Atlantic croaker calls, which may have been due to low statistical power (power=0.35; Table 2). There was a high degree of spatial overlap between measures of Atlantic croaker density derived from trawl CPUEs and calling index, especially in the mid-river habitat during August. With CART analysis we identified a significant relationship between calling index and CPUE (Fig. 3). Taken together, these data indicate that passive acoustic techniques have the potential to be a means of assessing trends in abundance and habitat selection for soniferous fishes. However, the relationships between data derived from passive acoustics and those from traditional capture methods are not simple. Further development of analytical methods may clarify these relationships. Use of a calling index with a resolution greater than four levels; the ability to quantify the Atlantic croakers' contribution to ambient noise levels with greater precision; and a better understanding of how the acoustic source levels of individual fish are affected by dissolved oxygen concentration, temperature, and body size may result in an improved ability to estimate Atlantic croaker density based on passive acoustic data.

An important result of this study is the demonstration that environmental factors (e.g., dissolved oxygen, temperature, and habitat) appear to influence the relationship between acoustically derived indices of Atlantic croaker density and trawl CPUE. Many factors can influence calling behavior, spectral qualities of calls, and distance over which calls transmit. For example, Connaughton et al. (2000) showed that the sound pressure level, pulse repetition rate, and dominant frequency of disturbance calls made by weakfish (*Cynoscion regalis*) increased with increasing temperature and that dominant frequency varied inversely with fish size. Thus, temporal trends in peak frequency of Atlantic croaker calls likely resulted from changes in temperature and growth of the juvenile fish (Fig 2, A and E). Changes in

Table 4

Williamson's index of spatial overlap between trawl catch per unit of effort of Atlantic croaker (CPUE) and two passive acoustic measures of Atlantic croaker (*Micropogonias undulatus*) density, calling index (CI) and received sound level of calls (RL), in the Neuse River estuary from June to October of 2000 (* indicates $P<0.01$).

	CI and CPUE	RL and CPUE
Jun–Oct	1.150*	1.002
Jun	1.083	1.036
Aug	1.264*	0.959
Oct	1.092	1.012
Creeks	1.019	1.018
River edge	1.132*	0.989
Mid-river	1.478*	1.061

dissolved oxygen concentration may have also affected calling behavior, but this topic has not been well studied in sciaenids.

Propagation of sound is affected by absorption, spreading loss, and scattering (reflection and refraction) (Richardson et al., 1995, p. 27–30); therefore, factors such as water depth, temperature gradients, density gradients, turbulence, suspended particles, bottom topography, and substrate type affect how sound travels and how efficiently sound is detected. That the highest spatial overlap values between CPUE and calling index (up to 1.48) occurred during August in the mid-river habitat likely reflects the high degree of patchiness in Atlantic croaker distribution related to the patchiness of hypoxia in this habitat. Also, the mid-river habitat was deeper and had smoother bathymetry than the other habitats, which may have resulted in a more uniform transmis-

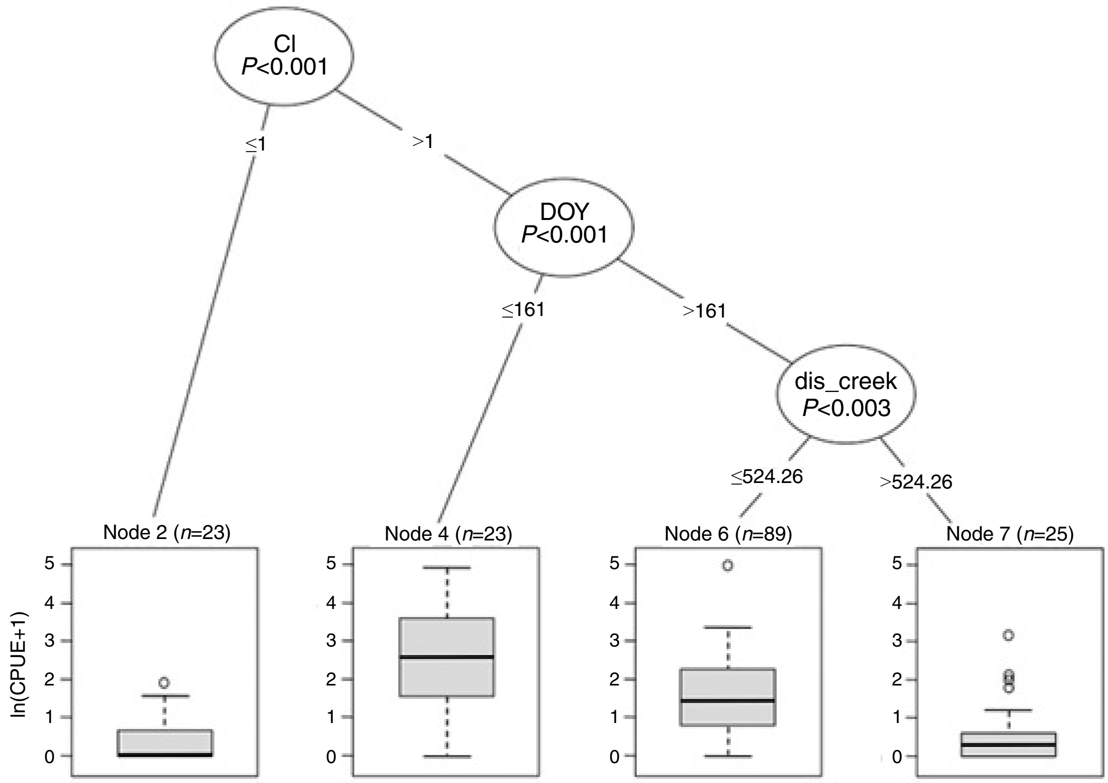

Figure 3

Diagram of the classification and regression tree (CART) showing the relationship between the dependent variable "trawl CPUE of Atlantic croaker" (ln (x+1) transformed) and eight predictor variables: day of year (DOY); dissolved oxygen concentration; temperature; salinity; depth; distance to creek (m) (dist_creek); received sound level; and calling index (CI) in the Neuse River estuary during summer 2000. Only the significant predictor variables are shown.

sion environment. This study illustrates the importance of planning passive acoustic surveys carefully to control for the confounding effects of environmental variables.

One major assumption used in this article is that the trawl data reflect actual Atlantic croaker densities in a consistent manner over time and across habitats. All fish sampling techniques have biases. For example, the catch efficiency of our trawl gear may have varied among the three habitats, by fish size, or between calling and noncalling fish. Therefore, some of the variation in the relationship between catch rates and sound production may have been due to variation in the catch efficiency of our trawl net, as well as in the variation in acoustic parameters. Because trawling is the most common method for assessing the abundance of demersal marine fishes, it is important to investigate the relationship between passive acoustic measures of abundance and those obtained from trawling.

Either the physical presence of, or the sound produced by, our boat could have caused a temporary disturbance of Atlantic croaker. For example, it may have caused the fish to flee from the immediate area or to change their acoustic behavior, which could have biased our results. Any disturbance would likely have been more pronounced in shallow water. We did not specifically investigate whether our boat may have affected our results. But there are a few points to consider. First, playback experiments of boat sounds in tanks elicited no significant change in swimming speed, turning rate, or depth in the water column of Atlantic croaker (D. Gannon, unpubl. data). Second, field recordings from the Neuse River that included the sounds of passing boats did not indicate an acoustic response from Atlantic croaker. The croaker sounds seemed to continue uninterrupted as boats passed by (but these boat sounds often masked the croaker sounds, and therefore such recordings were eliminated from our analysis). Third, the Neuse River estuary is highly turbid. Secchi depths during the summer are usually less than 1 m, which would limit visual detection of the boat. Finally, calling indices, received sound levels, and trawl catches were generally higher at shallow sampling stations (creek and river edge) than at deep ones (mid-river). In future studies an investigation of potential biases caused by disturbance from the sampling vessel would be worthwhile.

Although they tend to yield coarse data, there are some potential advantages to passive acoustic methods (see reviews by Rountree et al., 2006; Gannon, 2008; and Luczkovich et al., 2008.). In comparison with traditional fishery survey techniques (e.g., trawl surveys), passive acoustic surveys can be accomplished more quickly and cheaply with small vessels, few personnel, and low-cost equipment. Simple recording systems, such as the one used here, cost just a few hundred dollars and, in most cases, passive acoustic systems can be deployed with ease by one or two people in a small boat. Acoustic data loggers and telemetered sensors also allow remote data collection over long periods of time. Passive acoustic methods can allow the sampling of soniferous species that live on the bottom, within protective structures, or at great depths, and therefore, may be more appropriate than traditional sampling nets or active acoustic methods in some cases. Because of its relatively low cost, the ease with which it can be used, and its ability to collect data remotely from several sites simultaneously, passive acoustics allows larger areas to be monitored at higher sampling intensities than would be possible with a traditional survey method. Finally, passive acoustic methods are noninvasive and do not damage the habitat. Traditional fishery sampling techniques often cause high mortality rates of captured species and damage benthic habitat. Also, there has been much concern recently regarding anthropogenic noise in the sea (e.g., Popper et al., 2005, 2007); therefore passive acoustics can be more desirable than active acoustics in some situations.

Passive acoustics holds promise as a supplement to capture-based methods for assessing trends in relative abundance and for describing spatiotemporal patterns in distributions of soniferous fishes. To our knowledge, this is the first published attempt to compare passive acoustic data with data on the relative abundance of a soniferous fish species derived from traditional fisheries sampling techniques. Future research effort in fish passive acoustics should 1) help us to develop methods of quantifying calling rates with greater precision; 2) improve measurements of the contribution of the target species' calls to the ambient sound; and 3) provide a better understanding of how the source levels of the calls of individual fish are affected by dissolved oxygen concentration, temperature, and body size.

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