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Bottom water hypoxia suppresses fish chorusing in estuaries[a]

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ABSTRACT:
Hypoxia in coastal ecosystems is increasing as a result of water quality declines from nutrient pollution. Hypoxia negatively affects fish populations and marine life, limiting their spawning habitats, population size, and growth. In this study, two approaches were used to understand the effect of hypoxia on the chorusing and reproductive behavior of fishes in estuaries. One approach used a water quality meter integrated with a prototype passive acoustic recorder, developed to monitor dissolved oxygen and fish chorusing simultaneously and continuously at sites with normoxic and hypoxic conditions. In a second approach, passive acoustic recorders were deployed near ambient water quality monitoring stations, monitored by the North Carolina agencies in estuaries where hypoxia occurs periodically. In both approaches, when hypoxia (dissolved oxygen < 4.0 mg/L) occurred, fish chorusing was diminished or ceased. A strong correlation was observed between bottom water dissolved oxygen and the power spectral density in a 100–200 Hz frequency band associated with red drum (*Sciaenops ocellatus*, Sciaenidae) calling. Passive acoustic monitoring stations and integrated passive acoustic and water quality meters should be used in estuarine hypoxia monitoring efforts to examine the expanding areas of hypoxia and its impact on fish critical spawning habitats.
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I. INTRODUCTION

Hypoxia in coastal systems is increasing worldwide (Breitburg, 2002; Diaz, 2001; Pezner *et al.*, 2023). Monitoring the use of essential fish habitat and how it is affected by hypoxia in estuaries is a growing concern because poor water quality conditions can constrain fish and invertebrate population growth, reduce aquatic animal reproduction, cause mortality, and affect overall ecosystem function (Baird *et al.*, 2004; Breitburg, 2002; Testa *et al.*, 2017; Vaquer-Sunyer and Duarte, 2008). In Pamlico Sound and other North Carolina (NC) estuaries used by drum fishes (Sciaenidae), summertime hypoxia is a regular event that causes fish kills and changes the distribution of fish and crabs (Breitburg, 2002; Paerl *et al.*, 1998; Stanley and Nixon, 1992). The availability of suitable habitat for these estuarine-dependent fish and invertebrates is, thus, reduced. Because these species are commercially valuable, hypoxic events significantly alter the ecosystem function while impacting recreational and commercial fisheries dependent on these ecosystems.

Hypoxic zones in coastal regions can reduce the overall populations of fish and invertebrates, some of which are commercially valuable. Initially, hypoxia was defined as water with dissolved oxygen concentrations below 2 mg/L (Bricker *et al.*, 1999), based primarily on studies addressing the lethal effects of hypoxia on fishes. However, a more recent review of the sublethal effects of low dissolved oxygen concentrations on a variety of marine and aquatic animals suggests that a higher threshold for hypoxia should be used for conservation purposes because many marine organisms show stress at concentrations < 5 mg/L (Vaquer-Sunyer and Duarte, 2008). Hypoxia in estuarine bottom waters is the result of the combined effects of water density stratification (natural vertical salinity gradients with dense saline water at the bottom) and nutrients that increase algal growth at the surface, which then die and decompose in the bottom waters; the bacterial decomposition reduces available dissolved oxygen, and it cannot be replenished from the air– water interface at the surface because of stratification. Increased water temperatures due to climate change are exacerbating hypoxia in estuarine, coral reef, and coastal bottom waters (Pezner *et al.*, 2023; Testa *et al.*, 2017). To combat hypoxia, scientists and estuarine managers have established nutrient reduction plans to limit the input of nitrogen, phosphorus, and other nutrients to these watersheds. As the nutrient reductions are now implemented, the hypoxic conditions in these estuaries have been monitored using data-logging water quality and dissolved oxygen meters (Diaz *et al.*, 1992; Paerl, 2006; Stanley and Nixon, 1992). In the current study, we have chosen 4 mg/L as the threshold for hypoxia based on the local NC Department of Environmental Quality water quality criteria.

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However, we do not understand how hypoxia affects spawning behavior, feeding, and habitat use of fishes in estuaries. Along with conditions resulting in hypoxic water quality, the abundance and distribution of fish should be monitored as a response to hypoxia. As the estuaries are restored to conditions that reduce hypoxia, managers need to demonstrate that fish abundance is returning to normal levels. For example, in the Neuse River Estuary (NC), where the current study was conducted, carbon-flow ecosystem models indicated that fish predators were depleted as a result of chronic hypoxia because benthic invertebrate abundances were low after widespread hypoxic events in 1997, causing a decline at higher trophic levels (Baird *et al.*, 2004). Although fish biomass accumulated during the summer as fish grew in this estuarine nursery area, there was less growth of pelagic fish biomass in 1997 (during the summer with widespread hypoxia) relative to a less hypoxic year in 1998 (137.5% increase in biomass during the summer of 1997 vs 223.7% increase in biomass in 1998). Demersal fish growth was affected to a greater extent (386.2% growth in 1997 vs 584.2% growth in 1998). Less widespread hypoxia leads to higher levels of benthic invertebrates, pelagic, and demersal fish predator growth metrics; these growth metrics are used as a goal for restoring ecosystem nursery function in this estuary. Monitoring fish abundance, especially large fish predators, in estuaries is a difficult undertaking, fraught with challenges ranging from gear avoidance of nets by the target fish species (Booth and Potts, 2006; Erzini *et al.*, 2003; Rudstam *et al.*, 1984), the inability to use certain gear types on particular habitats (bottom trawls cannot work over oyster reefs), and the inability to use visual and video assessments as is performed for reef fish communities (Edgar *et al.*, 2004; Halford and Thompson, 1994; Harvey *et al.*, 2004; Prato *et al.*, 2017) and which is not feasible in highly turbid estuaries. All of these traditional methods require high labor and vessel costs, which result in intermittent sampling or under-sampling. Worse, the sampling of fish stocks often does not occur at a frequency that allows one to study the response of fish to short-term hypoxic events (Stanley and Nixon, 1992).

Therefore, we have devised an acoustic monitoring program to listen to fish sounds as a proxy for their distribution and abundance in estuaries. Fishes in the Sciaenidae family use sound to communicate in dark turbid estuarine waters, and these sounds are associated with spawning activities (Biggs and Erisman, 2021; Locascio and Mann, 2008; Luczkovich *et al.*, 2008b; Luczkovich and Sprague, 2002; Luczkovich *et al.*, 1999; Montie *et al.*, 2015; Rowell *et al.*, 2017; Walters *et al.*, 2007). We modeled sounds produced by virtual aggregations of calling weakfish (*Cynoscion regalis*) and exhibited that ensemble sound pressure levels measured with a single hydrophone increased by as much as 25 dB as the density of calling fish (number of individuals per volume) in the model increased from 0.00018 to 0.12 fish/m^3 (Sprague and Luczkovich, 2011). Furthermore, recent work on spawning aggregations of Gulf corvina (*Cynoscion othonopterus*) recorded the received sound pressure levels of the calling fish and measured fish density with an echosounder, showing a linear positive relationship between sound pressure and fish density up to 0.04 fish/m^3 (Rowell *et al.*, 2017). We can infer from these results that increased sound pressure levels are associated with an increased abundance of calling fish.

Here, we have developed two approaches to correlate sound production by fishes with changes in hypoxic conditions in the estuary. In one approach, we combined a passive acoustic recorder with a dissolved oxygen, salinity, and temperature recorder to get simultaneous and continuous measurements of these parameters. In another approach, we have placed passive acoustic recorders near established long-term estuarine water quality monitoring stations to compare the audio recordings of the estuary soundscape (largely dominated by fish in the family Sciaenidae reproductive calls) with varying dissolved oxygen levels in the estuary. This two-pronged approach allows us to map the distribution and abundance of fish using passive acoustic methods and examine the impact of hypoxic events on estuarine soundscape and, thus, Sciaenidae fish reproductive biology.

A. Initial observation of the effect of hypoxia on fish-calling behavior

For our initial passive acoustic monitoring of Sciaenidae fish spawning, we developed an autonomous sonobuoy for fish passive acoustic research (Luczkovich *et al.*, 2008b). Our original sonobuoys recorded fish sounds for 90 s on analog cassette tapes at intervals of ½–1 h automatically during a single night and were deployed over a year at various locations in Pamlico Sound, NC. A small boat was used to deploy and recover ten sonobuoys at randomly selected sites within two grids daily, and the sound was surveyed biweekly for a summer in 1998. The sonobuoys recorded the activity associated with spawning in these areas during 24-h periods. With this system, we successfully recorded the sounds of weakfish *C. regalis*, spotted seatrout *Cynoscion nebulosus*, silver perch *Bairdiella chrysoura*, and red drum *Sciaenops ocellatus*, species that spawn in estuaries in NC in the summer. However, the sampling was limited to 24-h periods in this version due to the limitation of recording on analog tapes. In this initial work, we noticed that the sound production from weakfish and spotted seatrout decreased with increasing hypoxia (Luczkovich *et al.*, 1999; Luczkovich *et al.*, 2008b). Using recording sonobuoys, a listener scored a drumming index (DI) of these species, which was based on the listener's classification of the recording: if no fish were heard calling (DI = 0), if a single fish was heard (DI = 1), or if multiple fish could be heard (DI = 2), or if a chorus of many fish was heard (DI = 3; Luczkovich *et al.*, 2008b). These DI values were summed on a nightly basis. Weakfish *C. regalis* summed nightly DI was zero when bottom dissolved oxygen concentration measured the next day at each sonobuoy location was <4 mg/L. The lowest levels of fish drumming indices for all species occurred when dissolved oxygen levels fell below 4 mg/L during the middle of the summer. These observations led us to devise a new monitoring system that included fish sound recordings and hypoxia monitoring.

For the first approach to monitoring the effects of hypoxia, we devised a new monitoring passive acoustic recorder, the Fish Acoustic Buoy and Underwater Logging Systems (FABULS). We describe this device here briefly and report some of the observations. In the second approach, we used a continuous passive acoustic recording "smart" hydrophone deployed at various locations, calibrated the FABULS in the laboratory using this smart hydrophone as a reference, and compared the sound recordings with dissolved oxygen levels measured at different depths and times from our own and ongoing NC government estuarine monitoring programs.

II. METHODS

A. Approach 1: FABULS development and testing

We developed and tested a prototype FABULS system that would work with existing water quality data loggers commonly used by researchers in coastal oceanography and estuarine ecology. We constructed the monitoring device for recording the acoustic behavior of the Sciaenidae and other soniferous fishes in estuarine systems with dissolved oxygen, salinity, and temperature water quality sonde (YSI multi-parameter water quality sonde model 6600EDS, YSI Incorporated, Yellow Springs, OH). The FABULS device recorded sounds at a sampling frequency of 22 050 Hz to uncompressed WAVE files on a compact flash memory card. The device was programmed to make a 10 s sound recording every 900 s (15 min) and record the water quality parameters at the same time. See supplementary material SuppPub1.pdf for a full description of the FABULS device.

The FABULS was tested on several occasions in the NC National Estuarine Research Reserve (NERR), Rachel Carson Reserve, Middle Marsh site (latitude 34.69336° and longitude −76.61367° using the Coordinate Reference System WGS84), at the National Oceanic and Atmospheric Administration (NOAA) National Marine Fisheries Service Beaufort Laboratory dock (NMFS Beaufort dock, latitude 34.71978° and longitude −76.67139°), and in the mouth of the Bay River near Pamlico Sound (latitude 35.17741° and longitude −76.55201°) to determine if it could record fish sounds and associated water quality information in an unattended mode. We deployed the unit at these estuarine locations to test it in a wide range of physical (variable salinity and dissolved oxygen levels) and biological (fish sound production) conditions. The deployment schedule began at the various sites in June 2005 and ended in September 2005 as described in Table I. These deployments lasted between 22 and 55 h at each site.

TABLE I. FABULS test locations, dates, test duration, and hypoxia conditions at each site.

Location	Date	Duration	Comments on hypoxia
NMFS Beaufort dock	6/30/2005	44 h	No hypoxia
Rachel Carson NCNERR	7/22/2005	22 h	No hypoxia
Bay River, NC	9/20/2005	55 h	Episodic hypoxia

B. Approach 2: Passive acoustic recorders and water quality monitoring study

In this approach, we did not use the FABULS simultaneous water quality and passive acoustic recorder system but instead used passive acoustic recorders placed near ambient water quality stations [University of North Carolina (UNC) ModMon program] routinely monitored for stratification events causing hypoxia. To capture a periodic stratification event, one must monitor water quality conditions in the estuary at stations spaced over a wide area and lower a dissolved oxygen probe through the water column at time intervals (biweekly) to observe stratification caused by density changes (salinity and temperature measurements, which allows one to compute a water density at each depth). Hypoxia is associated with rapid changes in density as a function of water depth, showing a sharp change or cline in the vertical salinity plot. Bottom water hypoxia (<4 mg/L dissolved oxygen) is associated with such sharp changes in density. Sound recordings were made at locations close in space and time to the water quality vertical profiles. Passive acoustic monitoring was performed using bottom-mounted hydrophones and acoustic recording. Dissolved oxygen and other water quality parameters were measured at the time of deployment. If bottom water hypoxia was observed at a nearby water quality monitoring station, we hypothesized that it should be associated with declines in fish sound production (as was observed in the first approach using the FABULS). Also, in this approach, a different passive acoustic recording system was used to record ambient sounds at estuarine locations in Pamlico Sound, the icListen HF (Ocean Sonics, Inc., Truro Heights, Nova Scotia, Canada), which is a calibrated hydrophone and data logger. The icListen SB2-ETH hydrophone sensitivity at 26 Hz is −168.3 dB re 1 V/μPa. A map of the locations where FABULS and icListen passive acoustic recorders were deployed in the NC Estuaries is provided in the supplementary material (SuppPub3.pdf). The icListen hydrophone was programmed to make continuous recordings with a sample rate of either 64 000 or 32 000 Hz stored the data in uncompressed broadcast WAVE files.

C. Spectral analysis

We performed spectral analysis of the recorded files. Details of the spectral analysis are given in our recent papers (Luczkovich and Sprague, 2022, 2023). We produced composite spectrograms from the FABULS recordings from average power spectra for each 10-s recording. To compute each average power spectrum, we produced a spectrogram of the recording using 1024-point Hanning windows with overlaps of 512 points and averaged each spectral component over all the time windows in the recording. Thus, each power spectrum (vertical slice) in the FABULS composite spectrogram is an average power spectrum of the entire 10-s recording. We produced composite spectrograms of the icListen recordings from average power spectra of 900 s

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(15 min) segments of each recording. As in the previous method, we computed each average power spectrum from a spectrogram of the recording segment produced from 1024-point Hanning windows with overlaps of 512 points and averaged each spectral component over all the time windows in the recording. Thus, each spectrogram in the icListen composite spectrogram is an average power spectrum of a 900 s recording segment. Composite spectrograms produced in this way do not show the small time details of fish calls and noise in the recordings, rather they show longer time trends in different frequency bands.

We used power spectral band (PSB) sums to identify species-specific contributions to the recordings. PSB sums were computed for specific frequency bands associated with known fish sounds. Table II shows the frequency range associated with different fish species. These frequency bands were chosen as indicators of calls by the various species. The bands do not contain all frequencies in the calls. The PSB sum, S_{PSB}, is the sum of all power spectrum components, P_n, for frequencies in the band $f_{\min} \le f_n \le f_{\max}$ such that

$$S_{\mathrm{PSB}}(f_{\min}, f_{\max}) = \sum_{n=n_{\min}}^{n_{\max}} P_n \Delta f, \tag{1}$$

where $n_{\min}$ is the index of the smallest frequency component in the band, $n_{\max}$ is the index of the largest frequency component in the band, and Δf is the frequency interval in the power spectrum. The average PSB sum was computed for each band in Table II using the average power spectra in the composite spectrograms described above. The PSB sums were plotted vs time to identify times in the recordings likely to contain fish sounds. One indication of fish activity is when the PSB sum for a frequency band increases with a different pattern than those for the other bands.

In addition to examining the composite spectrograms and PSB sums, we listened to each recorded track to identify species making the sounds and other sound sources (vessel noise, chain noise, and flow noise) such that we could annotate the composite spectrograms.

TABLE II. The frequency bands used in the power spectra analysis (PSB sums with frequency max and min) and the fish species that are dominant within each frequency band. A similar table with different band numbers was previously published (Luczkovich and Sprague, 2022). Atlantic croaker is assigned to band IV here with a wider frequency range than what was used in the previous study.

Frequency band (Hz)	Common name, species (family)
Band I: 100–200	Red drum, *Sciaenops ocellatus* (Sciaenidae)
Band II: 200–300	Oyster toadfish, *Opsanus tau* (Batrachoididae)
Band III: 300–600	Spotted seatrout, *Cynoscion nebulosus* (Sciaenidae)
	Weakfish, *Cynoscion regalis* (Sciaenidae)
Band IV: 300–1000	Atlantic croaker, *Micropogonias undulatus* (Sciaenidae)
Band V: 600–1500	Silver perch, *Bairdiella chrysoura* (Sciaenidae)
Band VI: 1500–2000	Striped cusk eel, *Ophidion marginatum* (Ophidiidae)

D. Water quality data

The FABULS recorded water temperature, salinity, and dissolved oxygen using the onboard water quality sonde. Immediately after each sound recording, the sonde was queried for water quality data six times at 10 s intervals. All values were recorded to the FABULS compact flash card, and the fifth reading taken during each sample was used for comparison.

The icListen does not have an onboard water quality instrument. Instead, water quality (temperature, salinity, and dissolved oxygen) was collected on vertical profiles as a part of the Mod Mon Program (UNC, which is available online[1]) and the NC Department of Environmental Quality (NCDEQ). Water quality stations closest to our passive acoustic monitoring stations [ModMon Neuse River Station NR-180, NCDEQ Ambient Water Quality Pamlico River Station O982500C, UNC Pamlico Sound 1 (PS 1), and UNC Pamlico Sound 2 (PS 2)] at the same approximate time of year (August and September) from sampling performed in 2016–2021 were used for comparisons. Hypoxia was determined when dissolved oxygen levels fell below 4.0 mg/L. Water quality collection details are given in the supplementary material, SuppPub4.pdf and SuppPub5.pdf.

All statistical analysis (correlation coefficients) and plotting of water quality data were performed in *R* version 4.3.1 and Python programming languages.

E. Calibration of the FABULS recorder

We calibrated the FABULS acoustic recording system described in Sec. II A by comparison with the icListen HF hydrophone described in Sec. II B. We deployed both hydrophones in a swimming pool and played sounds at frequencies of 100–3000 Hz using an underwater speaker (Clark Synthesis AQ339, Littleton, CO). The ratio of the recorded levels of the two hydrophones varied by less than 1.7 dB throughout the frequency range and less than 0.7 dB for frequencies 100–1000 Hz. We used the ratio of the recorded levels at 500 Hz as a calibration value for the FABULS recordings in this study because this is approximately the middle frequency of the fish sounds that we recorded. This calibration value directly compares the sound levels recorded by the FABULS and the icListen.

III. RESULTS

A. Approach 1: FABULS passive acoustic and hypoxia simultaneous monitoring study

The FABULS recorded audio data from the hydrophone and water quality data from the water quality sonde simultaneously. The audio and water quality query text files were recorded to the disk in time-stamped files at the same time. Later, these files were examined for patterns in sonograms to identify spawning fish and dissolved oxygen levels. We deployed the FABULS in the high-salinity (Luczkovich *et al.*, 2000) and low-salinity regions (Luczkovich *et al.*, 1999), where we had previously worked and recorded Sciaenidae fishes.

1. Tests in high salinity waters near Beaufort, NC

In general, no evidence of hypoxia was expected to occur or was apparent on the water quality sonde records of the NCNERR or in previous years (Fear, 2008), therefore, hypoxia was not expected to be observed during either of our first two tests at the NMFS Beaufort dock and the Middle Marsh site. As a first test, we deployed the unit at the NMFS Beaufort dock without submersing the FABULS housing and only lowering the water quality sonde and hydrophone below the surface. This was performed to test the unit's software and set recording levels. Dissolved oxygen levels were >10 mg/L [Fig. 1(A)], water temperatures were warm [27 °C–29 °C; Fig. 1(B)], and salinities were high [30 ppt or g/kg; Fig. 1(C)]. We could hear silver perch (*B. chrysoura* family Sciaenidae), Atlantic croaker (*Micropogonias undulatus* family Sciaenidae), spotted seatrout (*C. nebulosus* family Sciaenidae), and oyster toadfish (*Opsanus tau* family Batrachoididae) calling on these recordings. A composite spectrogram of the resulting test is shown in Fig. 1(D), where labels indicate the silver perch, Atlantic croaker, spotted seatrout, and oyster toadfish nocturnal chorusing events in the vicinity of the NMFS Beaufort dock. As this NMFS Beaufort dock was adjacent to an active boat channel, vessel noise was also recorded and is labeled in the plot. The hydrophone cabling on this initial test was not properly secured, resulting in flow noise during tide changes. This unsecured cable was corrected during the day on 2005-07-01 (resulting in a gap in the plots and spectrogram in Fig. 1).

Next, we deployed the FABULS housing, hydrophone, and water quality sonde and left them unattended at the Middle Marsh field site. Once more, we observed no evidence of hypoxia[Fig. 2(A)], with similar temperatures [Fig. 2(B)], and salinities [Fig. 2(C)] as found at the NMFS Beaufort Dock. We recorded silver perch producing chorusing sounds at the Middle Marsh site [Fig. 2(D)]. Flow noise was detected again and required further securing of the mooring chain after this deployment. These unwanted hydrophone cable and mooring chain sounds were problematic on this initial test of the FABULS because they occurred in the same frequency bands as fish chorusing and can be confused unless discriminated by a human listener.

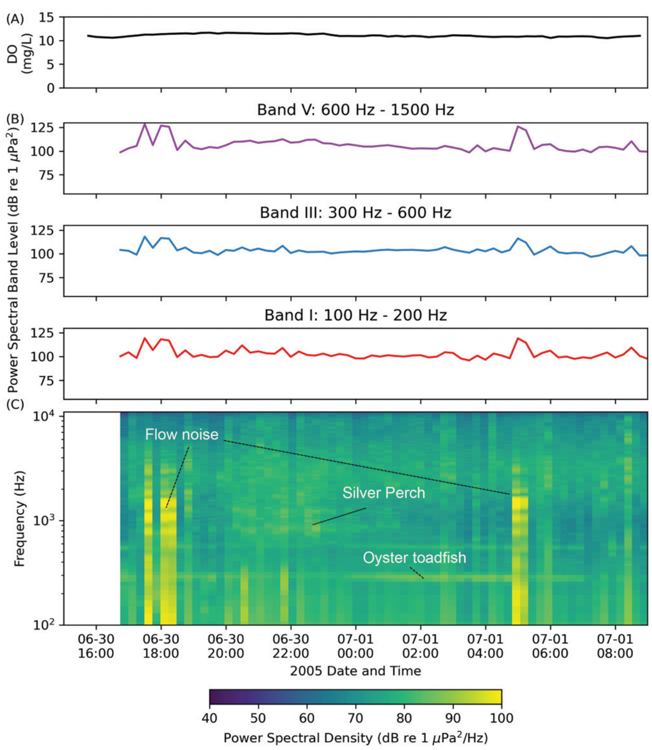

FIG. 1. Variation in fish sounds and water quality recorded by the FABULS deployed at the NMFS Beaufort dock from 2005-06-30 through 2005-07-02. (A) The dissolved oxygen (mg/L) was measured every 900 s from the water quality sonde during the deployment. (B) The PSB sums for bands I, II, and V are displayed. (C) The composite spectrogram of the power spectral density (dB re 1 μPa^2/Hz) as computed using the averages of 10-s sound recordings made every 900 s. The labels indicate the characteristic sound signatures of spotted seatrout, Atlantic croaker, and oyster toadfish choruses. Vessel noise is the result of outboard motorboats passing the site. An unsecured hydrophone cable was responsible for flow noise during tidal current changes at the site, and this was corrected during the test shown.

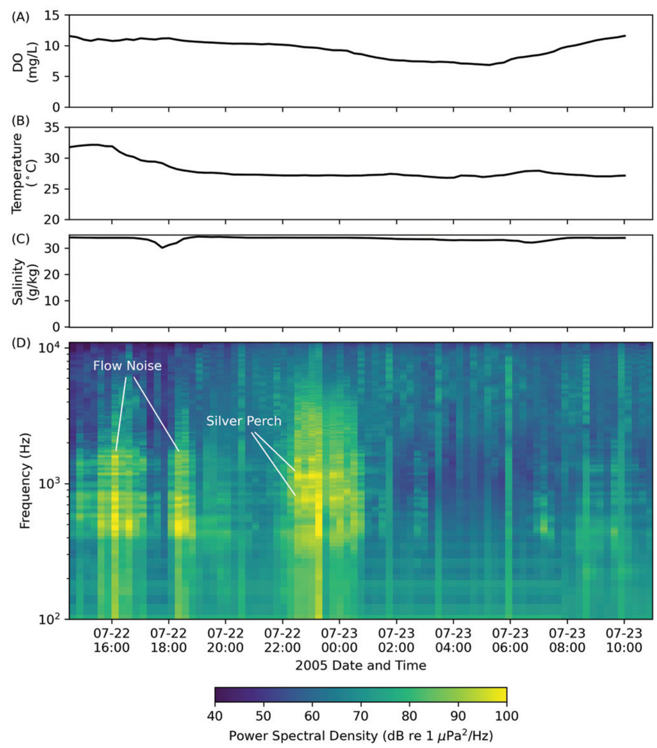

FIG. 2. Deployment of the FABULS at the Middle Marsh site, Rachel Carson NCNERR 2005-07-22 22:00 (UTC-4). (A) Dissolved oxygen levels (mg/L), (B) temperature (°C), (C) salinity (ppt) measurements, and (D) the composite spectrogram of the concurrent sound recordings, where labels indicate the chorusing of silver perch, are shown. Flow noise and rubbing sounds were recorded from an unsecured chain on the deployment frame.

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2. Tests in low-salinity and hypoxic areas of Pamlico Sound

We deployed the FABULS in September and October at the Bay River, NC site, where we have previously recorded red drum (*S. ocellatus* family Sciaenidae) and other sound-producing sciaenid fishes (Luczkovich *et al.*, 1999; Luczkovich *et al.*, 2008b). This site was where we have also observed periodic hypoxia in the past. On 2005-09-20 and 2005-09-29, we deployed the FABULS after it had been adjusted for sound levels and the software modified to record six dissolved oxygen readings during a 60-s interval on each 15-min sample cycle [Figs. 3(A)–3(D)]. In the first of these deployments (2005-09-20), weakfish (*C. regalis* family Sciaenidae) were recorded [Fig. 3(D)] on the FABULS, and periodic hypoxia did occur [Fig. 3(A)]. These hypoxic events were coincident with the influx of cooler waters [Fig. 3(B)] with higher salinities [Fig. 3(C)]. We plotted the dissolved oxygen measured concurrently with the sound recordings along with the band III PSB sum levels, which are associated with weakfish calling (Fig. 4). As the dissolved oxygen dropped from approximately 8 mg/L at the start of the deployment (2005-09-20 19:45 local time) to 2.5–2.7 mg/L later that night [2005-09-20 20:59 local time; Fig. 4(A)], a concomitant decline in weakfish sound production occurred, from 97 to 65 dB re 1 re μPa^2 [Fig. 4(B)]. Later, during the deployment, oxygen levels increased to over 7 mg/L and then decreased again to 0.9 mg/L on 2005-09-21 at 08:26 local time, showing the episodic nature of hypoxia occurrence and the fluctuating estuarine water quality conditions at this site. Normoxic conditions (dissolved oxygen > 4 mg/L) returned later in the day on 2005-09-21 at 14:29 local time, and the weakfish chorusing returned the following night but at a diminished level relative to the previous night [PSB sum = 88.2 dB re 1 re μPa2 at 2005-09-21 at 20:00; Fig. 4(B)]. Normally, weakfish are heard calling between sunset 19:00 and 04:00 nightly in June and July (Luczkovich *et al.*, 2008b). It is not known if the weakfish were more distant from the hydrophone, moving away from the hypoxic conditions, or if they were fewer, but this episode of hypoxia captured by the water quality instrumentation at this site had a definite effect on the diminished chorusing of weakfish as measured by the FABULS passive acoustic recorder.

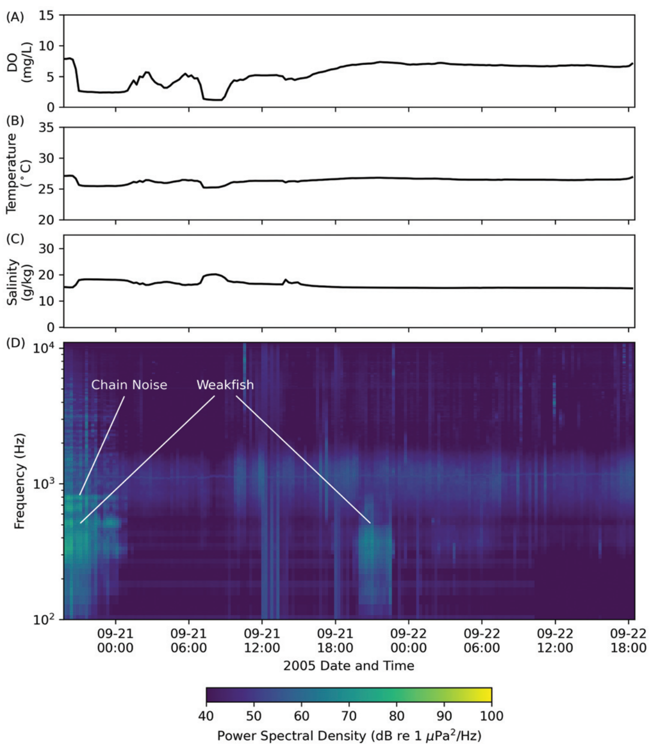

FIG. 3. FABULS deployed at the Bay River 2005-09-20 19:45 (UTC-4). Hypoxia occurred as shown in (A) dissolved oxygen (mg/L), (B) temperature (C°) decreased during hypoxic events, (C) salinity (ppt) increased during hypoxic events, (D) and the spectrogram indicated that weakfish chorusing declined during hypoxic events. Normally, weakfish are heard calling between sunset 19:00 and 04:00 nightly in June and July (Luczkovich *et al.*, 2008b).

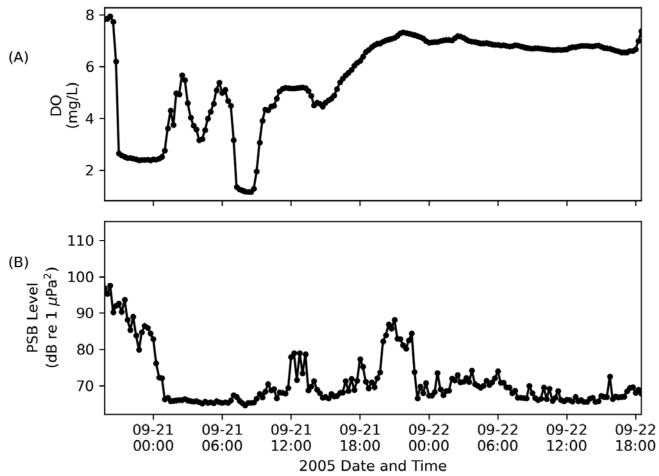

FIG. 4. (A) The dissolved oxygen (mg/L) is shown as it varies with the weakfish chorusing as measured by PSB sum for band III in (B) at the Bay River NC test site. Measurements of each response variable were taken at 15-min intervals by the FABULS and water quality sonde, starting at 2005-09-20 19:30 (UTC-4) and ending at 2005-09-22 18:02 (UTC-4). The site became hypoxic (<4 mg/L) between 2005-09-20 20:00 (UTC-4) and 2005-09-21 09:27 (UTC-4) in association with the influx of saltier water (20 ppt) from Pamlico Sound.

This set of deployments concluded our testing of the FABULS prototype, which captured variations in fish sound production and dissolved oxygen. We showed a decrease in fish sound production while simultaneously documenting a hypoxic event occurring at the low-salinity Bay River site. We did not see any decline in fish sound production at other sites where normoxic conditions were measured. When oxygen was high or above 4 mg/L, we recorded chorusing of fishes belonging to the Sciaenidae family (silver perch, spotted seatrout, and weakfish) and other soniferous species such as oyster toadfish *O. tau* in the family Batrachoididae. The Bay River site exhibited periodic hypoxia, which was likely caused by an increase in salinity stratification and increased bottom water salinity, possibly the result of weather-driven events, such as when river water entered the estuary, bringing in freshwater and nutrients, and cutting off the supply of oxygen from the atmosphere. This episode was a hypoxia event due to stratification at the Bay River, a low-salinity estuarine site [Fig. 3(C)], which did not occur at the Beaufort high-salinity sites [Figs. 1(C) and 2(C)] because of uniform salinity profiles.

B. Approach 2: Dissolved oxygen profiles and passive acoustic recording study

We have shown that locations with episodic hypoxia events had low fish sound production with a water quality logger that is near the hydrophone. In this second approach, we compare fish sound recordings in areas of normoxic water with recordings made at hypoxic sites based on nearby water quality records from ongoing water quality monitoring. As the first normoxic example, there was a very distinct red drum *S. ocellatus* fish chorus recorded [peak level during the red drum chorus PSB band I = 128 dB re 1 μPa^2; Fig. 5(A)] at the Neuse River at Gum Thicket Shoal (latitude 35.05999° and longitude –76.59756°) during the night of 2021-09-22 to 2021-09-23 during normoxic conditions [composite spectrogram of recordings; Fig. 5(B)]. Dissolved oxygen was normoxic on the bottom as measured before deployment (8.7 mg/L at 2021-09-21 19:23 UTC-4:00) and at the nearby ModMon Neuse River station 180 (average dissolved oxygen concentration during a vertical profile in depths of 0.0–6.7 m was 7.57 mg/L on 2021-09-27 09:53 UTC-4:00). In contrast, during a hypoxic event on 2016-09-27 to 2016-09-28 at the Pamlico River Pamlico Beach site (latitude 35.33515° and longitude −76.5730°) only Atlantic croaker could be heard calling at a diminished level [90 dB re 1 μPa^2 in band IV; Fig. 6(A)] and an Atlantic croaker chorus is indicated on two nights in the composite spectrogram [Fig. 6(B)]. No other Sciaenidae were heard calling. Hypoxia was documented by our team at the time of the icListen deployment (bottom water dissolved oxygen was 1.79 mg/L at 2016-09-26 16:58 UTC-4:00). At this location at deployment, the water temperature was 25.8 °C and salinity was 10.5 ppt; these values are similar to those measured at deployment at Gum Thicket Shoal in the Neuse River during normoxic conditions reported above (25.8 °C and 12.49 ppt). Both of these stations would be expected to have spawning red drum present at this time of year (mid-September). At the nearest water quality monitoring station to Pamlico Beach, 3 km away (NC DWQ Station 21NC03WQ-O982500C), water quality samples were taken

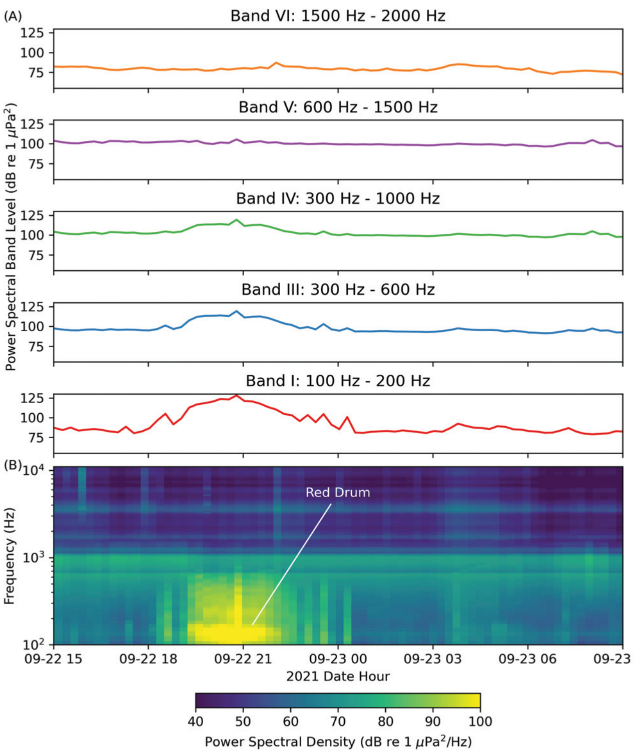

FIG. 5. Recording made with icListen passive acoustic recorder at the Neuse River Gum Thicket Shoals site on 2021-09-22 15:00 (UTC-4:00) through 2021-09-23 09:00 (UTC-4:00) during normoxic conditions. (A) The PSB sum plots for the dominant frequency bands associated with red drum "knocking" (band I, 100–200 Hz), spotted seatrout "grunting" (band III, 300–600 Hz), weakfish "purring" sounds (band IV, 300-1000 Hz), Atlantic croaker "popping" sounds (band V, 600–1500 Hz), and silver perch "chirping" sounds (band VI, 1500–2000 Hz). Note that the PSB levels range from 75– to 120 dB re 1 μPa^2. (B) Composite spectrogram (24-h composite of averaged 60-s power spectra) of the Sciaenidae chorus is shown on the dates and times (UTC-4) indicated. The bright yellow area between 19:00 and 22:00 is the result of a chorus of red drum, where individual fish produced calls 128 dB re 1 μPa^2.

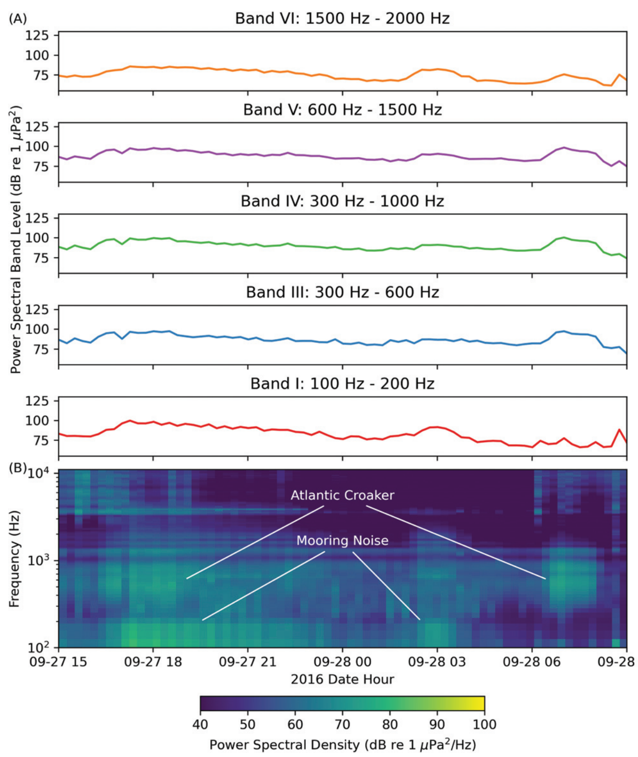

FIG. 6. Passive acoustic recordings made with the icListen at the Pamlico Beach site on 2016-09-27 15:00 (UTC-4:00) through 2016-09-28 09:00 (UTC-4:00) during hypoxia. (A) The PSB sum plots for the dominant frequency bands associated with red drum knocking (band I: 100–200 Hz), spotted seatrout grunting (band III: 300–600 Hz), weakfish purring sounds (band IV: 300–1000 Hz), Atlantic croaker popping sounds (band V: 600–1500 Hz), and silver perch chirping sounds (Band VI: 1500–2000 Hz) are shown. Note that the PSB levels range from 60 to 100 dB re 1 μPa^2. (B) Composite spectrogram of the same recordings (24-h composite of 60-s averaged power spectra) show the Atlantic croaker choruses on the dates and times (UTC-4:00) indicated.

20 days before and 22 days after the hydrophone deployment. Samples were taken in the daytime when oxygen is greatest due to daylight primary production. The bottom water at this monitoring station was normoxic in September and October 2016 (7.6 mg/L on 2016-09-06 and 6.1 mg/L on 2016-10-18). However, because the lowest values of dissolved oxygen at this site occur in August–October, episodes of hypoxia are most likely to occur then, especially at night or in the dark when primary production has ended (Fig. 7). Because the dissolved oxygen recorded at the time of deployment of the icListen hydrophone was hypoxic, far below the 4 mg/L hypoxia cutoff (Fig. 7), we have associated this low dissolved oxygen event with the decreased fish chorusing during deployment (Fig. 6).

The PSB sum plots shown above each spectrogram of normoxic (Fig. 5) and hypoxic events (Fig. 6) present the summed power spectral densities in each of the frequency bands that are dominant for red drum "knocking" (band I, 100–200 Hz), spotted seatrout "grunting" and weakfish "purring" sounds (band III, 300–600 Hz), Atlantic croaker "popping" sounds (band IV, 300–1000 Hz), and silver perch "chirping" sounds (band V, 600–1500 Hz). All of these frequency bands (associated with red drum, weakfish/spotted seatrout, Atlantic croaker, and silver perch) were of lower magnitude at 40–100 dB in hypoxic conditions vs 100–130 dB during peak chorusing in normoxic conditions (compare PSB sum plots in Figs. 2 and 3 and Figs. 5 and 6). From the comparisons of the PSB levels across sites and diminished levels in hypoxic conditions, we can infer that these Sciaenidae fishes were more distant from the hydrophone, had less intense or frequent calling behaviors, or were reduced in abundance due to hypoxia, producing lower PSB levels in each species' frequency band.

Finally, a positive relationship exists between high oxygen concentrations and fish reproductive calls. There was a significant correlation ($r = 0.823$, correlation t-test $= 6.9413$, and $P < 0.000\,000\,5$) between dissolved oxygen concentration in the bottom water and the sound production in band I (100–200 Hz, dominated by red drum chorusing in that band; Fig. 8). When hypoxia is occurring (<4 mg/L), the average PSB sum in band I is below 90 dB re 1 μPa^2. However, when the oxygen concentrations in the bottom waters are ≥6 mg/L (normoxic conditions), the average PSB levels increased above 90 dB and reached 110 dB re 1 μPa^2. These results suggest that hypoxia can limit the vocalization behavior of red drum during spawning events in Pamlico Sound and may interfere with reproductive signaling in this species of fish. This may also indicate that the red drum avoided areas of hypoxia, and this limits their critical spawning habitats.

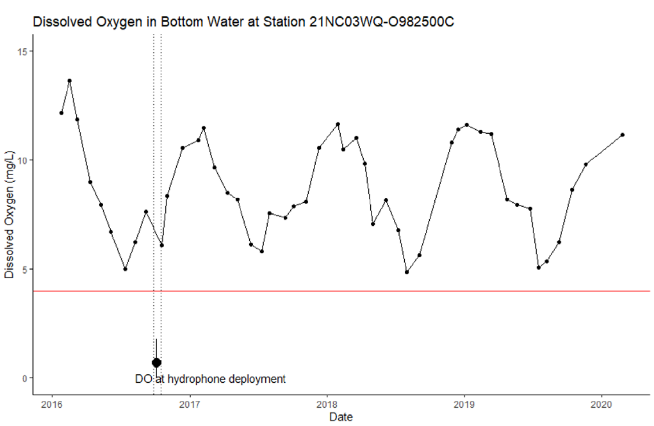

FIG. 7. (Color online) A plot of the dissolved oxygen (mg/L) in the bottom water of the Pamlico River Station 21NC03WQ-O982500C, which is 3 km away from the icListen deployment at Pamlico Beach, NC. The horizontal red line is the 4 mg/L cutoff used for defining hypoxia in this study. The vertical dashed line indicates the deployment and recovery date of the icListen hydrophone recordings. The dissolved oxygen (DO) at the time of deployment of the hydrophone is shown with error bars indicating the variation at the time of deployment.

IV. DISCUSSION

This set of experimental deployments shows the great potential for passive acoustic monitoring and the ambient noise from fish spawning to be used for monitoring these soniferous fish distributions and abundance remotely and continuously as dissolved oxygen and water quality conditions change in estuaries.

The area over which monitoring of fish calls and dissolved oxygen can be measured and correlated will require additional measurements to specify. We estimate this region to be in the range of 2–50 m, but it will depend on the background sound levels and source levels of fishes making sounds. Areas of estuarine hypoxia are larger than this in September and October, extending for 1000–5000 m along the bottom due to cross-channel upwelling events in the Neuse River Estuary hypoxic zones (Buzzelli *et al.*, 2002). The fish call detectability range that we report here is based

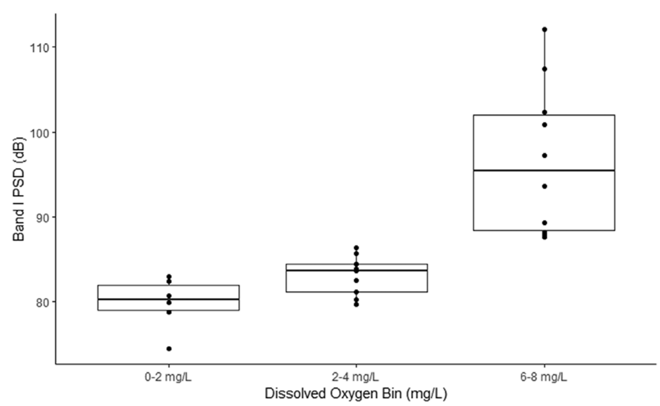

FIG. 8. The association between dissolved oxygen concentration (mg/L) in the bottom water measured at the time of deployment at our sites and the nearby water quality monitoring stations in August and September (2016–2021) nearest in space and time to the passive monitoring stations. The points indicate the measured values of the PSB sum levels in dB re 1 μPa^2 for band I (100–200 Hz), which is the frequency band that increases when red drum knocking and chorusing occur in August and September. Dissolved oxygen levels were grouped into three bins for plotting: 0–2 mg/L, 2–4 mg/L, and 6–8 mg/L (there were no observations in the range of 4–6 mg/L). Boxplots show the median values of the band I PSB as a horizontal line, the 25th–75th percentile ranges (interquartile ranges) inside the box, and the whiskers extend to 1.5 multiplied by the interquartile range above and below the median.

on the r_{max} distance of 50 m that we estimated using a cylindrical spreading model and assuming the call must be detectable above the background noise (Urick, 1983) for weakfish calling at 127 dB with a background sound level of 110 dB (Luczkovich *et al.*, 1999). In another study, we estimated the r_{max} distance for silver perch calling at 128 dB to be 2 m, assuming a background sound level was 125 dB due to other silver perch calling in the background (Sprague and Luczkovich, 2004). Greater ranges (44–281 m) were measured for spotted seatrout, which have greater source levels (137–140 dB) and were recorded in quiet conditions (calm seas) over varying substrates (Biggs and Erisman, 2021). Because we do not know how far away the fish are from the single hydrophone and how far the hypoxia extends without more thorough spatial samples being taken, we cannot state this range for certain. A 50 m radius is likely for the "vicinity" of the effect. A hydrophone array with multiple elements and dissolved oxygen sensors at each one could help answer this question.

Our study would have been improved if we had normoxic and hypoxic conditions measured at the same station, as was performed in approach 1 (with the FABULS making simultaneous recordings of sounds and dissolved oxygen); this would show that the fish are present and calling in normoxia but not hypoxia, where all other conditions are similar. Hypoxia can occur unpredictably in this study area; areas with red drum normally calling can, at other times, have these fish show avoidance and stop calling. When we deployed the icListen hydrophone at the Pamlico Beach location in September 2016 (Fig. 6), we expected red drum and other fish in the Sciaenidae to be present and calling, just as they were at Gum Thicket Shoals in September 2021 (Fig. 5). Other water quality conditions were similar at the two sites: both were made during the peak time of red drum spawning season (Luczkovich *et al.*, 2008b), and both recordings were made in September (deployment in the Pamlico River hypoxia 2016-09-16 vs deployment in the Neuse River during normoxic conditions 2021-09-10), in similar water depths (2–4 m), with similar salinity (10.5–12 ppt), and temperature measurements (both sites were 25.8 °C). Both locations normally have red drum calling at this time of year. Fishers capture red drum in both locations. It is due to the difference in hypoxia that we conclude that red drum were present and calling at the Neuse River normoxic location in September 2021 but were absent at the hypoxic location in Pamlico River in September 2016.

As hypoxia becomes more widespread in coastal ecosystems (Breitburg, 2002; Diaz, 2001), fishes and their habitats with normoxic conditions are being impacted, and the spawning habitat used by Sciaenidae fishes is contracting. Passive acoustic monitoring of fish reproductive calls has become commonplace around the world (Ibrahim *et al.*, 2018; Lowerre-Barbieri *et al.*, 2008; Luczkovich *et al.*, 2008a; Rountree *et al.*, 2006; Rountree and Juanes, 2020; Tellechea *et al.*, 2017) and can fill the gap in current water quality monitoring programs in terms of understanding how fish respond to changes in the estuarine water conditions. In the first approach that we described, we were successful in integrating a standard and widely used water quality meter with a prototype passive acoustic recorder of our design. A great advantage of this first approach is the simultaneous and continuous recording of the ambient sound and water quality conditions in the vicinity (∼100 m) of the hydrophone. In the second approach, we observed hypoxia occurring episodically in the region where red rum and other Sciaenidae species regularly spawn and produce mating sounds. We noted a diminished sound production when hypoxia was present in these samples of the soundscape. This agreed with previous work in this study area, where we noted that Sciaenidae species rarely produced sounds when hypoxia was severe (Luczkovich *et al.*, 2008b). Approach 1, using a simultaneous recording system for sound production by Sciaenidae and dissolved oxygen, is preferable to approach 2 in which non-contemporaneous measurements of these variables were made. Because fish can move around and avoid low dissolved oxygen conditions (Bell and Eggleston, 2005), Sciaenidae fishes (especially red drum and weakfish) recorded at a fixed location can have sound levels that vary dramatically as distance to the calling fish changes, even when other conditions (temperature and salinity) remain constant.

Episodic, short-term hypoxia has been documented here to be associated with changes in the estuarine soundscape. Specifically, the sounds produced by Sciaenidae fishes like weakfish and red drum were diminished, indicating that they were more distant from the hydrophone, changed their sound-producing behavior, or were fewer during spawning choruses. This reduction in sound levels associated with the spawning choruses of Sciaenidae fishes can serve as an early warning of more severe and longer-lasting hypoxia that results in fish kills (Paerl *et al.*, 1998; Testa *et al.*, 2017). Because our FABULS device was bottom mounted, and hypoxia affects the bottom waters under stratified water column conditions (Stanley and Nixon, 1992), this approach recorded changes in oxygen conditions near the benthic environment where these fishes spawn. Another possible way to deploy a FABULS is on a vertical profiling station in the middle of the estuary such that the soundscape is studied as the hydrophone is lowered and raised along with the water quality sonde. A profiling system could be useful in understanding top and bottom water differences in density, hypoxia, and fish sound propagation. Such profiling buoys and platforms exist in the ocean and estuarine monitoring now, but passive acoustic monitoring could be easily added to these profilers (Gomáriz *et al.*, 2015; Ostrovskii *et al.*, 2020; Reynolds-Fleming *et al.*, 2002).

We recommend that water quality monitoring stations should include passive acoustics systems to measure changes in fish reproductive calls. Such passive acoustic recording and signal processing technology exists and can be easily implemented into existing estuarine monitoring programs. These acoustic systems need not store long sound recordings with large file sizes. Instead, they could compute and store calibrated PSB sums like those used in this study.

Alternately, these passive acoustics systems could log calibrated decidecade or millidecade band power spectra, which could be combined appropriately to produce PSB sums.

V. CONCLUSION

We recommend that a device like our FABULS be further developed and widely implemented in estuarine and ocean water quality monitoring programs to gain insight into how hypoxia is affecting fishes and their spawnings. Our FABULS was a prototype device, and although it worked well, requires further development in terms of using newer and more powerful microprocessors, improved power consumption for long-term deployments, improvements in the user interfaces, onboard signal processing for PSB bands, and integration with the commercial water quality instrumentation.

SUPPLEMENTARY MATERIAL

See the supplementary material for details on the FABULS, UNC ModMon, and NCDEQ water quality monitoring protocols, hypoxia conditions, and maps of the study sites.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Ethics Approval

Work with fishes was approved by the East Carolina University (ECU) Institutional Animal Care and Use Committee (https://iacuc.ecu.edu/) under Animal Use Protocol No. D267b.

DATA AVAILABILITY

The data that support the findings of this study are openly available in https://dataverse.unc.edu/dataverse/GliderSoundscape2021.

[1]See https://paerllab.web.unc.edu/modmon/ (Last viewed March 5, 2024).

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