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Temporal soundscape patterns and processes in an estuarine reserve

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ABSTRACT: Underwater acoustic recordings can be used to measure the distribution and activity of sound-producing species and investigate variability in the physical and biological characteristics of marine ecosystems. This study characterized the summer soundscape of a coastal estuarine reserve, Middle Marsh, near Beaufort Inlet, North Carolina, USA. Passive recorders were deployed at 8 sites, within a mixture of seagrass, saltmarsh, oyster reef and soft-bottom habitats, and sampled for 2 min every 20 min between June and August 2014. Sound pressure levels (SPLs) in a high-frequency band (7–43 kHz) exhibited a periodicity of once per day, being 11 dB higher during the nighttime. This pattern is correlated with snapping shrimp sounds, with an average excess of ~12% more snaps detected at night. The same analysis for SPLs in a low-frequency band (150–1500 Hz) revealed a periodicity of twice per day, with diurnal sound levels varying by up to 29 dB. Temporal variability in the low-frequency soundscape is correlated with fish chorusing, as well as tidal water level, which may influence both the presence and absence of fish and the propagation of sound in the water column. The greatest SPLs are observed in association with periods of high biological activity during nighttime high tides. Sampling marine animals and their activities over ecologically relevant time scales is challenging using conventional techniques (trawls and throw traps) within complex shallow water habitats, particularly at night. Soundscape monitoring provides an additional method to assess spatiotemporal variation in essential fish habitat use within a complex mosaic of habitat types.

KEY WORDS: Passive acoustics · Habitat-related sound · Soundscapes · Estuarine habitats

INTRODUCTION

A soundscape is a composite of all biological, physical, and anthropogenic sound sources within a given habitat or area (Pijanowski et al. 2011). Soundscape ecology is an emerging field that includes studies to characterize the spatial and temporal variation in the sounds of an environment, investigate how those sounds affect organisms living in that environment, and use characteristics of the soundscape to infer habitat quality (Farina 2014, Lillis et al. 2014). Soundscapes also can be used to infer which organisms are present, their relative abundance, and their activities or behavioral interactions.

Early marine soundscape characterization work has focused on temporal patterns, which are key to understanding how organisms use a particular habitat(s) and why (Radford et al. 2008, Staaterman et al. 2014). While many soundscape investigations have used recordings from discrete blocks of time (e.g. dusk) (Radford et al. 2008, Lillis et al. 2014), there is increasing evidence from a wide-range of marine ecosystems — such as subtropical coral reefs in Australia (Radford et al. 2014), New Zealand (Radford et al. 2010),

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Panama (Kennedy et al. 2010), and the Florida Keys, USA (Staaterman et al. 2014), as well as oyster reefs in North Carolina, USA (Bohnenstiehl et al. 2016) — that soundscapes change over different time scales (days, weeks, months, lunar cycles) in response to environmental conditions. Better understanding of these temporal patterns requires long-term, simultaneous sound recordings made with high temporal fidelity (Staaterman et al. 2014, Bohnenstiehl et al. 2016).

Few studies, to our knowledge, have examined temporal patterns in the soundscape of a mosaic of intertidal, estuarine habitats. These environments are essential fish habitat for ecologically important species, like the silver perch *Bairdiella chrysoura*, and economically important species, like the spotted seatrout *Cynoscion nebulosus* (Mok & Gilmore 1983, Locascio & Mann 2008, Luczkovich et al. 2008). Passive acoustics provides a cost-effective way to obtain information on biodiversity and habitat characteristics (e.g. structural complexity, diversity of habitat types) that are potentially critical for ecological management and fisheries research (Sueur et al. 2008, Pekin et al. 2012, Lillis et al. 2014). This study investigates the soundscape of sites throughout Middle Marsh, an estuarine marsh ecosystem within the Rachel Carson Estuarine Research Reserve (RCERR) in Back Sound, North Carolina, USA, which has been well studied from a faunal and water quality perspective (Fear 2008). The goals were to (1) characterize temporal patterns in the summer soundscape in this coastal estuarine reserve at multiple sites, and (2) identify which processes and environmental variables influence temporal soundscape patterns across the reserve.

MATERIALS AND METHODS

Site selection and characterization

Soundscape characterization was conducted within Middle Marsh, part of the RCERR, a National Estuarine Research Reserve located in Back Sound, near Beaufort Inlet, North Carolina (Fig. 1). Middle Marsh is subject to semi-diurnal tides, and is located ~5 km from the nearby Beaufort Inlet that connects Back Sound to the Atlantic Ocean. Mean salinity at the site is high year round and ranges from >34 psu in summer to 32 psu in winter, with relatively little influence of the nearby North River estuary (Elis 1998). Water depth in Middle Marsh varies from 0.1 to 0.4 m at low tide to 1.2–1.5 m at high tide, except in several deeper channels adjacent to the shallower habitats

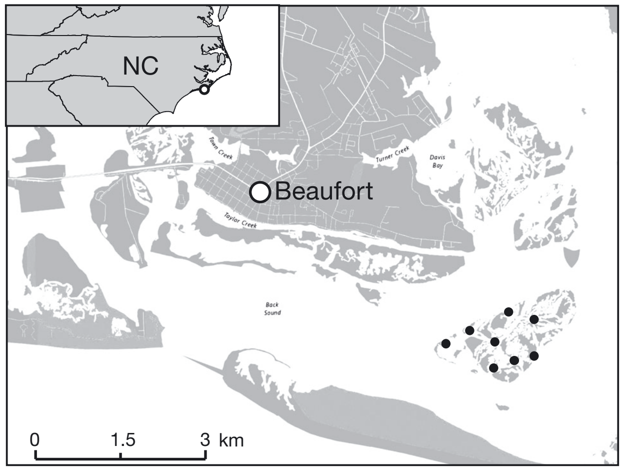

Fig. 1. Southeast US Coast (inset) and study sites (small black dots) in Middle Marsh in the Rachel Carson Estuarine Research Reserve (RCERR). Beaufort, North Carolina (NC), is located at 76.658° W, 34.718° N. See Table 1 for exact site locations

(Eggleston et al. 1999). The reserve contains a variety of estuarine habitats including seagrass beds, salt marshes, oyster reefs, and unstructured soft bottom (Fear 2008).

In 2006, the North Carolina Estuarine Research Reserve (NCNERR) conducted habitat-mapping analysis in the RCERR (http://portal.ncdenr.org/web/crp/habitat-mapping). Using the updated, ground-truthed habitat data from 2013 (NCNERR), and a submerged aquatic vegetation (SAV) map from 2008 (NCDENR and Albermarle-Pamlico National Estuary Partnership (APNEP), http://data.nconemap.gov/downloads/vector/sav.zip), we generated a spatially referenced map of the different habitat types in the RCERR (Fig. 2). This map was then refined through subsequent field reconnaissance, resulting in selection of 16 Middle Marsh sites that were suitable for hydrophone deployment. Eight of 16 possible sites were then randomly chosen as deployment sites for the duration of the study. Our main criteria for suitability were that (1) the hydrophones remained submerged at maximum low tide, and (2) the sites were separated by sufficient distance to be acoustically independent (i.e. the sounds produced by an individual or shoal of animals were not detectable across multiple recording stations). Each site is encircled by marshlands that are only partially submerged at high tide and/or dense seagrass beds that limit the detection range of biological signals to a few meters to tens of meters (e.g. Mann 2006, Wilson et al. 2013) (Figs. 1 & 2). This distance is small relative to the 330–480 m separation between each site and its nearest neighbor.

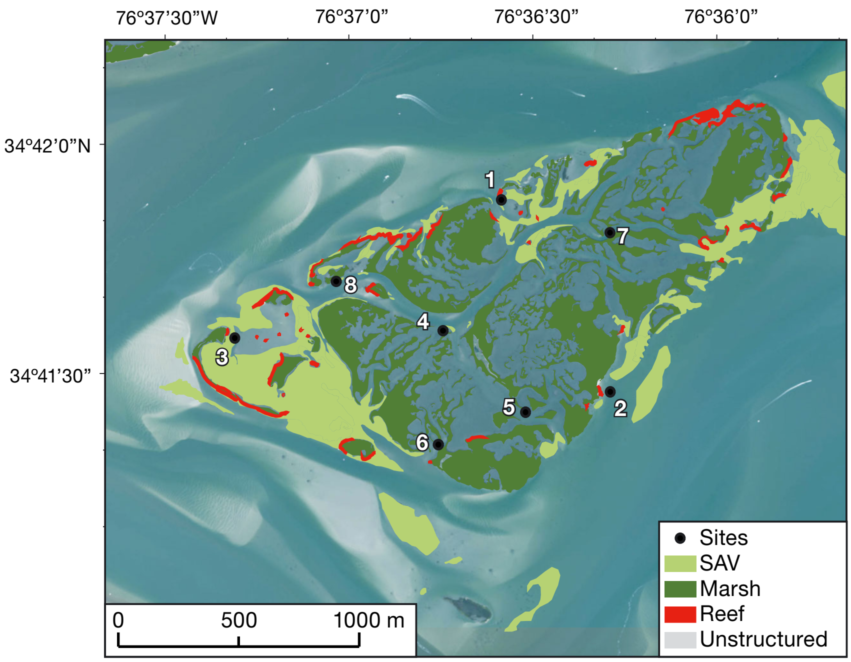

Fig. 2. Middle Marsh in the Rachel Carson Estuarine Research Reserve (RCERR) showing habitat layers and deployment sites. SAV: submerged aquatic vegetation

Acoustic sampling

To allow characterization of differences in the soundscape over time scales relevant to environmental variation (e.g. diurnal, lunar), underwater sound was recorded at the 8 sites simultaneously during monthly deployments in Summer 2014, with scheduled 2 min recordings taken once every 20 min at a sample rate of 96 kHz. At each site, an underwater acoustic recording system (SoundTrap, Ocean Instruments New Zealand) was strapped vertically to a metal post and positioned ~0.15 m above the seafloor and in water of sufficient depth so that it remained submerged during spring low tides. At low tide, the SoundTraps were submerged at least 0.15 m, and at high tide, they were submerged in ~1 m of water, depending on the magnitude of the tide. Sampling occurred during June, July, and August 2014 in 3 deployments, with gaps in the data representing times where the SoundTraps were not recording due to malfunction or maintenance (Table 1). The SoundTrap analog signal is digitized at a fixed rate of 288 kHz. A digital anti-alias filter, with a cutoff frequency of 0.45 times the desired sample rate, is then applied before decimation. Consequently, at our sample rate of 96 kHz, the useable (−3 dB) bandwidth of these recordings is 0.020–43.0 kHz.

Table 1. Site locations and deployment end dates and times for each site (start dates and times are given in parentheses). Dates given as mm/dd/yy; times given in h. –: SoundTraps not recording due to malfunction or maintenance

Site	Location	End date		
		Deployment I (6/12/14, 17:00)	Deployment II (7/9/14, 17:00)	Deployment III (8/8/14, 17:00)
1	34.69761° N, 76.60992° W	07/08/14, 07:00	07/28/14, 17:00	08/31/14, 05:00
2	34.69059° N, 76.60568° W	07/07/14, 20:00	08/07/14, 09:00	08/31/14, 05:00
3	34.69283° N, 76.62214° W	07/07/14, 20:00	08/07/14, 09:00	08/31/14, 05:00
4	34.69292° N, 76.61269° W	06/23/14, 14,:00	08/07/14, 09:00	08/31/14, 05:00
5	34.68986° N, 76.60917° W	07/03/14, 00:00	08/04/14, 20:00	08/31/14, 05:00
6	34.68878° N, 76.61304° W	07/08/14, 07:00	08/04/14, 20:00	–
7	34.69621° N, 76.60536° W	07/08/14, 07:00	–	08/31/14, 05:00
8	34.69472° N, 76.61743° W	07/08/14, 07:00	08/04/14, 20:00	–

Acoustic analysis

Each 2 min recording was processed in MATLAB using purpose-written code. These recordings were then partitioned into a lower (150–1500 Hz) and a higher (7–43 kHz) frequency band to examine variation in sound pressure levels (SPLs) within bandwidths of ecological interest. The low-frequency band includes the range in which most fish vocalizations occur, including the frequency range of the silver perch *Bairdiella chrysoura*, a dominant sound producer in this area (Mok & Gilmore 1983, Ramcharitar et al. 2006), and excludes low-frequency water turbulence noise, which typically peaks at frequencies between 1 and 100 Hz (Wenz 1962, Urick 1983). The high-frequency band was selected to exclude energy associated with perch chorusing frequencies (which extend up to ~5 kHz), and to include energy in frequencies associated with snapping shrimp (Au & Banks 1998), also a major contributor of acoustic energy to estuarine soundscapes (Lillis et al. 2014, Bohnenstiehl et al. 2016). The spectrum between the 2 analysis bands includes frequencies where fish calls and snaps overlap significantly, making it difficult to disentangle their contributions to SPL. These break points also were selected to exclude the ~2500–3000 Hz frequency band, where a small notch is observed in the frequency response of some of the SoundTraps. This 'notch issue' has been addressed by the manufacturer, and there is no indication that this malfunction influences the recording capabilities within our analysis bands.

To quantify temporal patterns in the soundscape of Middle Marsh, the 8 sites were used as sub-samples of the overall soundscape. We used the median value of each of 3 acoustic variables: (1) SPL in the high-frequency band, (2) SPL in the low-frequency band, and (3) snap rate among the 8 sites, for each recording time, to produce a time series for each of these 3 variables. We examined spatial coherence in SPLs among sites using Pearson's correlation coefficients to justify pooling among sites for temporal analyses of SPLs (Tables S1 & S2 in the Supplement; available at: www.int-res.com/articles/suppl/m550p025_supp.pdf). The range of correlation coefficients between SPLs in the low band at each site was from 0.41 to 0.75. Correlation coefficients between SPLs in the high-frequency band at each site ranged from 0.43 to 0.79, with the exception of Site 2 and Site 7, which had less snapping shrimp activity than other sites. Since the acoustic time series are likely to be influenced by time-varying biotic and abiotic factors, wavelet scalograms were produced to determine strength and persistence of the periodicity in SPLs in both frequency bands (Grinsted et al. 2004).

Spectrograms of individual recordings were used to help identify soniferous species and patterns in fish chorusing. Visual inspection of spectrograms and auditing of recordings was used to detect fish chorusing and, where possible, identify the chorusing species via comparison to archived sound files (e.g. www.dosits.org/science/soundsinthesea/commonsounds/) and available descriptions of acoustic characteristics of calls for certain species (Fine & Lenhardt 1983, Mok & Gilmore 1983). The spectrogram and spectra for each 2 min recording were examined for presence or absence of fish chorusing, as well as for peaks in frequencies associated with fish calls. Silver perch *B. chrysoura* and oyster toadfish *Opsanus tau* calls could be identified using this method, and all other fish species that could not be identified at the time of analysis were grouped into the 'other fish' category.

To further examine temporal and spatial patterns in snapping shrimp acoustic activity, individual snaps were identified using the envelope correlation method described by Bohnenstiehl et al. (2016). The snap kernel was derived from a suite of snaps recorded locally within Middle Marsh, with the procedure operating on a 7–43 kHz band passed waveform. An amplitude threshold of 107 dB re 1 µPa (peak-to-peak) was applied to the detection catalog; this corresponds to the 90% quantile of the background sound levels observed throughout the monitoring period for all sites.

To quantify diurnal patterns in the acoustic activity of snapping shrimp, the number of snaps detected during the day is compared to those detected at night. For each day, the percent excess snaps occurring at night is calculated as: $100 \times (N_o - N_e)/N_t$, where N_o is the number of snaps observed at night and N_e is the expected number given the fraction of nighttime recordings and the total number of snaps detected daily (N_t) (Bohnenstiehl et al. 2016). Positive values of percent excess snaps indicate greater snap activity at night, whereas negative values indicate greater activity during daylight hours. Daytime and nighttime periods are defined based on local sunrise and sunset times.

Environmental data collection and analysis

The local soundscape can be modulated, in part, by abiotic variables such as temperature, wind speed and direction, tidal currents and tidal water levels (Urick 1983). Water level data sampled at 6 min intervals were retrieved from a nearby NOAA station in Beaufort, North Carolina (Station ID: 8656483), located ~5 km away from Middle Marsh. These data were interpolated every 20 min to match the sampling interval of the acoustic data. The correlation between tidal water level and SPLs was determined for both the low and high-frequency analysis bands. A tidal phase angle was assigned to the time of each

2 min recording, with measurements made at high tide representing a phase of 0° and those made at low tide representing a phase of 180°/−180° (Fig. 3) (Stroup et al. 2007).

Water temperature data (°C) sampled at 15 min intervals were retrieved from the NERRS Shackleford Banks water quality monitoring site, located ~2.5 km from Middle Marsh (34.687° N, 76.644° W). These records were also interpolated to match the 20 min sampling interval of the acoustic data and correlated with SPLs in the low- and high-frequency analysis bands.

Wind speed and direction data sampled at hourly intervals were collected from the same NOAA station as the water level data and also interpolated to match the 20 min sampling interval of the acoustic data. The wind speed and direction data were converted to an alongshore wind velocity (U) and a cross-shelf wind velocity (V), and a cross-correlation analysis was performed using the SPLs in both the low and high-frequency bands. Local currents at sites within Middle Marsh were not monitored.

Because lunar phase might influence the activity of soniferous organisms, and the loudest soundscapes were recorded at night, we tested the difference in nighttime SPLs among lunar quarters. For each deployment day, the mean of nighttime (sunset to sunrise) SPLs in both the low- and high-frequency bands were determined and grouped by lunar quarter (LQ1 [new] = lunar days 27–4; LQ2 [first quarter] = lunar days 5–11; LQ3 [full] = lunar days 12–19; LQ4 [third quarter] = lunar days 20–26) (Eggleston et al. 1998). A non-parametric Kruskal-Wallis test was used to evaluate whether or not the nightly SPLs differed significantly between lunar quarters.

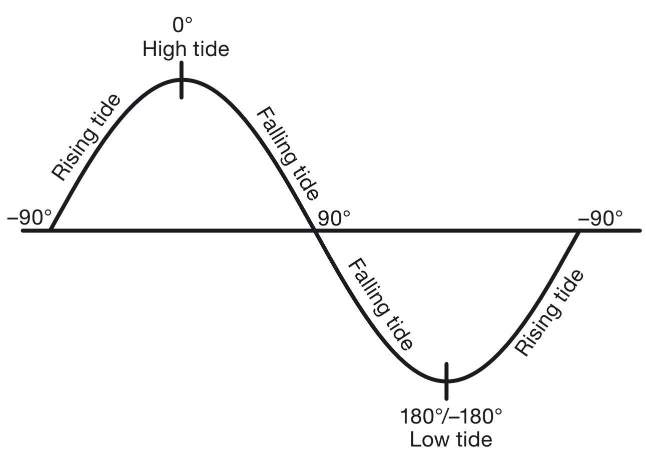

Fig. 3. Definition of tidal phase angle. High tide is assigned the phase angle of 0°. Falling to low tide, the phase angle increases to 180°. Rising to high tide, the phase angle is negative and increases from −180° to 0°

RESULTS

General temporal patterns in SPLs

SPLs in Middle Marsh varied over diurnal timescales. Within the low-frequency band (150–1500 Hz), SPLs exhibited an average daily range between maximum and minimum SPLs of 19 ± 0.5 dB with a periodicity of 2 cycles per day (Fig. 4). Within the high-frequency band (7–43 kHz), SPLs exhibited an average daily range between maximum and minimum SPLs of 11 ± 0.4 dB with a periodicity of 1 cycle per day and maximum SPLs occurring at night (Fig. 5). Wavelet analysis showed that the strength and persistence of these periodicities varied throughout the 3 deployments (Figs. 4 & 5). In Deployment I, the periodicity in both the high- and low-frequency bands was interrupted during the days following Hurricane Arthur, a Category 2 storm that made landfall in Beaufort, North Carolina, the evening of July 3, 2014, and continued through the early morning hours of July 4, 2014 (Figs. 4 & 5). In Deployment II, the periodicity in both bands was lessened during the neap tide after the new moon, when there was a low range in water levels (Figs. 4 & 5). There was a break in periodicity in the low-frequency band and a lessening in the high-frequency band periodicity during the new moon in August.

Processes underlying temporal patterns in SPLs

Abiotic variables

The low-frequency band had a strong positive correlation with water level, a relationship that was strongest for Deployment I (average $r = 0.754 \pm 0.005$, $p < 0.01$, Table S3 in the Supplement at www.int-res.com/articles/suppl/m550p025_supp.pdf). The high-frequency band had a very weak positive correlation with water level (average $r = 0.08 \pm 0.01$, $p < 0.01$, Table S4 in the Supplement). SPLs in the low-frequency band vary systematically with water level, being ~15 dB greater around high tide (0° tidal phase) than at low tide (180°/−180° tidal phase) (Fig. 6A). In addition, SPLs during a falling tide (0 to 180° tidal phase) were slightly elevated relative to those during a rising tide (−180 to 0° tidal phase). SPLs in the high-frequency band did not vary by more than a few decibels throughout a tidal cycle (Fig. 6B).

Water temperatures exhibited a quartile range of 24–29°C (21–36°C full range) throughout the 3

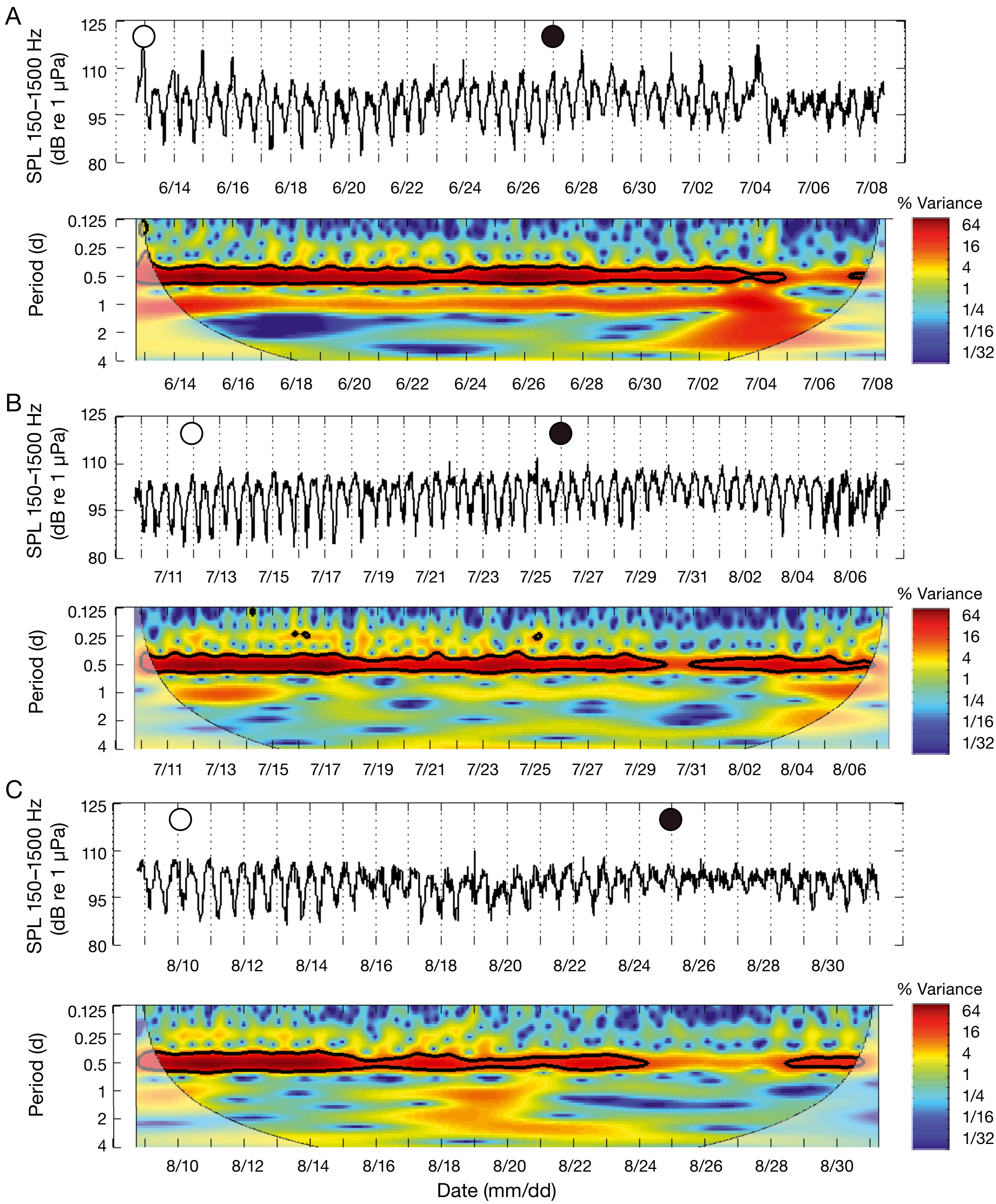

Fig. 4. Sound pressure level (SPL) in the lower frequency band (150–1500 Hz) over time with associated scalogram for Deployments (A) I, (B) II, and (C) III. In the SPL over time panel, vertical dashed lines denote midnight of each day and open/filled circles indicate full/new moons, respectively. Scalograms illustrate periodicity in the time series with areas in red showing stronger periodicities, and cooler colors representing weaker periodicities. Areas outlined in black are significant at the 95% confidence level. Lighter shaded areas on the edges represent data that cannot be resolved due to the length of the time series. These scalograms show the periodicity of peaks in SPLs in days (period of 0.5 = peak in SPL twice per day)

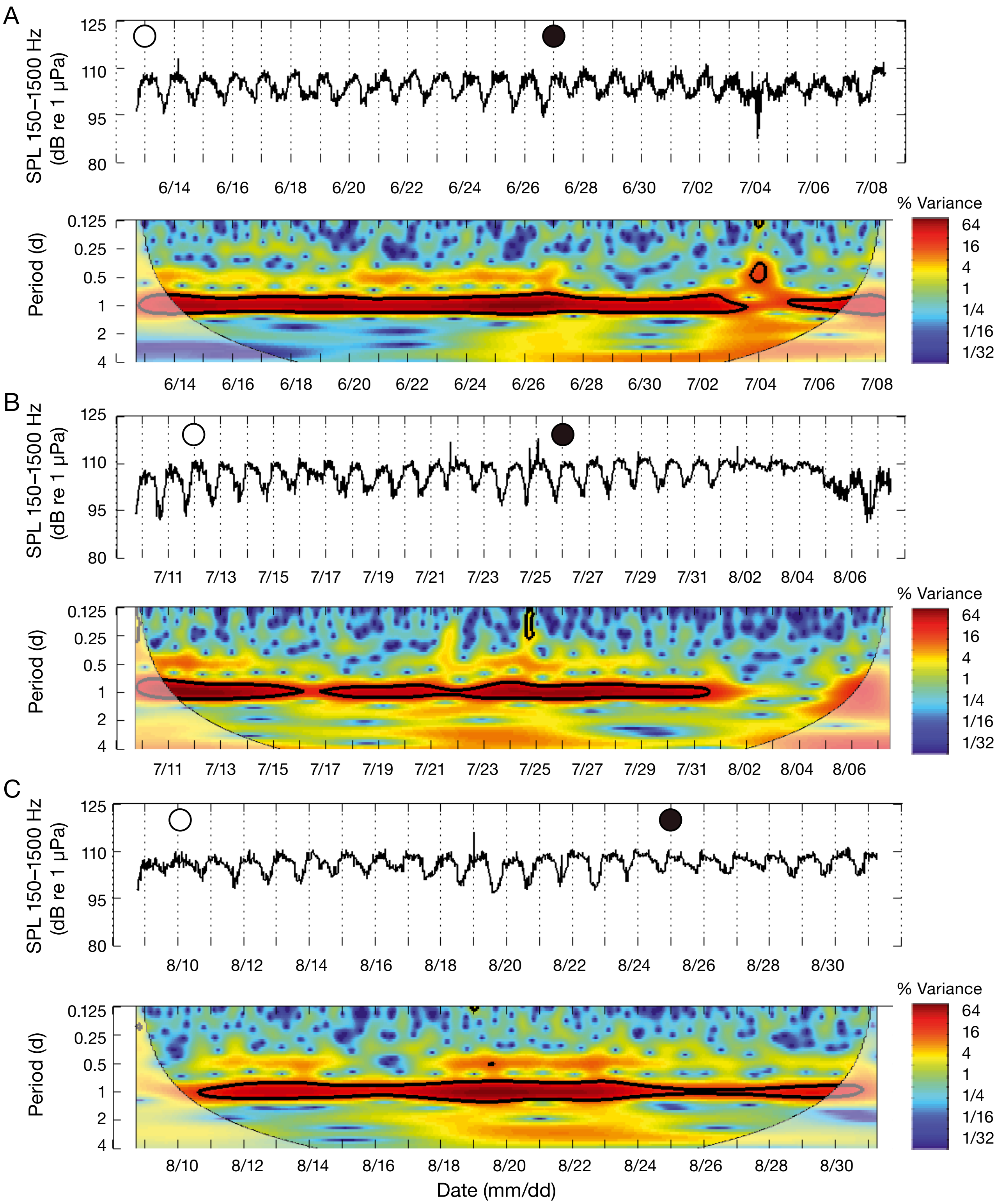

Fig. 5. Sound pressure level (SPL) in the higher frequency band (7–43 kHz) over time with associated scalogram for Deployments (A) I, (B) II, and (C) III. In the SPL over time panel, vertical dashed lines denote midnight of each day and open/filled circles indicate full/new moons respectively. Scalograms illustrate periodicity in the time series with areas in red showing stronger periodicities, and cooler colors representing weaker periodicities. Areas outlined in black are significant at the 95% confidence level. Lighter shaded areas on the edges represent data that cannot be resolved due to the length of the time series. These scalograms show the periodicity of peaks in SPLs in days (period of 0.5 = peak in SPL twice per day)

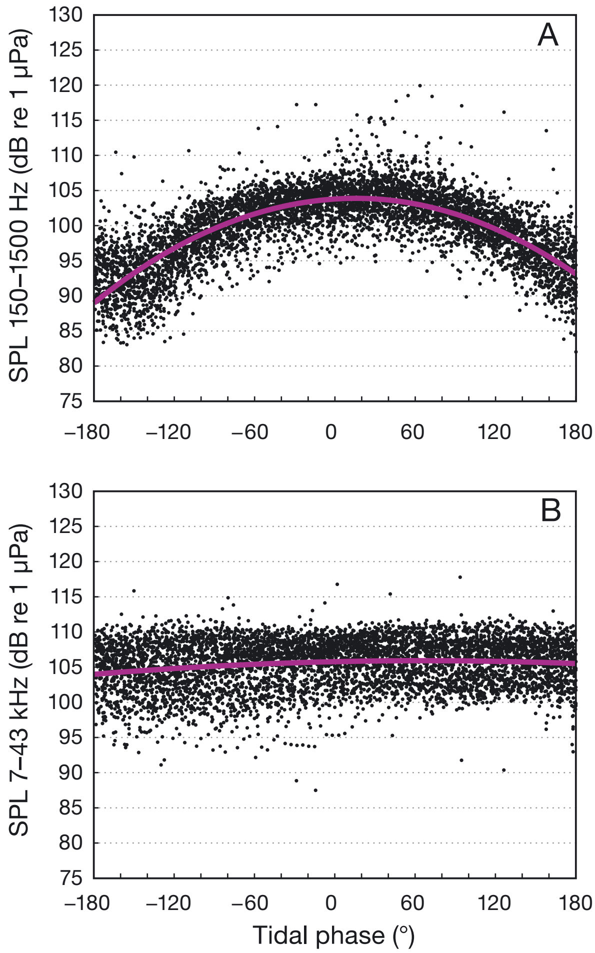

Fig. 6. Sound pressure levels (SPLs) in the (A) lower frequency band (150–1500 Hz) and (B) higher frequency band (7–43 kHz) vs. phase angle over 3 deployments for all sites. Quadratic trend line was fit to the median SPL at each phase angle

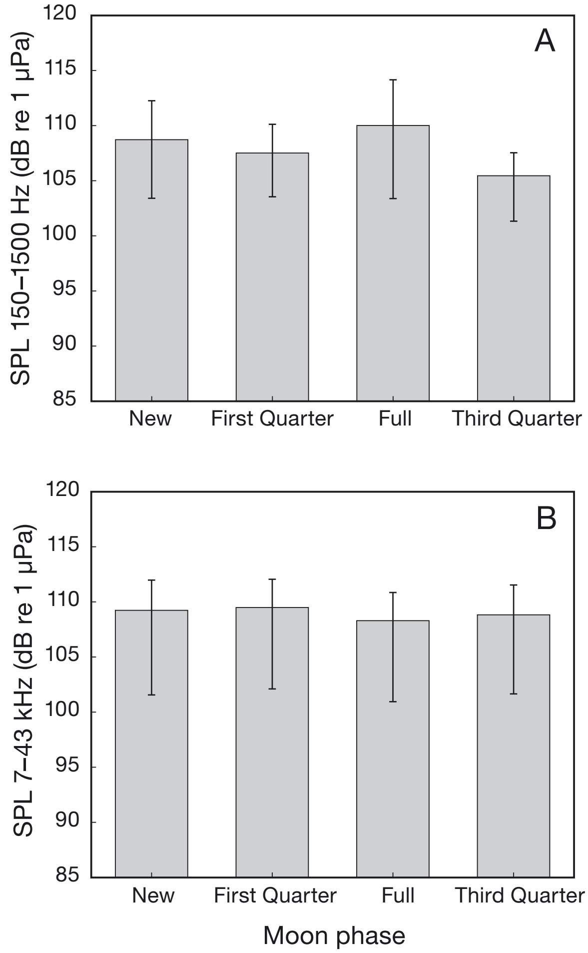

Fig. 7. Mean nightly dB sound pressure levels (SPLs) in the (A) lower frequency band and (B) higher frequency band for all sites for each moon phase (lunar quarter) in Deployments I, II, and III. Error bars are the 68% confidence interval

deployments, with an average temperature of 27°C. The low-frequency band had a weak, yet positive, relationship with water temperature ($r = 0.05 \pm 0.02$, $p < 0.01$). Both the high-frequency band and snap rate had relatively weak, negative relationships with water temperature ($r = -0.31 \pm 0.01$, $p < 0.01$, $r = -0.19 \pm 0.01$, $p < 0.01$, respectively).

There was a slightly negative correlation between SPLs and cross-shelf (V) wind velocity for both the high-frequency ($r = -0.26 \pm 0.02$, $p < 0.01$) and the low-frequency ($r = -0.09 \pm 0.02$, $p < 0.01$) bands. The relationship between alongshore wind velocity (U) and SPLs was not significant for the high-frequency band and was negatively correlated with the low-frequency band ($r = -0.15 \pm 0.03$, $p < 0.01$).

There was no significant difference in mean nightly SPLs in the low-frequency band among lunar quarters, though nighttime SPLs were slightly elevated during new (LQ1) and full moons (LQ3) (Fig. 7A, $p = 0.73$). Mean nightly SPLs in the high-frequency band were not significantly different among lunar quarters (Fig. 7B, $p = 0.43$).

Biotic sound sources: fish chorusing

The largest peaks in SPLs in the low-frequency band coincided with choruses characteristic of large aggregations of soniferous fish species. Silver perch choruses occurred during falling high tides (0–100° tidal phase), with the most choruses present at 20° to 40° tidal phase (Fig. 8). Oyster toadfish choruses occurred on both sides of the high tide but were present in similar amounts from −60° to 60° tidal phase

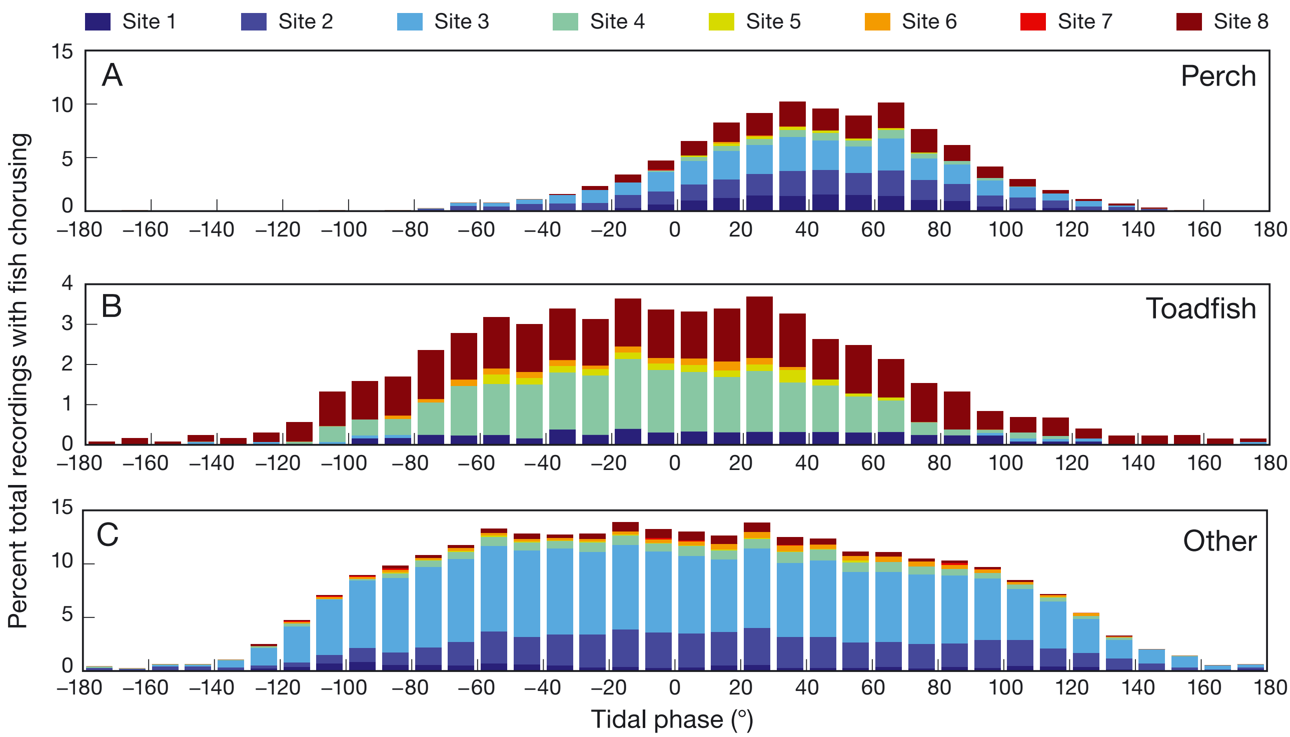

Fig. 8. Percent of total recordings with fish chorusing at each phase angle of the tidal cycle (0° = high tide, 180°/–180° = low tide) for all sites over Deployments I, II, and III. Each color represents a different site (see Table 1). Choruses represented are produced by (A) silver perch *Bairdiella chrysoura*, (B) oyster toadfish *Opsanus tau*, and (C) other or unidentified fish. Presence of fish chorusing was identified through inspection of spectrograms and auditing of recordings

(Fig. 8). Other, unidentified fish choruses also occurred on both sides of the high tide (Fig. 8). This 'other' category included calls from at least 3 fish species, and future research will attempt to identify and distinguish patterns for individual species within this category.

There were changes in the amount of fish chorusing by each group (perch, toadfish, other/unidentified) throughout the study period. Silver perch were most prominent in Deployment I, with choruses dying off by mid-August (Fig. 9). The perch choruses may also have been influenced by lunar phase, as fewer choruses were present between the full and new moon phases but would increase in duration and abundance around the new and full moon (Fig. 9). Perch choruses occurred at night, after sunset, through the early morning hours, and were not present during daytime recordings (Fig. 10). Persistent toadfish choruses were not observed in Deployment I and were most numerous during Deployment II, peaking in mid-July (Fig. 9). Toadfish chorusing was present at all times of day, though there were fewer occurrences during the mid-day hours (10:00–14:00 h) than in evening and early morning hours (21:00 to 06:00 h) (Fig. 10). Other, unidentified fish choruses were present through all 3 deployments, with fewer fish choruses between the full and new moon phases (Fig. 9). These fish choruses were identified at all times of day but were observed most from 06:00 to 21:00 h, with fewer observations during the early morning hours (00:00 to 05:00 h) and with the onset of perch chorusing (21:00 to 00:00 h) (Fig. 10).

Biotic sound sources: snapping shrimp

The high-frequency band was selected to represent snapping shrimp activity (Lillis et al. 2014, Bohnenstiehl et al. 2016). There was a strong, positive correlation between snap count and the high-frequency band, indicating that changes in SPLs in the high-frequency band can be explained by snapping shrimp activity (average $r = 0.728 \pm 0.006$, Table S5 in the Supplement). Mean snap rate was greater in the evening and early morning hours than in daylight hours, (Fig. 11) a pattern that intensified from Deployment I to Deployment III. Median snap amplitude remained relatively constant throughout a 24 h period for all 3 deployments. Percent excess shrimp snaps was positive for all 3 deployments, indicating that more snaps occurred at night than during the day (Fig. 12).

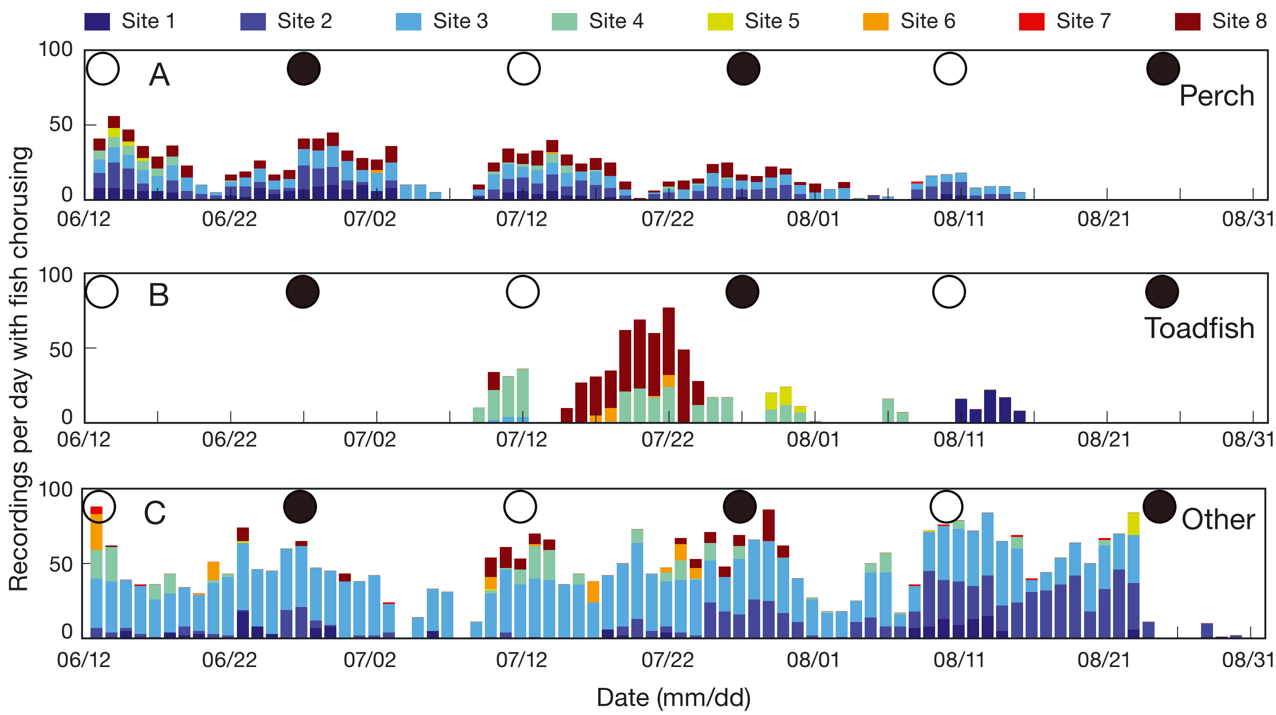

Fig. 9. Recordings per day (72 total recordings for each recording day at each site) of fish chorusing for each day of Deployments I, II, and III for all sites. Choruses represented are from (A) silver perch *Bairdiella chrysoura*, (B) oyster toadfish *Opsanus tau*, and (C) other or unidentified fish. Open/filled circles indicate full/new moons, respectively. Presence of fish chorusing was identified through inspection of spectrograms and auditing of recordings

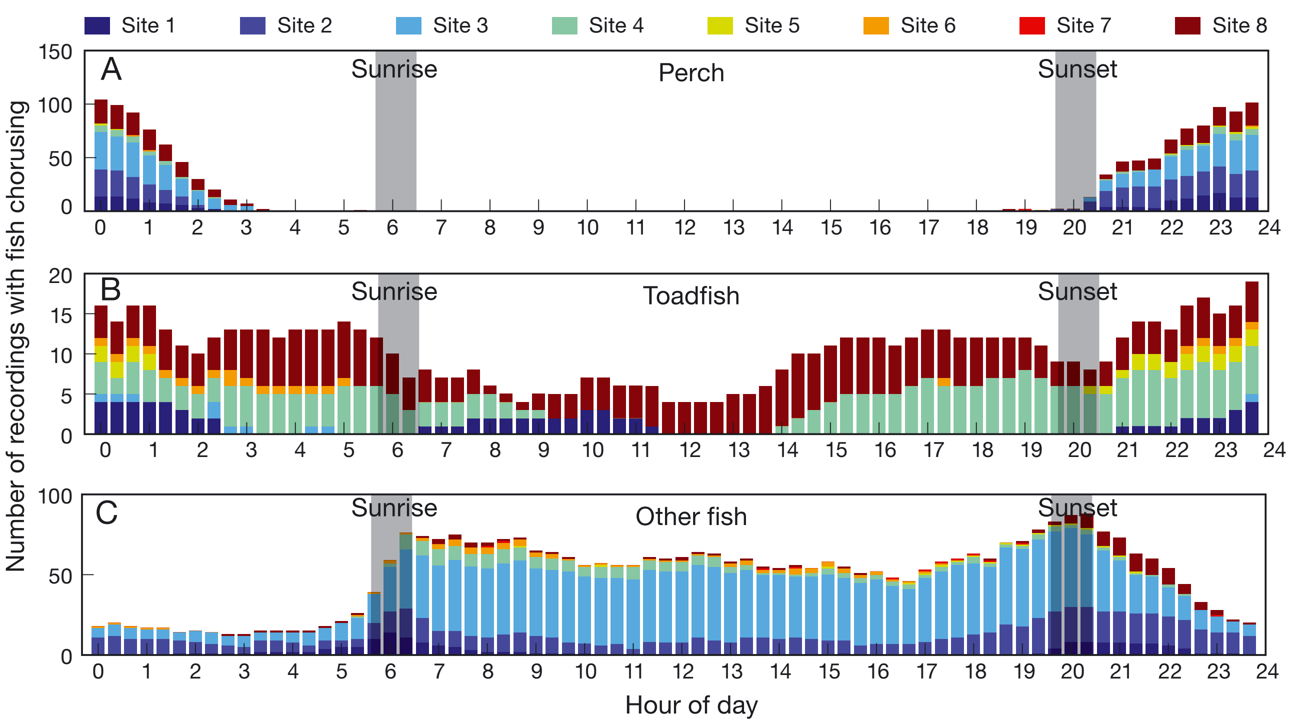

Fig. 10. Files with fish chorusing for each time of day (00:00 to 23:40 h, every 20 min) for all sites through Deployments I, II, and III. Shaded gray boxes illustrate the range in sunrise or sunset times throughout the 3 deployments. Choruses represented are from (A) silver perch *Bairdiella chrysoura*, (B) oyster toadfish *Opsanus tau*, and (C) other or unidentified fish. Presence of fish chorusing was identified through inspection of spectrograms and auditing of recordings

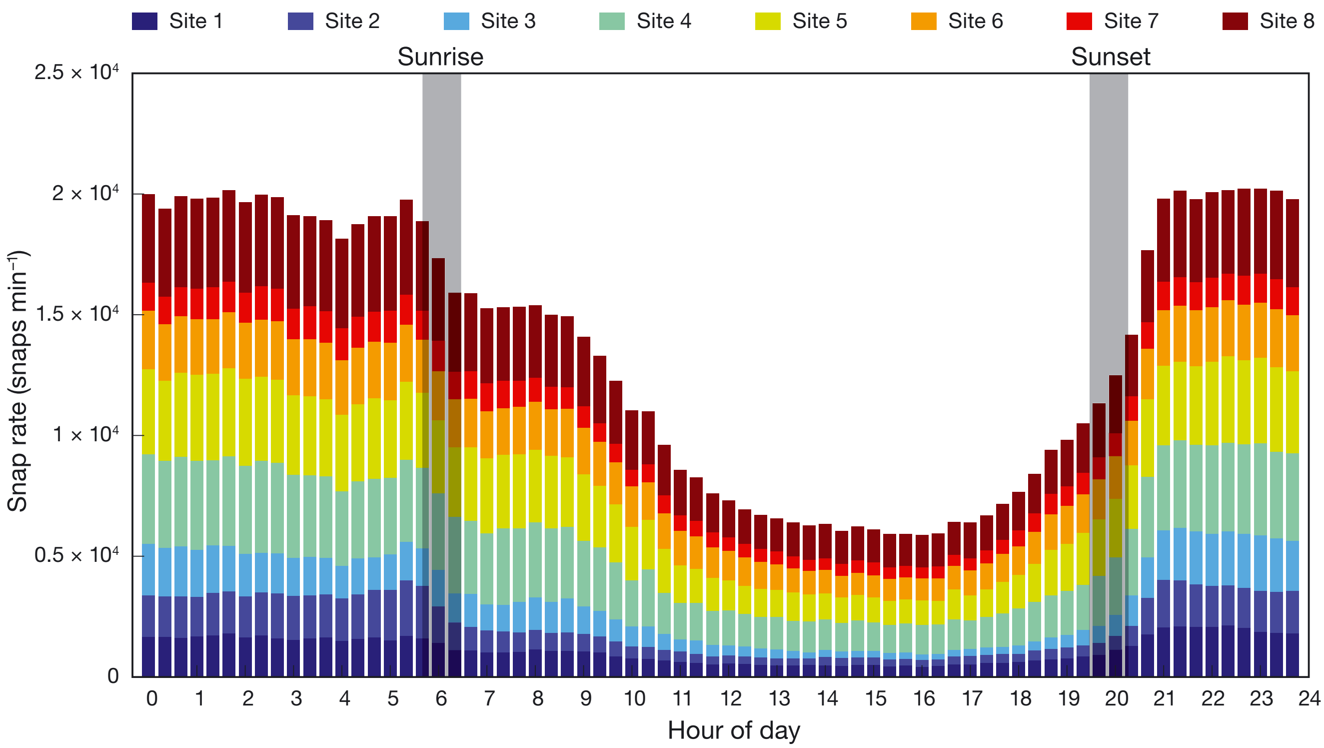

Fig. 11. Mean snap rate for each time of day (00:00 to 23:40 h, every 20 min) for all sites. Shaded gray boxes illustrate the range in sunrise or sunset times throughout the 3 deployments

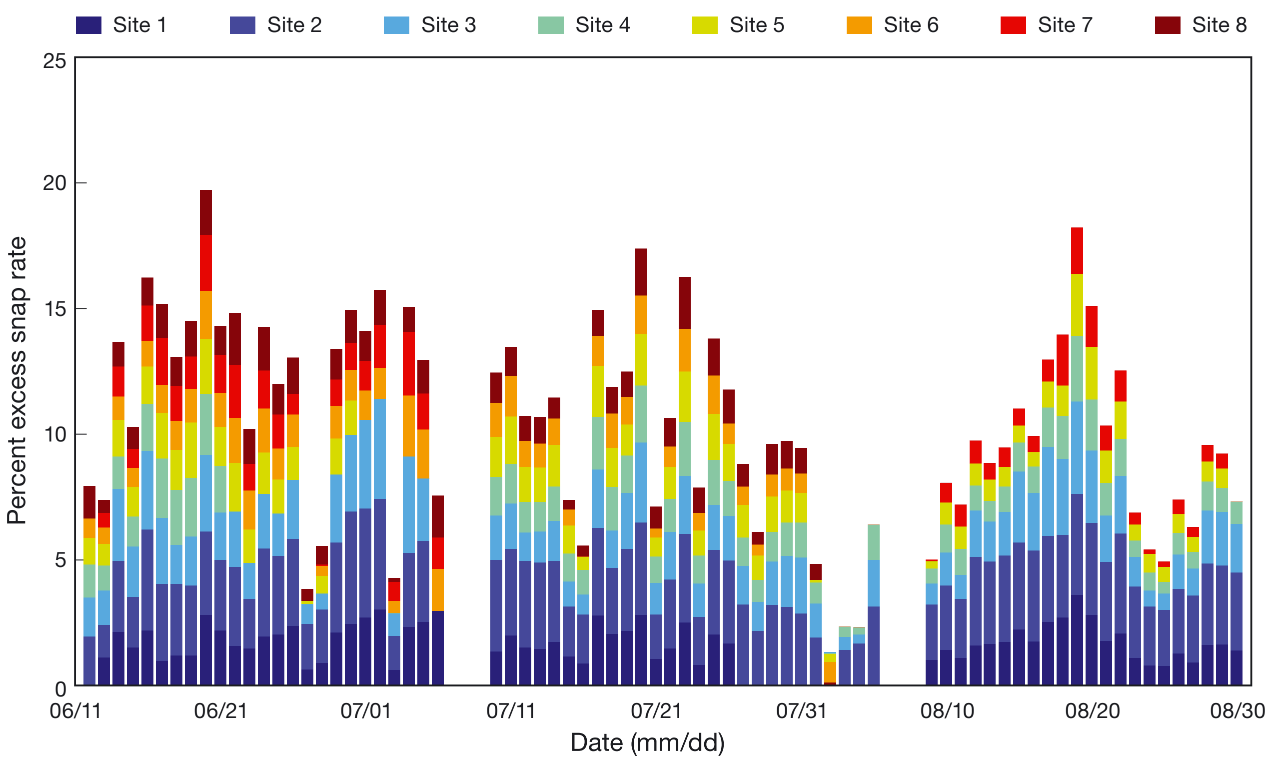

Fig. 12. Percent excess snap rate throughout Deployments I, II, and III for all sites. Bars show the daily percent excess values that are significant at the 95 % confidence level. Gaps in the graph are times when the SoundTraps were taken out to offload data

DISCUSSION

The temporal patterns and processes in the soundscape of Middle Marsh, North Carolina, USA illustrate the complexity of soundscapes in a mosaic of intertidal estuarine habitats. Sound pressure levels in the high-frequency band peaked once per day, consistently at nighttime, driven by diurnal activity of snapping shrimp, whereas sound pressure levels in the low-frequency band peaked twice per day, driven by the interactive effects of changing water levels and fish chorusing.

Processes underlying temporal patterns in SPLs

Abiotic factors (wind speed, water temperature, lunar quarter) appeared to have much less influence on the summer soundscape in Middle Marsh compared to diurnal biological activity. Sound produced by wind was expected to influence the low-frequency soundscape, and the negative relationship observed between wind velocity and low-frequency SPLs could indicate that the increase in SPLs associated with nightly fish chorusing is much greater than the increase in SPLs associated with greater wind speeds associated with daytime sea breezes (Staaterman et al. 2014). Soundscape characteristics, such as snap rate and SPLs, have been correlated with water temperature; however, due to the relatively short duration of this present study, these patterns were not evident (Staaterman et al. 2014, Bohnenstiehl et al. 2016). Previous studies have observed differences in soundscape characteristics, particularly when it comes to lunar phase, so it is surprising that lunar quarter had no influence on soundscape characteristics in this study, perhaps due to the fact that this study took place in an estuary where turbidity is higher than in rocky and coral reef systems (Radford et al. 2008, Staaterman et al. 2014).

Sound pressure levels in the low-frequency band peaked twice per day coinciding with the higher tidal amplitudes and periods of fish chorusing, possibly the result of both tidally influenced fish calling activity and movement and higher sound propagation at high tide. Even at high tide, the dominant frequency at which most fish call fell below the cutoff frequency (f_c) for sound propagation within the marsh's sandy-bottomed channels (e.g. f_c = 1.0 kHz at the 1 m water level and f_c = 4.0 kHz at the 0.25 m water level) (Mann 2006, Au & Hastings 2008). This suggests that the fish calls detected were sourced near the hydrophone recorders (ranges less than a few 10s m) and that the decrease in low-frequency SPLs observed at low tide is at least partially driven by greater transmission loss. This phenomenon, however, cannot explain an asymmetry in call patterns with respect to tidal phase (Fig. 8A), and therefore some species may also be moving in and out of the marsh or vocalizing more during different portions of the tidal cycle.

Other studies of fish activity in intertidal environments reveal greater fish activity occurring at higher water levels (Kleypas & Dean 1983, McGrath & Austin 2009, Meyer & Posey 2009). For example, in other marsh systems mummichog *Fundulus heteroclitus* and white perch *Morone americana* move into the flooded marsh with the tide for spawning and to take advantage of food resources (McGrath & Austin 2009, Meyer & Posey 2009). Chorusing is associated primarily with spawning (Mok & Gilmore 1983), and fish species spawning in shallow water or intertidal habitats may take advantage of higher water levels to ensure a greater propagation distance of their low-frequency calls (Bass & Clark 2003). Midshipman fish, for example, had greater vocal behaviors when the high tide was deeper (Bass & Clark 2003). This is similar to the pattern observed for soniferous fish species in Middle Marsh.

Unlike toadfish and other fish chorusing, perch chorusing occurred only at nighttime and predominantly during falling tides. Synchronized spawning during high water of spring tides is common for many marine fish and invertebrate species and is thought to reduce predation pressure on eggs, as they will disperse out of the habitats with the receding tide (Cronin & Forward 1982, Gibson 1992). Silver perch are nocturnal feeders and use flooded habitats as foraging areas and are also nocturnally active on seagrass banks at night and around high tide (Kleypas & Dean 1983, Sogard et al. 1989). Though sound production by silver perch is associated with spawning and not feeding, previous studies illustrate that perch use and move into flooded habitats during nighttime and high tide, suggesting that they may do the same when spawning. A previous study found that both maximum daily sound production, as well as appearance of eggs and larvae for the silver perch, occurred between 17:00 and 22:00 h, further supporting the fact that silver perch are active at night in these estuarine habitats (Mok & Gilmore 1983).

Diurnal patterns in snapping shrimp activity modulated SPLs in the high-frequency band. More snaps were observed at night than during the day, which is consistent with other summertime recordings of snapping shrimp activity (Radford et al. 2008, Bohnenstiehl et al. 2016). Radford et al. (2008) suggest snapping shrimp are more active at night to avoid predation in daylight hours. Snapping shrimp activity is also elevated in summer as a result of warmer water temperatures that increase their activity (Radford et al. 2008, Bohnenstiehl et al. 2016). Recent work within subtidal habitats in nearby Pamlico Sound, North Carolina, however, shows that in winter months the diurnal pattern of snapping shrimp acoustic activity reverses, with more snaps occurring during the day than at night (Bohnenstiehl et al. 2016).

In both the high-frequency and the low-frequency sound bands, a peak in SPLs occurred at night, indicating greater acoustic activity for some fish and invertebrates producing sounds in Middle Marsh. Soniferous fish, such as silver perch, produce sounds associated with spawning to attract mates (Mok & Gilmore 1983). This loud, nightly chorus of species-specific sound production represents an advantage for these fishes, most of which are broadcast spawners, allowing them to come together and spawn in darkness via the use of acoustic communication (Locascio & Mann 2008).

Passive acoustic recordings provide a cost-effective, non-invasive way to identify critical spawning habitats for soniferous species, and knowledge of spawning habitat and stock estimates is essential for conservation of fish stocks (Locascio & Mann 2008, Luczkovich et al. 2008, Rowell et al. 2015). Traditional sampling methods for determining spawning sites and times (e.g. collecting larvae, gonadal condition assessment) require tremendous labor, may not allow high spatial or temporal coverage, and are often not reliable as there is uncertainty of species identification at early life stages and variation in gear efficiency between habitats (Kellison et al. 2003, Luczkovich et al. 2008, Rowell et al. 2015). The sounds produced by soniferous fish are species- and activity-specific, which allows not only for fish identification, but also characterization of their activity (Mok & Gilmore 1983, Luczkovich et al. 2008, Rowell et al. 2015). Though there have been studies aimed at identifying fish spawning sites through passive acoustics, methods were not consistent, and there does not appear to be a consensus on how these surveys should be conducted. A benefit of longer-term soundscape studies with high temporal and spatial resolution, like the present study, is the ability to get a more complete picture of when and how various soniferous species are using the study area. Short, snapshot recordings at multiple sites may miss important aspects of acoustic activity; for example, the influence of nighttime and tidal phase on silver perch chorusing would be missed with a single nightly recording window. This single season study limits our ability to test for seasonal patterns (e.g. snapping shrimp activity; Bohnenstiehl et al. 2016) and missed peak spawning periods of other soniferous fish species that spawn in late spring (May), such as the weakfish, or late summer (September), such as the red drum (Luczkovich et al. 2008). It is necessary to have multiple, simultaneous, recordings over several seasons to understand how and when these fish species are using these habitats, to better inform management of stocks, and conservation of these essential habitats.

Underwater soundscapes are inherently complex, and relationships between acoustic and environmental variables are difficult to disentangle for complex, shallow estuarine habitats. Despite these difficulties, soundscape analysis illustrates the importance of these habitats as essential fish spawning habitats. Patterns in the low-frequency band and fish chorusing revealed the interacting role of nighttime and tidal phase on fish behavior and habitat use in Middle Marsh, while the diurnal pattern in snapping shrimp activity adds to our understanding of this ubiquitous sound-producer. Future characterizations of estuarine soundscapes must consider the influence of water level and other environmental variables. Moreover, the development of fish call detectors will greatly advance the use of passive acoustics in management and conservation of essential fish habitat.

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