

1 **Title:** Soundscapes of natural and artificial temperate reefs: similar temporal patterns but distinct
2 spectral content

3

4 **Authors:** Rebecca V. Van Hoeck^{1*}, Avery B. Paxton^{2,3}, DelWayne R. Bohnenstiehl⁴, J.
5 Christopher Taylor², F. Joel Fodrie⁵, Douglas P. Nowacek⁶, Christine M. Voss⁵, Charles H.
6 Peterson⁵

7

8 **Affiliations:**

9 ¹Department of Biology, University of North Carolina at Chapel Hill, 120 South Road, Chapel
10 Hill, NC 27599, USA.

11 ²National Ocean Service, National Centers for Coastal Ocean Science, National Oceanic and
12 Atmospheric Administration, 101 Pivers Island Road, Beaufort, NC 28516, USA.

13 ³CSS Inc., 10301 Democracy Lane Suite 300 Fairfax, Virginia 22030, USA; Under
14 NOAA NCCOS Contract # EA133C17BA0062.

15 ⁴Department of Marine, Earth, and Atmospheric Sciences and Center for Geospatial Analytics,
16 North Carolina State University, 2800 Faucette Dr., Raleigh, NC 27607, USA.

17 ⁵Institute of Marine Sciences, University of North Carolina at Chapel Hill, 3431 Arendell Street,
18 Morehead City, NC 28557, USA.

19 ⁶Nicholas School of the Environment & Pratt School of Engineering, Duke University Marine
20 Lab, 135 Duke Marine Lab Road, Beaufort, NC 28516, USA.

21

22 ***Correspondence:** rebeccavh@unc.edu

23

1 **Running page head:** Natural versus artificial reef soundscapes

2

3 **Abstract:** Marine soundscapes often differ among habitats; however, relatively little is known
4 about whether soundscapes on naturally occurring habitats differ from soundscapes on human-
5 made structures. To address this question, we investigated whether temporal and spectral
6 characteristics of biological sound production differ between natural and artificial offshore reefs.
7 Specifically, we analyzed recordings from five, week-long hydrophone deployments on two
8 natural rocky reefs and two artificial reefs on the North Carolina, USA, continental shelf.

9 Analysis of sound pressure level (SPL) on hourly and seasonal scales revealed similar temporal
10 patterns between the reef types. These patterns were largely driven by four dominant fish
11 vocalizers with seasonal chorusing patterns, including a toadfish species (*Opsanus spp.*). Despite
12 similar temporal patterns within reef type soundscape spectral content was more similar within
13 reef type than between, especially during the April deployment - which had the most acoustic
14 activity. Our findings suggest that the soundscapes of shipwreck artificial reefs may differ from
15 the soundscapes of natural rocky reefs, possibly due to differing community composition. As
16 sound plays an important role in the navigation and settlement of many marine species,
17 soundscape differences between natural and artificial habitats could affect ecosystem function
18 through species behavior and interactions.

19

20 **Keywords:** marine soundscape, artificial reef, temperate reef, spectral dissimilarity index,
21 bioacoustics

22

23

1 **1. INTRODUCTION**

2 The ways in which animals make and perceive sounds play an important role in the
3 ecology of many species. Individuals use sound to communicate and interpret a wide array of
4 social and ecological cues, including territorial aggression, group cohesion, mate attraction,
5 gamete release synchronization, navigation, and settlement (Suthers et al. 2004, Popper &
6 Hawkins 2019). Among the more than 30,000 extant fishes, over 800 teleost fish species,
7 representing 30 families, are known to produce species-specific calls for communication
8 (Rountree et al. 2006). Moreover, all fishes possess the morphological capability to perceive
9 acoustic particle motion and many species have specialized hearing due to connections between
10 the inner ear and gas-filled sacs, such as swim bladders (Ladich 2014). These connections can
11 facilitate lower hearing thresholds, broader frequency sensitivity, and detection of sound pressure
12 (Popper & Fay 2011).

13 The collection of biological sounds, in combination with geological and anthropogenic
14 sounds, across a landscape form the soundscape (Pijanowski et al. 2011). Early research in
15 marine soundscape ecology identified that ambient acoustic levels are elevated at structured
16 habitats compared to adjacent unstructured benthos (Radford et al. 2011, Lillis et al. 2014).
17 Furthermore, different habitat types have been documented to have different soundscape
18 characteristics, even when situated within a few kilometers of one another. For example, the
19 soundscape of sea urchin dominated reefs in New Zealand contain a greater number of snapping
20 shrimp snaps and increased acoustic activity in an 800 – 2500 Hz frequency band compared to
21 macro-algae dominated reefs and beach habitats (Radford et al. 2010). Additionally, Radford et
22 al. (2014) documented distinct temporal and spectral characteristics at adjacent fringing reefs,
23 back reefs, and lagoon sites.

1 Across numerous taxa and life stages, marine organisms respond to underwater sound.

2 For example, many species of coral reef fish larvae (Tolimieri et al. 2000, Tolimieri et al. 2004,
3 Leis & Lockett, 2005) and crab post-larvae (Radford et al. 2007) exhibit a directional swimming
4 response to broadcasted reef sounds, and juvenile and adult coral reef fish use sound to guide
5 nocturnal migrations (Radford et al. 2011). Similarly, among the planktonic larvae of sessile
6 invertebrates, oyster (Lillis et al. 2013) and coral (Vermeij et al. 2010) settlement increases in
7 response to reef sound. As a result of the broad use of sound as a navigational and settlement cue
8 among marine organisms, differences in broadcasted soundscapes among distinct habitats and
9 habitat types may affect recruitment processes, community structure, and habitat function.

10 Artificial reefs are frequently introduced to marine environments to increase habitat

11 availability and enhance fishery productivity (Pickering & Whitmarsh 1997). To understand the
12 success of artificial reef deployment for conservation and management goals, comparisons with
13 natural reefs are required (Carr & Hixon 1997). Following colonization by fish, artificial reefs
14 often support different community compositions and greater biomass than natural reefs. This
15 pattern of increased biomass is especially pronounced in planktivorous species, leading to an
16 altered trophic structure of artificial reef communities compared to natural reef communities
17 (Arena et al. 2007, Simon et al. 2013, Paxton et al. 2017). Differences in community
18 composition between artificial and natural reefs may produce distinct soundscapes on each reef
19 type, especially in terms of biophony. If marine organisms are using sound to navigate their
20 environment and make habitat selections, differences in the soundscapes of natural and artificial
21 reefs could lead to recruitment of different species, thereby affecting the function of artificial
22 reefs. A few studies have explored soundscape characteristics at artificial patch reefs, frequently
23 constructed out of cinder blocks (Ghazali et al. 2013, Lyon et al. 2019) though, to our

1 knowledge, the soundscape characteristics of artificial reefs have not been compared to natural
2 reefs.

3 In the present analysis, we evaluate whether temperate marine soundscapes differ
4 between natural and artificial reefs. Specifically, we document the fish vocalizers that exhibited
5 seasonal chorusing behavior as well as describe and compare the temporal and spectral
6 soundscape characteristics of four temperate reefs offshore of North Carolina – two natural and
7 two artificial. Lastly, we discuss the potential ecological implications of distinct soundscapes
8 broadcasted on natural and artificial reefs and on individual habitats more broadly.

9 **2. MATERIALS & METHODS**

10 **2.1. Study Sites**

11 Two natural and two artificial reefs in Onslow Bay, North Carolina (NC), USA, were
12 selected for soundscape description and comparison (Fig. 1a). Onslow Bay has a heterogeneous
13 seafloor consisting of sandy benthic substrates, hardbottom formed by rocky reef ledges and
14 pavements, as well as numerous artificial reefs, including historic shipwrecks, intentionally
15 scuttled ships, and other human-made structures (Department of the Navy, 2009). The reefs
16 included in this study range from 41.5 – 50.4 km from Beaufort Inlet, and 35.4 – 42.4 km from
17 the shelf break. The natural reefs include two rocky reef ledges: 210 Rock (34° 14.448' N, 76°
18 35.538' W) and West Rock (34° 19.368' N, 76° 36.396' W), located at 32 m and 30 m depths,
19 respectively. The artificial reefs include a 55.8 m U.S. Coast Guard Buoy Tender at 34 m depth,
20 *Spar* (34° 16.626' N, 76° 38.730' W), and a 133.8 m U.S. Navy Cable Layer at 35 m depth,
21 *Aeolus* (34° 16.698' N, 76° 38.592' W). Both artificial reefs were intentionally scuttled as part of
22 the North Carolina Division of Marine Fisheries Artificial Reef Program (AR-305; N. C.
23 Department of Natural Resources & Fisheries 1988). The *Spar* was sunk in June 2004 and is

1 fully intact. The *Aeolus* was sunk in July 1988 and consists of an intact bow and stern with a
2 region of rubble in the middle.

3 2.2 Acoustic Data Collection

4 Underwater sound was recorded concurrently on each site during up to five,
5 approximately week-long deployments between November 2015 and August 2016 (Table S1).

6 Both natural reefs and the *Spar* were sampled during all five deployments. We intended to
7 sample two artificial reefs during all deployments; however, strong current and sediment
8 movement at an initially selected artificial reef site impeded data collection. As a result, the
9 *Aeolus* was selected as a contingency site during the third through the fifth deployments.

10 Continuous recordings were made using calibrated, omni-directional hydrophones (SoundTrap
11 202 STD, Ocean Instruments, New Zealand) mounted 0.5 m above the seafloor on a weighted,
12 metal conical frame which was placed approximately 5 m from the habitat structure (Fig. 1b).
13 The positions of the hydrophones and frames were fixed across all deployments.

14 Sound pressure was recorded continuously at a rate of 96 kHz, with instruments
15 producing a flat (± 3 dB) frequency response between 0.020 – 43.0 kHz. To reduce computational
16 challenges associated with continuous recordings and facilitate rapid visual screening of acoustic
17 activity via spectrogram, the audio was initially subsampled to two-minute recordings every 15
18 minutes for the duration of the deployments. These subsamples mimic the typical duty cycle
19 employed in many marine soundscape ecology studies (e.g., Bohnenstiehl et al. 2018). All
20 acoustic processing was conducted in MATLAB using purpose-written code (MATLAB
21 R2019b). Each file was demeaned, and response corrected to μ Pa using the hydrophone specific
22 calibration value.

1 Time-series and spectral analysis of the two-minute subsamples identified sporadic,
2 anomalously large amplitude impulsive signals that drastically altered the sound pressure level
3 (SPL) time-series and power spectra (Fig. 2, Fig. S1). These impulsive signals may be produced
4 when a swimming animal collides with the instrument frame or hydrophone (i.e., fish bumps;
5 e.g. Buskirk et al. 1981, Bowman & Wilcock 2014). To remove the effect of the impulsive
6 signals and reduce the intrusion of anthropogenic noise, each two-minute recording was further
7 subsampled by extracting the eight quietest five-second duration, non-overlapping time windows
8 within the file. This resulted in a summary of 40 seconds of recorded audio every 15 minutes. To
9 accomplish this subsampling, a Fast Fourier Transform was applied to the full two-minute file
10 (NFFT = 2^{15} points, 0% overlap, and Hanning window). Next, the average root-mean-square
11 (RMS) bandwidth power of every five-second, non-overlapping time window within the two-
12 minute file was summarized and sorted from quietest to loudest. The average acoustic spectra for
13 each file were then calculated by summarizing only the eight quietest five-second windows (Fig.
14 2; see supplement for further explanation). Across a deployment, this acoustic summary resulted
15 in a matrix where each column represented the mean spectra of a recording and each row
16 contained the power at a given frequency (frequency resolution, $\Delta f = 2.92$ Hz). Band-limited
17 SPLs were then calculated by integrating the acoustic power over the appropriate rows in this
18 matrix. All SPL values are RMS and reported in units of dB referenced to 1 μ Pa.

19 2.3 Statistical Analyses

20 *2.3.1 Fish chorusing*

21 Spectrograms and acoustic spectra of individual recordings were visually inspected to
22 identify dominant fish vocalizations and chorusing as well as rapidly screen for anthropogenic
23 noise. The source of frequently observed sounds (biological, anthropological, or unknown) and

1 the species identity of biological vocalizers were confirmed by both aural and visual inspection
2 of the recording when possible. Observed vocalizations were compared to described fish calls in
3 bioacoustic catalogues (Fish & Mowbray 1970) and the peer-reviewed literature in attempt to
4 identify the species (Lobel et al. 2010, Staaterman et al. 2014, Mooney et al. 2016).

5 Representative calls and daily calling pattern of each type of dominant fish vocalization were
6 documented via spectrogram with an appropriate time and frequency resolution for each call
7 type. Average acoustic features of each call type were summarized using purpose-written code
8 by extracting call samples from all sites and deployments when calls were observed.

9 *2.3.2 Temporal Patterns*

10 To evaluate acoustic activity in an ecologically relevant manner, data were separated into
11 a low- and high-frequency band. The low-frequency band, from 0.1 – 2.0 kHz, was selected to
12 isolate sounds from fishes and minimize ambient noise from geologic origins, such as rain or
13 wind at the surface (Urick 1983, Hildebrand 2009). The high-frequency band, 7 – 20 kHz, was
14 selected to isolate invertebrate sounds, predominantly snapping shrimp (Everest et al. 1948). The
15 gap between selected frequency bands intentionally excludes intermediate frequencies, which
16 contain overlaps between fish and invertebrate sounds.

17 Temporal variation in SPL was examined on daily and seasonal scales. As week-long
18 recordings were made at up to five time points over the course of ten months, we refer to each
19 deployment by the month it occurred in and among deployment variation as seasonal variation.
20 To evaluate differences in observed SPLs among the reef-types, sites, and deployments we
21 conducted a two-way ANOVA for each frequency band. To identify which sites and
22 deployments were contributing to significant differences we conducted pairwise comparisons
23 using Tukey's honest significant difference tests. Due to observed diurnal patterns in SPLs and

1 their relation to the photoperiod, we isolated the recordings between sunset and astronomical
2 twilight (henceforth called dusk) when daily SPLs peaked across all sites and deployments, for
3 comparison. To account for temporal autocorrelation among the acoustic files, dusk SPLs were
4 averaged for each day within a deployment. As a result, the number of replicates included for
5 each site and deployment combination was equal to the number of days in a deployment.

6 For each frequency band, we evaluated the differences between reef types and among
7 sites separately for a total of four ANOVAs (low frequency by reef type, low frequency by site,
8 high frequency by reef type, high frequency by site). We first investigated differences in dusk
9 SPL aggregated by reef-type. The full model for each frequency band included dusk SPL as the
10 response variable and reef-type, deployment, and an interaction between reef-type and
11 deployment as predictor variables. The site-level model also included site, deployment, and an
12 interaction between site and deployment as predictor variables for each frequency band. For all
13 models (reef-type level and site level, for high- and low-frequency bands) removal of the
14 interaction term significantly worsened the fit of the model and inspection of normal Q-Q plots
15 demonstrated that the assumptions of normality were met; therefore, we proceeded with the full
16 model and Gaussian distributions for both frequency bands. All statistical analysis was
17 conducted using the programming software R version 3.6.0 (R Core Team, 2019).

18 *2.3.3 Spectral Content*

19 To evaluate dissimilarity in soundscape spectral content at each site, we used distance-
20 based redundancy analysis (dbRDA) – a multivariate method that uses pairwise ecological
21 distances to map variables in reduced dimensional space allowing visual assessment of patterns
22 in the data. DbRDA was conducted on each deployment individually, resulting in five
23 ordinations. The distance between pairwise samples was calculated using the spectral

1 dissimilarity index developed by Sueur et al., (2008). The spectral dissimilarity is calculated as
2 [Eq. 1]:

3 [Eq. 1]
$$D_f = \frac{1}{2} \sum_{f=1}^N |S_1(f) - S_2(f)|, \text{ with } D_f \in [0,1]$$

4 where D_f represents the dissimilarity between two samples on a scale from 0 to 1, f represents the
5 frequency bins over which the index is evaluated and $S_1(f)$ and $S_2(f)$ represent the probability
6 mass functions of the two spectra being compared. In our study, $S_n(f)$ and D_f were evaluated
7 over the low-frequency band (0.1-2.0 kHz) using the mean hourly spectra recorded during
8 nighttime hours. Only nighttime recordings were included because this was an observed period
9 of increased biological activity and reduced anthropogenic noise.

10 To identify what acoustic activity was driving sample separation, the proportion of total
11 acoustic power within select frequency bands was calculated for each sample. These frequency
12 bands were determined by viewing the spectra and identifying common peak frequencies (e.g.
13 0.1 – 0.3 kHz, 0.3 – 0.5 kHz, 0.5 – 0.8 kHz, and 0.8 – 2 kHz). The formula for each dbRDA was
14 the spectral dissimilarity distance matrix constrained by the proportion of acoustic power
15 contained within these smaller frequency bands. As such, the frequency bands driving sample
16 separation are indicated by the loading vectors and the eigenvalues of each ordination dimension
17 represent the amount of variability explained by the loading constraints.

18 To evaluate whether spectral content varied by reef-type, a multivariate Analysis of
19 Similarity (ANOSIM) was conducted on the spectral dissimilarity matrix from each deployment.
20 ANOSIM is a nonparametric test that evaluates the null hypothesis that there are no differences
21 in dissimilarity within and between groups. To evaluate this hypothesis, ANOSIM ranks all
22 pairwise dissimilarities from a distance matrix, summarizes the mean ranks between and within
23 groups, calculates a test statistic, and evaluates significance via Monte Carlo permutations ($n =$

1 1000). The test statistic, R , is expected to be 0 under the null hypothesis and 1 when all pairs
2 between groups are more dissimilar than pairs within groups (Clarke 1993). All multivariate
3 analyses were conducted using the “vegan” package in R (Oksanen et al. 2019).

4 **3. RESULTS**

5 **3.1 Fish Chorusing**

6 The dominant fish vocalizers that exhibited seasonal chorusing patterns consisted of
7 toadfish boatwhistles (*Opsanus* spp.), and three unidentified vocalizers described as a knock,
8 creak, and growl. (Fig. 3). Though the unidentified calls were compared to similar calls reported
9 in various fish call databases, there were not close enough matches to confidently report a
10 species identity. To facilitate future identification, temporal and spectral features of each call
11 type were summarized (Table 1).

12 Fish choruses were observed in November, April, and June and all chorusing species
13 were observed on both natural and artificial reefs. Toadfish chorusing was observed in April and
14 June but was most abundant in April. During April, toadfish calls were observed on all sites at all
15 times throughout the day, with the onset of chorusing usually observed at 20:00 EDT and lasting
16 until 06:00 EDT (Fig. 4a). The daily patterns in SPL in the low-frequency band in April can be
17 attributed largely to this calling behavior of toadfish across all sites (Fig. 5). Toadfish choruses
18 also were observed on all sites except West Rock in June, with chorus onset around 21:00 EDT
19 lasting until 05:00 EDT.

20 The unidentified knock was an impulsive sound frequently in sets of multiple knocks
21 (Fig. 3b). Choruses of the knocks were observed on all sites during April, while occasional
22 knocks were observed in all deployments. During April, the daily pattern consisted of a rapid

1 onset of a dusk chorus at 20:00 EDT that was maintained for approximately one hour with
2 occasional knocks continuing for up to four hours (Fig. 4a).

3 The unidentified creak was observed on the *Spar* and West Rock in November from
4 approximately 19:00 EDT – 06:00 EDT, with periods of most intense chorusing during
5 crepuscular periods (Fig. 4b). This call consisted of multiple pulse sets in a pulse train. Each
6 pulse set consisted of three pulses alternating in frequency (Fig. 3c). The first and third pulses
7 had a peak frequency of 1669.4 ± 101.7 Hz on average while the second pulse peaked at 2728.7
8 ± 156.1 Hz. Each pulse varied in duration, lasting 1.7 ± 0.8 ms, ms, and 2.1 ± 1.0 ms,
9 respectively. The first and second pulse were separated by 5.0 ± 1.6 ms and the second and third
10 were separated by 4.9 ± 1.5 ms.

11 Choruses of the unidentified growl, a low frequency sound with a 90-500 Hz bandwidth,
12 were also observed on all sites in November and January (Fig. 3d). The chorus had a rapid onset
13 at 19:00 EDT, lasted one hour with occasional growls observed until 07:00 EDT (Fig. 4b).

14 Similar to the creaking sound, the growl consisted of multiple pulse sets in a pulse train. Each
15 pulse set contained two pulses alternating in frequency with 26.5 ± 11.8 ms between the center of
16 each pulse. The first pulse had an average duration of 40.1 ± 11.7 ms and peak frequency of
17 100.8 ± 22.9 Hz, while the second pulse was 19.5 ± 4.9 ms long with a 160.9 ± 57.3 Hz peak
18 frequency.

19 3.2 Temporal Patterns

20 In general, the temporal patterns of biological sound production were similar across all
21 reefs. Within the low-frequency band (0.1 – 2 kHz), dominated by fish sounds, daily patterns
22 across all sites consisted of increased SPL at dusk and generally greater SPLs at night than
23 during the day (Fig. 5). Seasonally, the intensity of the dusk peak varied, corresponding with the

1 presence of fish chorusing. During November, there was also a peak in acoustic activity on the
2 *Spar* and West Rock at dawn that can be attributed to chorusing by the unidentified creaking
3 species.

4 Within the low frequency band, dusk SPL did not differ between natural and artificial
5 reefs (ANOVA, $F_{\text{reef type}}(1,113) = 2.63, p = 0.108$). All sites broadly exhibited similar seasonal
6 trends; however, dusk SPL significantly differed among sites (ANOVA, $F_{\text{site}}(3,105) = 3.37, p <$
7 0.021), deployment (ANOVA, $F_{\text{deployment}}(4,105) = 63.56, p < 0.001$), and an interaction between site
8 and deployment (ANOVA, $F_{\text{site*deployment}}(10,105) = 2.00, p = 0.040$). Post-hoc Tukey's HSD tests
9 revealed that differences in dusk SPL were driven largely by seasonal variation as there were no
10 significant differences among sites within a deployment (Fig. 6). Overall, dusk SPL decreased
11 significantly from November to January, increased drastically to a maximum in April, then
12 decreased to a minimum in August (Fig. 7). The April deployment, which coincided with the
13 most abundant fish chorusing, was significantly louder than all others. Lastly, SPLs between the
14 sites across the entire sampling period only significantly differed between two sites, with the
15 *Spar* supporting greater levels than West Rock (Tukey HSD, $p = 0.015$).

16 Within the invertebrate dominated high-frequency band (7 – 20 kHz), crepuscular peaks
17 in SPL and elevated SPL at night were observed in all sites and seasons (Fig. 8). Investigation of
18 dusk SPLs identified significant differences between the reef types, with artificial reefs
19 supporting louder high-frequency soundscapes (ANOVA, $F_{\text{reef type}}(1,113) = 99.55, p < 0.001$). SPLs
20 also varied by deployment (ANOVA, $F_{\text{deployment}}(4,113) = 19.89, p < 0.001$) and an interaction
21 between reef type and deployment (ANOVA, $F_{\text{reef type*deployment}}(4,113) = 8.73, p < 0.001$). Post-hoc
22 Tukey's HSD test revealed that artificial reef SPLs were significantly higher than natural reefs in

1 November ($p < 0.001$), January ($p < 0.001$), and April ($p < 0.001$), but not in June ($p = 0.587$) or
2 August ($p = 0.998$; Fig. 9).

3 Comparisons of dusk SPLs at the site level revealed significant differences among sites
4 (ANOVA, $F_{\text{site}(3,105)} = 342.85$, $p < 0.001$), deployments (ANOVA, $F_{\text{deployment}(4,105)} = 124.17$, $p <$
5 0.001), and their interaction ($F_{\text{site} \times \text{deployment}(10,105)} = 13.45$, $p < 0.001$). Pairwise comparisons
6 among the deployments revealed that seasonal variation was mostly driven by reduced SPLs
7 during January (Tukey HSD, Fig. 10a). While, variation among the sites was driven by increased
8 SPLs on the *Spar* and reduced SPLs at West Rock (Tukey HSD, Fig. 10b). Pairwise comparisons
9 among sites within deployment revealed many significant differences. Notably, dusk SPLs were
10 always higher on the *Spar* than the *Aeolus* (Tukey HSD, Apr. $p < 0.001$; Jun. $p < 0.001$; Aug. $p <$
11 0.001) and tended to be higher on 210 Rock than West Rock (Tukey HSD, Jan. $p < 0.001$; Apr. p
12 < 0.001 ; Aug. $p < 0.001$).

13 3.3 Spectral Content

14 During seasons with increased SPLs and fish chorusing events, specifically April and
15 June, the spectral content of each reef's soundscape became more distinct. This is shown by
16 tighter grouping of samples within site and greater separation between sites (Fig. 11). Moreover,
17 as each sample represents an hour, temporal trends in spectral activity can be observed over the
18 course of the night. Pairwise D_f values for each ordination ranged from 0.1 to 0.8 suggesting that
19 there were substantial differences between some pairwise spectral probability mass functions.

20 Evaluating low-frequency spectral differences using dbRDA allowed the ordinations to
21 be described in terms of the acoustic activity driving the differences between sites. The smaller
22 frequency bands used to constrain each ordination generally represented a unique dominant fish
23 caller in the average spectra. Activity in the 100-300 Hz range was usually attributed to

1 miscellaneous low-frequency sounds, and in some deployments a toadfish peak. The 300-500 Hz
2 band was indicative of toadfish, the 500-800 Hz band of the unidentified knock, and the 800-
3 2000 Hz band of the unidentified creaking call.

4 Comparison of spectral dissimilarities between natural and artificial reefs revealed that
5 spectral content significantly varied by reef type in all deployments except November
6 (ANOSIM, $R = 0.06$, $p = 0.19$). The separation between reef types was greatest during April
7 (ANOSIM, 0.64 , $p = 0.001$), with artificial reef position driven by activity in the 300-500 Hz
8 band while natural reefs were driven by 100-300 Hz activity. Separation between the reef types
9 was also significant in January (ANOSIM, $R = 0.39$, $p = 0.001$), June (ANOSIM, $R = 0.40$, $p =$
10 0.001), and August (ANOSIM, $R = 0.38$, $p = 0.001$).

11 Analysis of November showed that activity within the 100-300 Hz band was driving the
12 separation of 210 Rock from the other sites, while the overlap in *Spar* and West Rock samples
13 was driven by activity in the 800-2000 Hz band (Fig 11a). This 800-2000 Hz activity, on both a
14 natural and artificial reef, aligns with the timing of the unidentified creaking chorus and explains
15 the lack of significant difference between the reef types during November (Fig. 11b). Overall,
16 the loading vectors explained 93.8% of the variation among the samples, with 58.3% captured on
17 axis 1 and 35.5% captured on axis 2.

18 In January 2016, the average spectra of each site contain a unique peak that drove its
19 loading (Fig. 11d). The *Spar* and West Rock samples were each tightly clumped suggesting
20 minimal spectral change throughout the night, with the *Spar* being driven by the 300-500 Hz
21 band and West Rock the 800-2000 Hz band. The majority of 210 Rock samples plotted between
22 the *Spar* and West Rock; however, an increase in activity in the 500-800 Hz band from 2-6 hours
23 after sunset drove some separation of the samples (Fig. 11c, d). Overall, the loading vectors

1 explained 86.0% of the variation among the samples, with 60.0% captured on axis 1 and 26.0%
2 captured on axis 2.

3 Within April, each site showed distinct spectral separation from the other sites, although
4 a consistent temporal trend was observed among all sites (Fig. 11e). This temporal trend was
5 driven by an increase in activity in the 500-800 Hz band, attributed to the knocking chorus,
6 ranging from 1-4 hours after sunset, with the duration of the increase varying across sites (Fig.
7 11f). Overall, the loading vectors explained 84.7% of the variation among the samples in April,
8 with 48.4% of the variation captured on axis 1 and 36.3% captured on axis 2.

9 Within June, natural reef samples each ordinate closely within site, with the loadings of
10 210 Rock driven by activity in the 100-300 Hz band and West Rock driven by the 500-800 Hz
11 and 800-2000 Hz bands (Fig. 11g). Although within site grouping was apparent for the artificial
12 reefs, their samples broadly ordinated similarly with their loadings driven by minimal activity in
13 the 300-500 Hz, 500-800 Hz, and 800-2000 bands (Fig. 11g, h). Overall, the loading vectors
14 explained 91.2% of the variation among the samples in June, with 58.1% of the variation
15 captured on axis 1 and 33.1% captured on axis 2.

16 In August, the samples within each site clumped tightly together with minimal separation
17 among the sites (Fig. 11i). The loadings of West Rock were driven by a broad peak between
18 1500 and 1750 Hz and align strongly with the 800-2000 Hz vector (Fig. 11i, j). Among the other
19 three sites, the average spectra showed that there were few to no distinct spectral peaks
20 associated with a specific caller (Fig. 11j). Overall, the loading vectors explained 87.6% of the
21 variation among the samples in August, with 75.2% and 12.4% of the variation captured on axis
22 1 and 2, respectively.

23 **4. DISCUSSION**

1 Our research demonstrates that soundscape characterization is a novel approach towards
2 testing whether artificial reefs mimic natural reefs. We documented the soundscapes of four
3 temperate reefs, two natural and two artificial, during five sampling periods across a 10-month
4 period. Although the broad temporal patterns were consistent across all reefs regardless of reef
5 type, these patterns were driven largely by the timing of dominant sound sources. Further
6 analyses of finer details available in the spectral content revealed distinct soundscapes on each
7 site, with spectral differences generally greater between natural and artificial reefs than within
8 reef type. This separation between natural and artificial reefs was especially pronounced during
9 time periods with increased acoustic activity and higher SPLs. These spectral differences may be
10 the result of differing community compositions and trophic structures on natural and artificial
11 reefs. Moreover, consistent soundscape differences across reef types could affect artificial reef
12 function through species behavior and interactions in response to sound.

13 4.1 Comparisons between reef types

14 While the general pattern of crepuscular peaks in SPL aligning with the seasonal
15 photoperiod was similar between the reef types, high frequency dusk SPLs were significantly
16 higher on artificial reefs than natural reefs during three out of five deployments. More complex
17 habitat structures, such as those of healthy sponge-dominated reefs, are known to host higher
18 densities of snapping shrimp and are associated with higher observed snap rates and high-
19 frequency SPLs (Butler et al. 2016). One possible explanation for elevated SPLs on artificial
20 reefs is that the higher vertical relief and resulting habitat complexity of shipwrecks (Paxton et
21 al. 2017) could support higher densities of snapping shrimp than the comparatively diffuse
22 habitat structure of a natural rocky reef ledge.

1 Many marine soundscape studies have evaluated differences among sites or habitat types;
2 however, few have employed multivariate analyses such as dbRDA. The strong consistency
3 between the observed average spectra and separation of samples according to the ordination
4 loading vectors suggest that this method is appropriate and informative for evaluating differences
5 in soundscape spectral composition. Across most deployments, the samples grouped most
6 similarly within their site and were separated from samples of other sites, demonstrating that
7 each site exhibited a unique spectral composition. This is notable especially for the two artificial
8 reefs which are situated only 250 m from one another.

9 The April deployment, which consisted of the highest SPLs and most abundant low-
10 frequency acoustic activity, provided the most interesting result. During April, the night
11 soundscape of all sites contained choruses of toadfish and unidentified knocks. Despite the
12 presence of the same chorusing species on each reef, there was still substantial spectral
13 separation of the sites. The soundscapes also were separated by reef type, with the artificial reefs
14 exhibiting similar spectral content in the 300-500 Hz band and the natural reefs in the 100-300
15 Hz band.

16 Within our study system in Onslow Bay, NC, comparative surveys of natural and
17 artificial reefs have found that artificial reefs and specifically ships support greater fish biomass
18 than natural reefs, whereas other metrics such as species richness are similar between reef types
19 (Paxton et al. 2017, Paxton et al. 2019). Moreover, differences in community composition by
20 reef type are driven by greater abundances of large piscivores and water column planktivores,
21 such as jacks and scad respectively, on artificial reefs, leading to an altered trophic structure
22 (Paxton et al. 2017). Given the presence of the same dominant vocalizers across all reefs, the
23 spectral separation between the reef types is likely a result of differences in the proportion of the

1 total soundscape that the dominant vocalizers occupy, as well as differences in miscellaneous
2 acoustic activity, including less dominant calls and sounds associated with feeding, that are not
3 currently attributable to a certain vocalizer or behavior. As artificial and natural reefs frequently
4 support communities that differ in trophic structure (Arena et al. 2007, Simon et al. 2013, Paxton
5 et al. 2017), it is plausible that there would be differences in sounds associated with feeding on
6 natural and artificial reefs. It would be valuable to explore whether these non-vocalization
7 sounds can be attributed to specific sources or behaviors, possibly through the use of combined
8 visual surveys and multi-hydrophone localization arrays.

9 The differences in spectral composition documented on the temperate hardbottom reefs
10 included in this study may have important ecological implications. Multiple studies have shown
11 that different habitats and specifically different reef types broadcast distinct soundscapes
12 (Radford et al. 2010, 2014, Lillis et al. 2014a). We provide initial evidence that shipwreck reefs
13 may broadcast distinct soundscape from natural reefs, as well. Given the ability of fishes to
14 localize a sound source (Sand & Bleckmann 2008, Hawkins & Popper 2018) and marine
15 organisms' attraction to habitat-associated soundscapes, soundscape differences between habitats
16 may play a role in facilitating recruitment to reef habitats and could perpetuate differences
17 among reef types or benthic habitats more broadly. Models of sound propagation away from
18 reefs suggest that habitat-associated sounds, and specifically chorusing events, can be detected
19 on the order of kilometers away from a reef. (Radford et al. 2011b, Lillis et al. 2014b). We
20 propose that if a migrating individual encountered competing acoustic cues from adjacent
21 habitats and the soundscape of one reef has a dominant signal in a preferred frequency band,
22 such as one associated with conspecifics, the individual may be more likely to settle at that reef.
23 As different species of fishes have unique auditory sensitivities, these behavioral patterns could

1 ultimately support distinct communities on separate reefs. Future research on whether marine
2 animals are able to perceive small differences in acoustic signals and whether they are attracted
3 to acoustic activity in specific frequency ranges would facilitate a better understanding of
4 whether the spectral differences we observed across multiple reefs have a meaningful ecological
5 effect.

6 4.2 Comparisons among sites

7 We documented strong diurnal patterns in biological acoustic activity, with tight ties to
8 the photoperiod. Within the fish-dominated, low-frequency band these patterns were similar to
9 those documented in other marine soundscape studies with SPLs quietest during the day, loudest
10 at dusk, and remaining elevated through the night. While there were not significant differences
11 between the reef types, the diurnal pattern was generally consistent across all sites and seasons
12 with the level of the dusk peak varying seasonally accordant with the amount of fish chorusing
13 observed. Across all sites, the daily pattern was least apparent during January and August,
14 coinciding with the deployment with the quietest dusk SPLs. During these deployments, few
15 distinct fish calls were observed and there was no presence of fish chorusing. Alternatively, the
16 diurnal patterns in acoustic activity were most apparent in April and can largely be attributed to
17 frequent calling and chorusing by toadfish (*Opsanus spp.*) and the dusk chorus of the
18 unidentified knock.

19 Investigation of low-frequency spectral content identified distinct soundscapes on all
20 reefs, with separation among the sites most pronounced during April and June. As previously
21 summarized, the April soundscape consisted of choruses of toadfish and the unidentified knock.
22 The onset of the knock chorus, with a peak frequency around 650 Hz, occurred immediately after
23 sunset and continued most intensely for one hour, with occasional knocks observed until

1 approximately 4 hours after sunset. This pattern is clearly visible in the corresponding
2 ordination, with a temporal shift away from activity in the 500-800 Hz band as the night
3 progressed. This common temporal pattern among the dominant vocalizer yet maintained
4 spectral separation among the sites and reef types most clearly summarizes the finding of similar
5 temporal patterns but distinct spectral content on temperate reefs.

6 Within the invertebrate-dominated, high-frequency band, acoustic levels were
7 consistently lowest during the day, peaked at dawn and dusk, and were elevated at night. Similar
8 to the low-frequency band, the strength of this daily pattern exhibited strong seasonal variation
9 with the quietest SPLs observed across all sites sampled in January. Snapping shrimp acoustic
10 activity in shallow-water estuarine systems varies with abiotic variables, such as temperature
11 (Bohnenstiehl et al. 2016). The coldest temperatures in Onslow Bay, NC, are generally around
12 January, which may explain the decrease in acoustic activity during that deployment (Whitfield
13 et al. 2014). Additionally, comparisons among the sites revealed that dusk SPLs were always
14 higher on the *Spar* and 210 Rock than the *Aeolus* and West Rock, respectively. This relationship
15 between sites was also mirrored in the low-frequency band during multiple deployments.

16 It is interesting to consider whether there are site-level traits that could explain the
17 consistent pattern of higher SPLs on specific reefs within a reef type. Research in a variety of
18 other marine systems have identified correlations between habitat and community metrics, such
19 as density, abundance, species diversity, and coral cover, and increased SPLs in specific
20 frequency bands (Kennedy et al. 2010, Freeman & Freeman 2016, Staaterman et al. 2017). A few
21 possible mechanisms to explain the elevated SPLs on the *Spar* and 210 Rock are differences in
22 community composition, abundance, or trophic structure that relate to differences in habitat traits
23 such as complexity, vertical relief, size of the reef, or proximity to other reefs. Additionally, reef

1 location and context, such as proximity to the shelf break, prevailing current speed and direction,
2 or level of anthropogenic noise disturbance, could affect the community composition and
3 associated soundscape.

4 Overall, this consistent pattern in relative SPLs among sites in addition to the
5 documented spectral differences among the soundscapes of each reef warrants further
6 exploration of the relationship between habitat and community traits and the soundscape of
7 temperate natural and artificial reefs. As there are known differences in habitat metrics and
8 community composition on each reef we sampled, our findings provide further support that
9 marine soundscapes may be indicative of habitat and community metrics and could be a valuable
10 remote sensing tool to index fish communities. To gain a deeper understanding of whether the
11 documented soundscape differences are correlated with specific habitat or community features
12 further exploration, with a larger sample size, of soundscape characteristics paired with habitat
13 and community traits across multiple reefs within each reef type are needed.

14 4.3 Fish Chorusing

15 Animal vocalizations serve numerous social and ecological roles, and fish vocalizations
16 are frequently associated with reproductive or agonistic behaviors. For example, fish chorusing,
17 or periods of frequent to constant calling, are almost always associated with reproductive
18 behaviors and spawning (Bass & Rice 2010). The acoustic signature of spawning activity makes
19 passive acoustic monitoring and soundscape description a useful method for studying the spatial
20 and temporal variability of marine population dynamics, as well as evaluating habitat utilization.
21 Because our sampling events were spread across multiple seasons throughout the year, we were
22 able to capture some of the temporal variability potentially related to spawning activity on or

1 near the habitats studied. For a thorough understanding of temporal dynamics, long-term
2 recordings with minimal gaps are required.

3 The sonic behaviors of toadfish are among the most well-studied for any fish species.
4 Toadfish make their characteristic boatwhistle call, associated with mating and nest defense, by
5 rapid contraction of muscles lining the swim bladder (Fine & Lenhardt 1983). The oyster
6 toadfish, *Opsanus tau*, is the only documented toadfish in the inshore waters of North Carolina
7 and is known to make seasonal migrations from offshore wintering locations to inshore and
8 estuarine habitats for spawning in spring (Shwartz 1974). The late spring onset of toadfish
9 chorusing we observed on temperate offshore reefs matches seasonal chorusing onset
10 documented in a Chesapeake Bay oyster reef system, as well as on offshore reefs in Georgia and
11 Florida (Ricci et al. 2017, Rice et al. 2017). It is unclear whether the toadfish calls we
12 documented are from a resident offshore population that foregoes seasonal migrations inshore, or
13 if they are a species other than *O. tau*, such as the leopard toadfish, *Opsanus pardus*, which
14 inhabits deeper rocky reefs year-round in the Gulf of Mexico or an analogous undescribed
15 *Opsanus* species.

16 In attempt to identify the specific source of the unidentified choruses, the call features
17 were compared to documented vocalizers in other soundscape studies and soniferous fish
18 collections in the Western North Atlantic. The observed knock vocalization is similar in pulse
19 duration and frequency range to known pomacentrid calls, such has the dusky and bicolor
20 damselfish (*Stegastes adusus* and *S. partitus*), both of which are present on the studied reefs
21 (Spanier 1979). However, pomacentrids are generally more acoustically active during the day,
22 while the knock chorus was observed at dusk (Lobel et al. 2010). In laboratory recordings,
23 tomtate (*Haemulon aurolineatum*) which are abundant on the studied reefs and have a spring

1 spawning season, have also been documented making a similar impulsive vocalization. However,
2 tomtate vocalizations have a longer pulse duration (40-130 ms) and more of a grunt quality than
3 a knock.

4 The unidentified creak was compared to vocalizations of the striped cusk eel (*Ophidion*
5 *marginatum*), but inspection of the frequency spectrum revealed the cusk eel pulse is centered on
6 only one frequency (Mooney et al. 2016), while the creak pulses alternate between two
7 frequencies. For the unidentified growl, gray snapper (*Lutjanus griseus*) adult and larvae make a
8 similar low frequency growl (Staaterman et al. 2014), though to our knowledge there has not
9 been documentation of gray snapper choruses in field or laboratory settings. As a result, visual
10 confirmation of the growl source is required to confidently assign a species identity. While it
11 would be ideal to identify each vocalizer to species, or even family, to enhance understanding of
12 the ecological role of marine soundscapes and their interaction with individuals, it is still
13 possible to explore these interactions without specific identification. Moreover, documentation of
14 the unidentified vocalizations in the literature is critical to facilitating future identification.

15 4.4 Caveats

16 In the current study, we did not evaluate how the soundscapes varied in response to
17 abiotic factors, such as lunar phase, temperature, wind, or sea state. As the sites included in this
18 study are geographically close to one another and range in depth from 30-37m, it is unlikely that
19 wind is a substantial contributor to the soundscape differences documented. Future research
20 investigating how these abiotic factors affect the soundscape of temperate reefs would help to
21 infer whether differences in acoustic levels are site-level differences that can be attributed to
22 ecological differences among the habitats. Additionally, it is important to note that the distance
23 between the sound source and the hydrophone will affect observed SPL, and due to the unique

1 geometries of each reef we were unable to fully standardize the position of the hydrophone
2 relative to the extent, quantity or characteristics of each reef habitat.

3 Lastly, we acknowledge that the sample size of the present study limits our ability to
4 generalize how the soundscapes of artificial reefs relate to that of natural reefs more broadly.
5 However, the consistent spectral differences we observed between the reef types, as well as
6 among all sites warrants further exploration. To date, research on how marine soundscapes vary
7 across habitat and community traits has resulted in promising, but equivocal results. Artificial
8 reefs vary greatly and measurably in area, vertical relief, and heterogeneity, with documented
9 differences in the communities they support. With appropriately designed studies, artificial reefs
10 could be a useful system to better understand the mechanistic relationships between soundscape
11 variation and habitat and community traits.

12
13
14
15
16
17
18
19
20
21

22 **Acknowledgements:** We thank R.C. Rosemond, H.R. Lemoine, E. Ebert, L. Bullock, A. Pickett,
23 D.W. Freshwater, J. Fleming, J. Hughes, M. Kenworthy, G. Sorg, E. Pickering, G. Safrit, S.

1 Davis, C. Lewis, JR. Purifoy and crew from Olympus Dive Center, and T. Leonard and crew
2 from Discovery Diving for diving and boating assistance. We also thank C.M. Tucker and J.
3 Umbanhower for statistical consultation on this project. This research was supported by funding
4 from the Bureau of Ocean Energy Management under cooperative agreement M13AC00006, a
5 Duke Energy Foundation Graduate Fellowship to RVV through UNC's Institute for the
6 Environment, a National Science Foundation Graduate Research Fellowship to ABP under Grant
7 No. DGE-1144081, a P.E.O. Scholar Award to ABP, and a Carol & Edward Smithwick Royster
8 Society of Fellows Dissertation Completion Award to ABP. The views and conclusions
9 contained in this document are those of the authors and should not be interpreted as representing
10 the opinions or policies of the US Government, nor does mention of trade names or commercial
11 products constitute endorsement or recommendation for use.

12
13
14
15
16
17
18
19
20
21

22 **Literature Cited**

23 Arena PT, Jordan LKB, Spieler RE (2007) Fish assemblages on sunken vessels and natural reefs

1 in southeast Florida, USA. *Hydrobiologia* 580:157–171.

2 Bass AH, Rice AN (2010) Vocal-Acoustic Communication in Fishes: Neuroethology. In: *In:*

3 *Encyclopedia of animal behavior. Oxford: Academic Press.* p 558–567

4 Bohnenstiehl DR, Lillis A, Eggleston DB (2016) The Curious Acoustic Behavior of Estuarine

5 Snapping Shrimp: Temporal Patterns of Snapping Shrimp Sound in Sub-Tidal Oyster Reef

6 Habitat. *PLoS One* 11:e0143691.

7 Bohnenstiehl DRD, Lyon RP, Caretti ON, Ricci SW, Eggleston DB (2018) Investigating the

8 utility of ecoacoustic metrics in marine soundscapes. *J Ecoacoustics* 2: #R1156L.

9 Bowman DC, Wilcock WSD (2014) Unusual signals recorded by ocean bottom seismometers in

10 the flooded caldera of Deception Island volcano: volcanic gases or biological activity?

11 *Antarct Sci* 26:267–275.

12 Buskirk RE, Frohlich C, Latham G V., Chen AT, Lawton J (1981) Evidence that biological

13 activity affects Ocean Bottom Seismograph recordings. *Mar Geophys Res* 5:189–205.

14 Butler J, Stanley JA, Iv MJB, Butler MJ (2016) Underwater soundscapes in near-shore tropical

15 habitats and the effects of environmental degradation and habitat restoration. *J Exp Mar Bio*

16 *Ecol* 479:89–96.

17 Carr MH, Hixon MA (1997) Artificial Reefs: The Importance of Comparisons with Natural

18 Reefs. *Fisheries* 22:28–33.

19 Clarke K (1993) Non-parametric multivariate analyses of changes in community structure. *Aust*

20 *J Ecol* 18:117–143.

21 Department of the Navy (2009) Essential Fish Habitat Assessment for the Environmental Impact

22 Statement/Overseas Environmental Impact Statement

23 Everest FA, Young RW, Johnson MW (1948) Acoustical Characteristics of Noise Produced by

1 Snapping Shrimp. *J Acoust Soc Am* 20:137–142.

2 Fine ML, Lenhardt ML (1983) Shallow-water propagation of the toadfish mating call Sonic
3 system of deep-sea cusk-eels (Ophidiidae) View project SHALLOW-WATER
4 PROPAGATION OF THE TOADFISH MATING CALL " Artic Comp Biochem
5 Physiol Part A Physiol Camp Eiochmz Ph~sroi 76:2–231.

6 Fish MP, Mowbray WH (1970) Sounds of Western North Atlantic Fishes: A Reference File of
7 Biological Underwater Sounds. Johns Hopkins Press, Baltimore, MD.

8 Freeman LA, Freeman SE (2016) Rapidly obtained ecosystem indicators from coral reef
9 soundscapes. *Mar Ecol Prog Ser* 561:69–82.

10 Ghazali SM, Montgomery JC, Jeffs AG, Ibrahim Z, Radford CA (2013) The diel variation and
11 spatial extent of the underwater sound around a fish aggregation device (FAD). *Fish Res*
12 148:9–17.

13 Hawkins AD, Popper AN (2018) Directional hearing and sound source localization by fishes. *Cit*
14 *J Acoust Soc Am* 144:3329.

15 Hildebrand J (2009) Anthropogenic and natural sources of ambient noise in the ocean. *Mar Ecol*
16 *Prog Ser* 395:5–20.

17 Kennedy E V., Holderied MW, Mair JM, Guzman HM, Simpson SD (2010) Spatial patterns in
18 reef-generated noise relate to habitats and communities: Evidence from a Panamanian case
19 study. *J Exp Mar Bio Ecol* 395:85–92.

20 Ladich F (2014) Fish bioacoustics. *Curr Opin Neurobiol* 28:121–127.

21 Leis JM, Lockett MM (2005) LOCALIZATION OF REEF SOUNDS BY SETTLEMENT-
22 STAGE LARVAE OF CORAL-REEF FISHES (POMACENTRIDAE). *Bull Mar Sci*
23 76:715–724.

1 Lillis A, Eggleston D, Bohnenstiehl D (2014a) Estuarine soundscapes: distinct acoustic
2 characteristics of oyster reefs compared to soft-bottom habitats. *Mar Ecol Prog Ser* 505:1–
3 17.

4 Lillis A, Eggleston DB, Bohnenstiehl DR (2013) Oyster Larvae Settle in Response to Habitat-
5 Associated Underwater Sounds. *PLoS One* 8:e79337.

6 Lillis A, Eggleston DB, Bohnenstiehl DR (2014b) Soundscape variation from a larval
7 perspective: the case for habitat-associated sound as a settlement cue for weakly swimming
8 estuarine larvae. *Mar Ecol Prog Ser* 509:57–70.

9 Lobel PS, Kaatz IM, Rice AN (2010) Acoustical Behavior of Coral Reef Fishes. In:
10 *Reproduction and Sexuality in Marine Fishes: Patterns and Processes*. Cole KS (ed)
11 University of California Press, p 307–348

12 Lyon RP, Eggleston DB, Bohnenstiehl DR, Layman CA, Ricci SW, Allgeier JB (2019) Fish
13 community structure, habitat complexity, and soundscape characteristics of patch reefs in a
14 tropical, back-reef system. *Mar Ecol Prog Ser* 609:33–48.

15 MATLAB (2019) version 9.1.0.441655 (R2019a). Natick, Massachusetts: The MathWorks Inc.

16 Mooney TA, Kaplan MB, Izzi A, Lamoni L, Sayigh L (2016) Temporal trends in cusk eel sound
17 production at a proposed US wind farm site. *Mar Ecol Prog Ser* 24:201–210.

18 North Carolina Department of Natural Resources & Community Development (1988) North
19 Carolina Artificial Reef Master Plan

20 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara
21 RB, Simpson GL, Solymos P, Stevens MHH, Szoeecs E, Wagner H (2019) Vegan:
22 Community Ecology Package.

23 Paxton AB, Peterson CH, Taylor JC, Adler AM, Pickering EA, Silliman BR (2019) Artificial

1 reefs facilitate tropical fish at their range edge. *Commun Biol*
2 2:<https://doi.org/10.1038/s42003-019-0398-2>.

3 Paxton AB, Pickering EA, Adler AM, Taylor JC, Peterson CH (2017) Flat and complex
4 temperate reefs provide similar support for fish: Evidence for a unimodal species-habitat
5 relationship. *PLoS One* 12:e0183906.

6 Pickering H, Whitmarsh D (1997) Artificial reefs and fisheries exploitation: A review of the
7 'attraction versus production' debate, the influence of design and its significance for policy.
8 *Fish Res* 31:39–59.

9 Pijanowski BC, Villanueva-Rivera LJ, Dumyahn SL, Farina A, Krause BL, Napoletano BM,
10 Gage SH, Pieretti N (2011) Soundscape Ecology: The Science of Sound in the Landscape.
11 *Bioscience* 61:203–216.

12 Popper AN, Fay RR (2011) Rethinking sound detection by fishes. *Hear Res* 273:25–36.

13 Popper AN, Hawkins AD (2019) An overview of fish bioacoustics and the impacts of
14 anthropogenic sounds on fishes. *J Fish Biol* 94:692–713.

15 R Core Team (2019) R: A language and environment for statistical computing. Version 3.6.0.

16 Radford CA, Jeffs AG, Montgomery JC (2007) Directional swimming behavior by five species
17 of crab postlarvae in response to reef sound. *Bull Mar Sci* 80:369–378.

18 Radford CA, Stanley JA, Jeffs AG (2014) Adjacent coral reef habitats produce different
19 underwater sound signatures. *Mar Ecol Prog Ser* 505:19–28.

20 Radford CA, Stanley JA, Simpson SD, Jeffs AG (2011a) Juvenile coral reef fish use sound to
21 locate habitats. *Coral Reefs* 30:295–305.

22 Radford CA, Stanley JA, Tindle CT, Montgomery JC, Jeffs AG (2010) Localised coastal habitats
23 have distinct underwater sound signatures. *Mar Ecol Prog Ser* 401:21–29.

1 Radford CA, Tindle CT, Montgomery JC, Jeffs AG (2011b) Modelling a reef as an extended
2 sound source increases the predicted range at which reef noise may be heard by fish larvae.
3 Mar Ecol Prog Ser 438:167–174.

4 Ricci SW, Bohnenstiehl DR, Eggleston DB, Kellogg ML, Lyon RP (2017) Oyster toadfish
5 (Opsanus tau) boatwhistle call detection and patterns within a large-scale oyster restoration
6 site. PLoS One 12:e0182757.

7 Rice AN, Soldevilla MS, Quinlan JA (2017) Nocturnal patterns in fish chorusing off the coasts of
8 Georgia and eastern Florida. Bull Mar Sci 93:455–474.

9 Rountree RA, Gilmore RG, Goudey CA, Hawkins AD, Luczkovich JJ, Mann D. (2006)
10 Listening to fish: Applications of passive acoustics to fisheries science. Fisheries 31:433–
11 446.

12 Sand O, Bleckmann H (2008) Orientation to auditory and lateral line stimuli. In: *Fish
13 Bioacoustics*. Springer New York, p 192–240

14 Shwartz FJ (1974) Movements of the Oyster Toadfish (Pisces: Batrachoididae) about
15 Solomons, Maryland. Chesap Sci 15:155–159.

16 Simon T, Joyeux J-C, Pinheiro HT (2013) Fish assemblages on shipwrecks and natural rocky
17 reefs strongly differ in trophic structure. Mar Environ Res 90:55–65.

18 Spanier E (1979) Aspects of Species Recognition by Sound in Four Species of Damselfishes,
19 Genus Eupomacentrus (Pisces: Pomacentridae). J Comp Ethol 51:301–316.

20 Staaterman E, Ogburn MB, Altieri AH, Brandl SJ, Whippo R, Seemann J, Goodison M, Duffy
21 JE (2017) Bioacoustic measurements complement visual biodiversity surveys: Preliminary
22 evidence from four shallow marine habitats. Mar Ecol Prog Ser 575:207–215.

23 Staaterman E, Paris CB, Kough AS (2014) First evidence of fish larvae producing sounds. Biol

1 Lett 10:2–6.

2 Sueur J, Pavoine S, Hamerlynck O, Duvail S (2008) Rapid acoustic survey for biodiversity
3 appraisal. *PLoS One* 3:1–9.

4 Suthers RA, Fitch WT, Fay RR, Popper AN (2004) Vertebrate sound production and acoustic
5 communication. In (Fay RR & Popper AN (ed) *Springer Handbook of Auditory Research*.
6 Springer, Switzerland.

7 Tolimieri N, Haine O, Jeffs A, McCauley R, Montgomery J (2004) Directional orientation of
8 pomacentrid larvae to ambient reef sound. *Coral Reefs* 23:184–191.

9 Tolimieri N, Jeffs A, Montgomery JC (2000) Ambient sound as a cue for navigation by the
10 pelagic larvae of reef fishes. *Mar Ecol Prog Ser* 207:219–224.

11 Urick RJ (1983) *Principles of Underwater Sound*. McGraw-Hill Inc., New York, NY.

12 Vermeij MJA, Marhaver KL, Huijbers CM, Nagelkerken I, Simpson SD (2010) Coral larvae
13 move toward reef sounds. *PLoS One* 5:e10660.

14 Whitfield PE, Muñoz RC, Buckel CA, Degan BP, Freshwater DW, Hare JA (2014) Native fish
15 community structure and Indo-Pacific lionfish *Pterois volitans* densities along a depth-
16 temperature gradient in Onslow Bay, North Carolina, USA. *Mar Ecol Prog Ser* 509:241–
17 254.

18

19

20

21

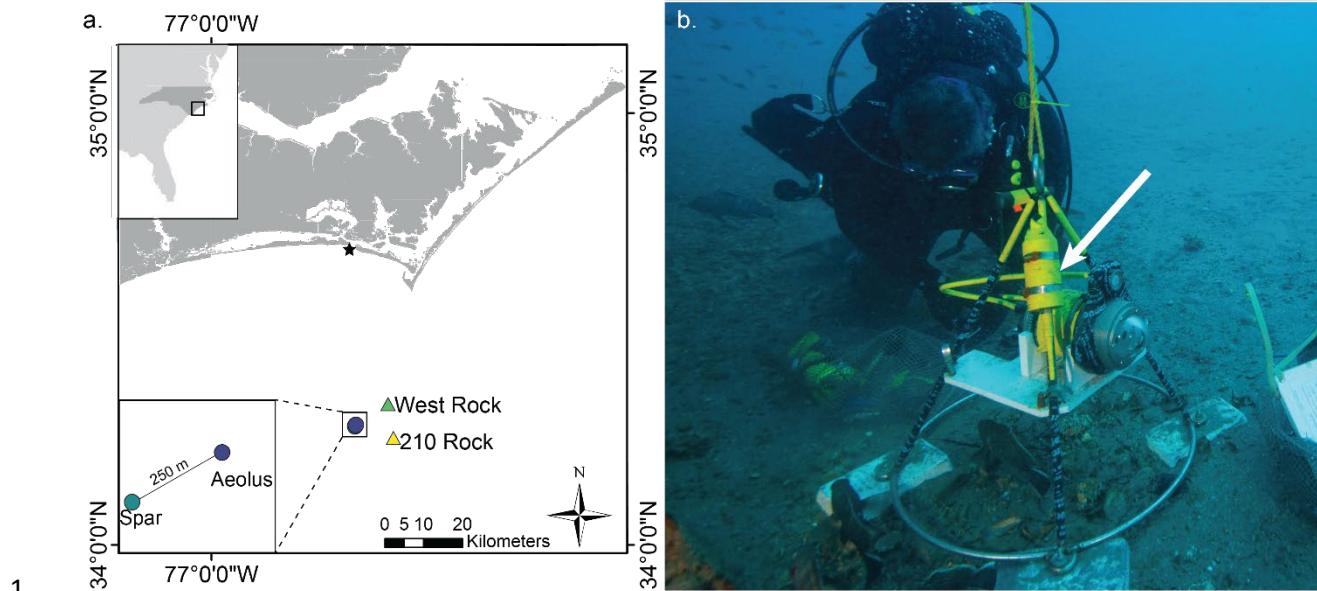
22 **Tables**

1 **Table 1.** Acoustic features of fish calls that exhibited seasonal chorusing. Sample calls and
 2 pulses were extracted from each site and deployment chorusing was observed. The distribution
 3 of toadfish fundamental frequency was bimodal as such the mean and standard deviation of each
 4 mode is reported. The multiple values of bandwidth and peak frequency for the creak and growl
 5 describe each pulse in a pulse set.

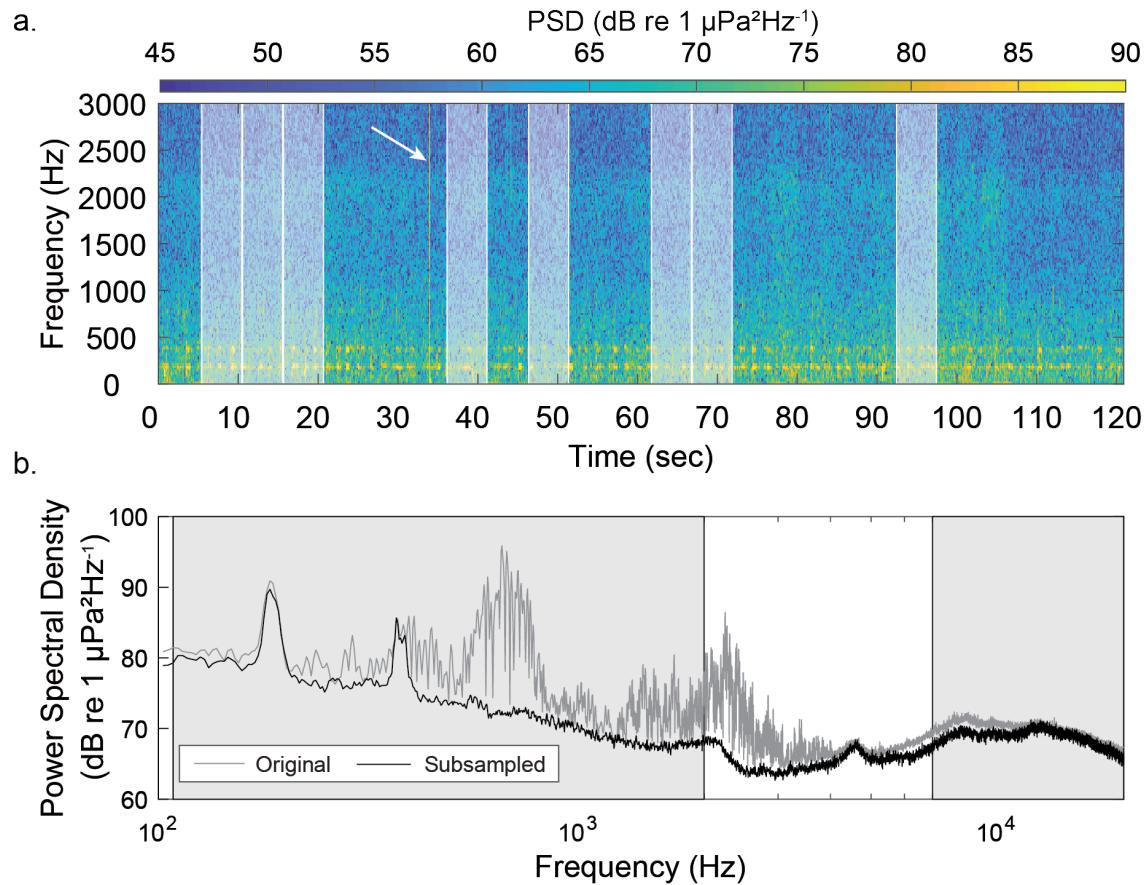
Features	Toadfish	Knock	Creak	Growl
Duration (ms)	508.3 ± 106.4	7.7 ± 6.3	2281.1 ± 488.5	3350.1 ± 229.5
Fundamental Frequency (Hz)	147.9 ± 13.7	--	--	--
No. Harmonics	$2 (1 - 4)$	--	--	--
Bandwidth (Hz)	--	553.4 ± 138.1	326.8 ± 100.6 420.2 ± 137.7	146.5 ± 24.7 367.8 ± 96.0
Peak Frequency (Hz)	--	653.4 ± 153.2	1669.4 ± 101.7 2728.7 ± 156.1	100.8 ± 22.9 160.9 ± 57.3
No. pulses in set	--	6.7 ± 4.0	56.3 ± 11.8	111.0 ± 7.8
Pulses per second	--	7.8 ± 8.9	24.8 ± 1.4	33.2 ± 1.1
No. calls summarized	161	399 pulses, 10 sets	646 pulse sets, 75 pulse trains	94 pulse sets, 23 growl trains

6
 7
 8
 9
 10
 11
 12
 13
 14
 15
 16
 17
 18

Figures



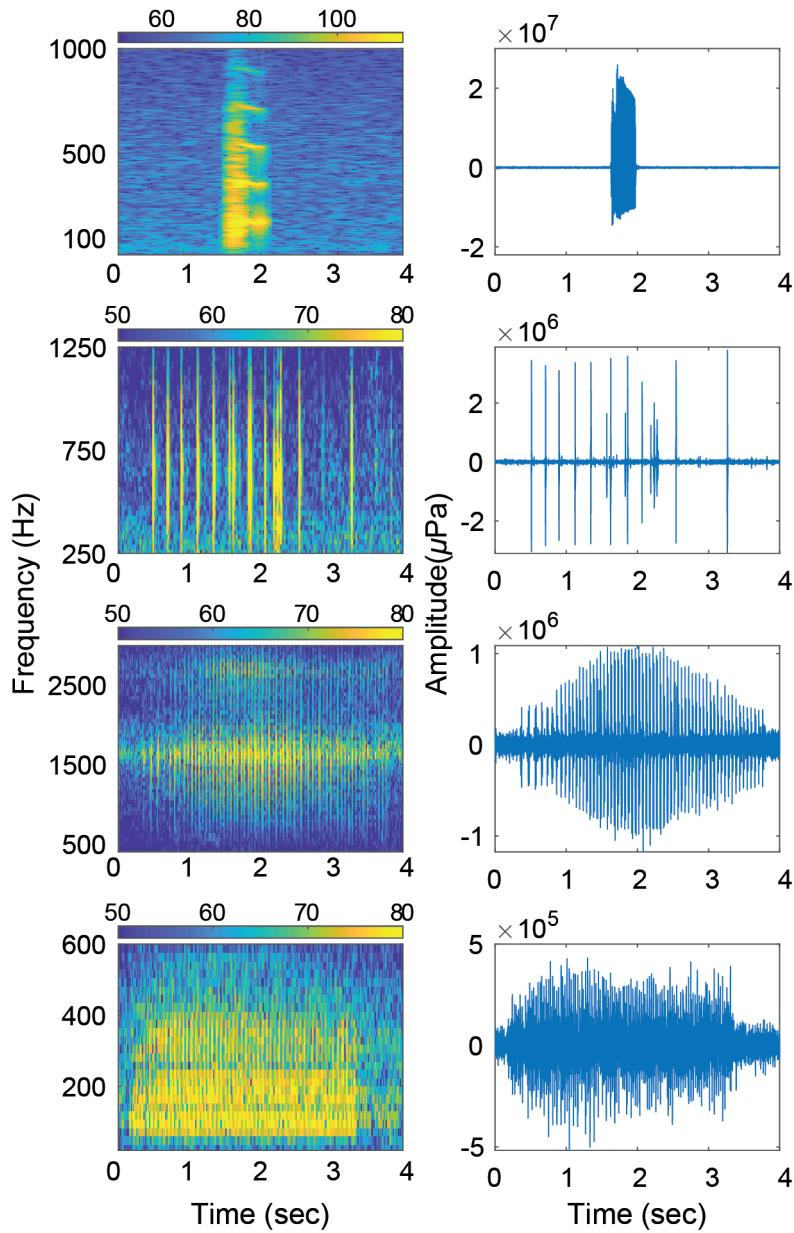
5 **Fig. 1.** a. Reef sites offshore of Onslow Bay, NC. Triangles are natural reefs, circles are artificial reefs, and Beaufort Inlet is indicated by the star. b. Image of hydrophone deployment set-up, the arrow indicates the position of the SoundTrap. Photo credit: J. McCord / CSI



1
 2 **Fig. 2.** Demonstration of the effect of a "fish bump" on the average power spectral density of a
 3 two-minute audio file. (a) Spectrogram of a representative file recorded on 210 Rock in April
 4 2016. The arrow points to an impulsive signal likely the result of an animal collision with the
 5 hydrophone. The white shaded boxes indicate the eight quietest, five-second subsamples
 6 extracted to remove the effect of the fish bump. (b) Plot of power spectral density demonstrating
 7 that the subsampling methodology preserves the toadfish peaks while removing the noise due to
 8 the fish bump. The gray shaded boxes indicate the frequencies summarized in each frequency
 9 band.

10

11



1
2 **Fig. 3.** Spectrogram (left panel) and waveform (right panel) of dominant fish calls observed.
3 Note variations in y-axes scales. The colorbar is power spectral density ($\text{dB re } 1 \mu\text{Pa}^2\text{Hz}^{-1}$) (a.)
4 Toadfish boatwhistle (spectrogram NFFT = 2^{15} , 90% overlap); (b) unidentified knocks
5 (spectrogram NFFT = 2^{12} , 90% overlap); (c) unidentified creak (spectrogram NFFT = 2^{11} , 90%
6 overlap); (d) unidentified growl (spectrogram NFFT = 2^{12} , 90% overlap)

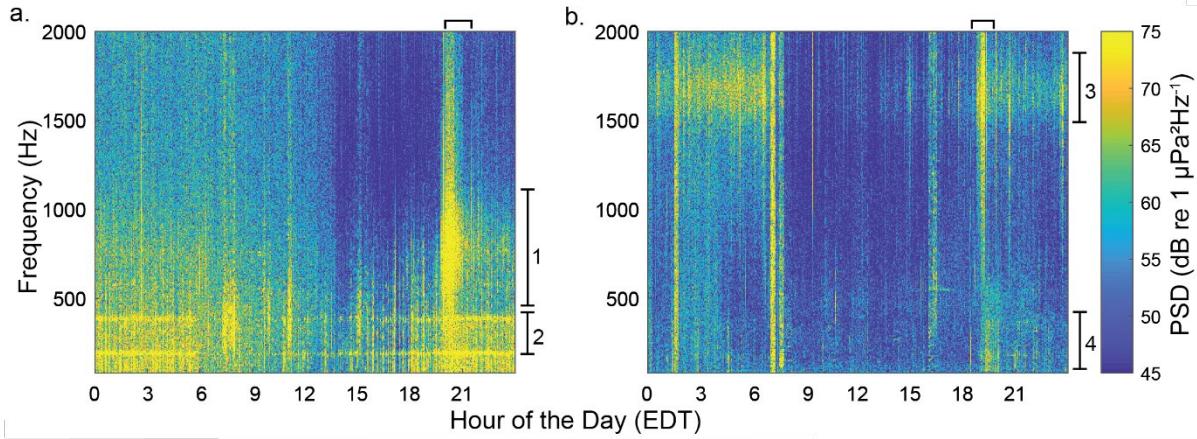
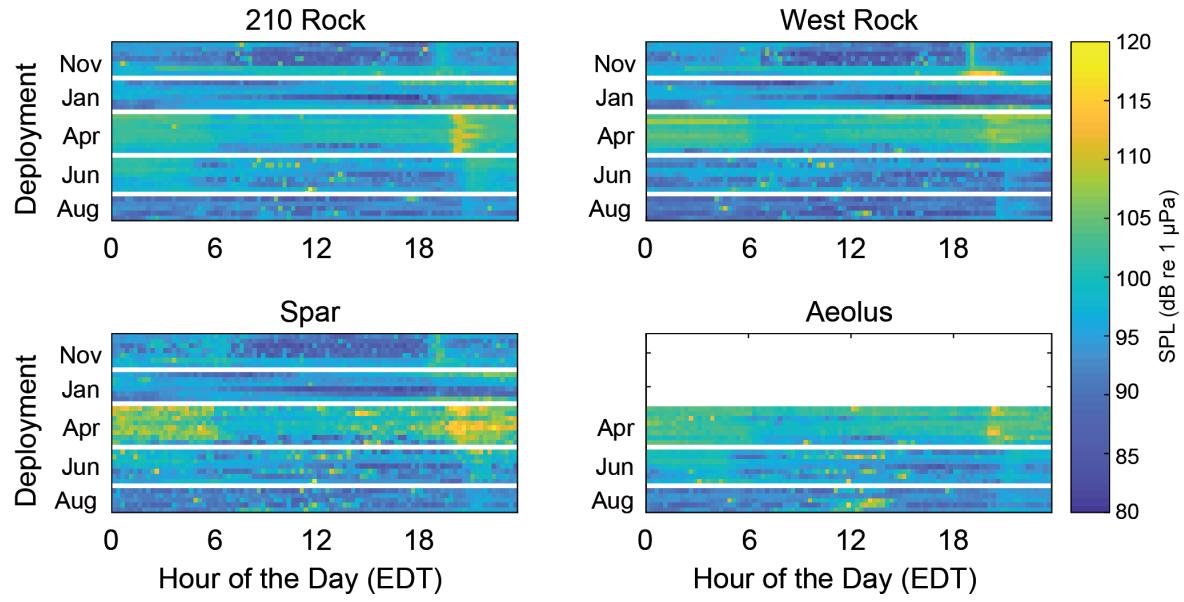
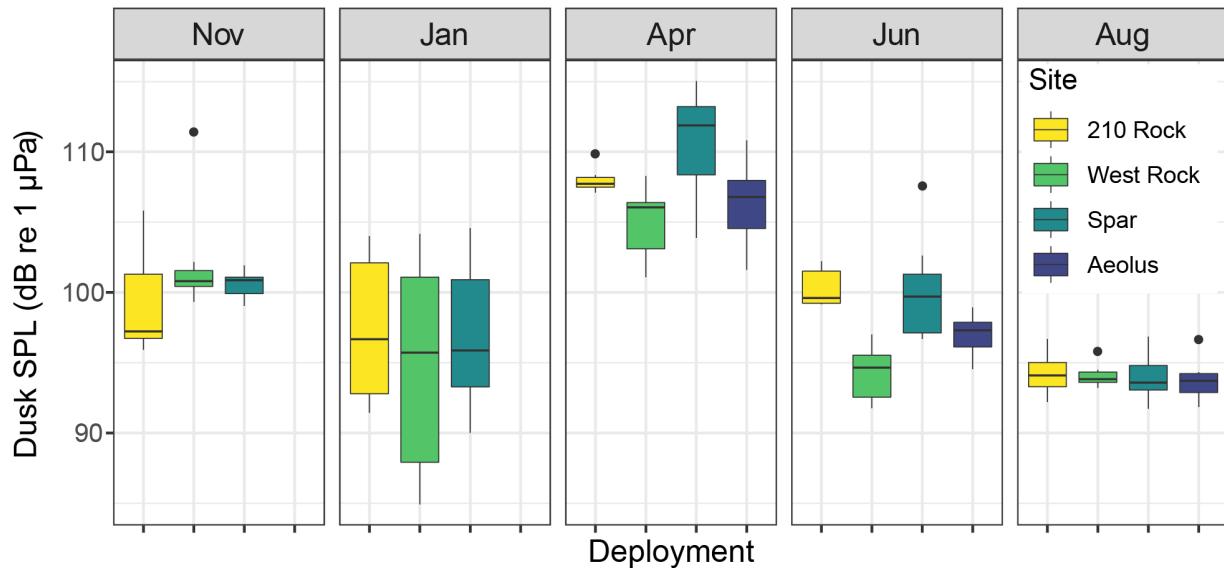


Fig. 4. Daily patterns of fish chorusing events during April and November. Each figure is a stacked spectrogram of all two-minute subsamples from a single day with representative fish chorusing events ($\text{NFFT} = 2^{15}$). The bracket on top of each panel identifies dusk and the numbered brackets on the right side identify the frequency bands of interest for each vocalizer. (1) knock, (2) toadfish, (3) creak, (4) growl. (a) Knock and toadfish choruses recorded during April on 210 Rock. Toadfish vocalizations were present throughout the day but peak during dusk, while the unidentified knock chorus was only present at dusk. (b) Creak and growl choruses recorded during November on West Rock. The creak chorus peaked during crepuscular periods while the growl chorus was only present at dusk.

11
12
13
14
15
16
17
18
19

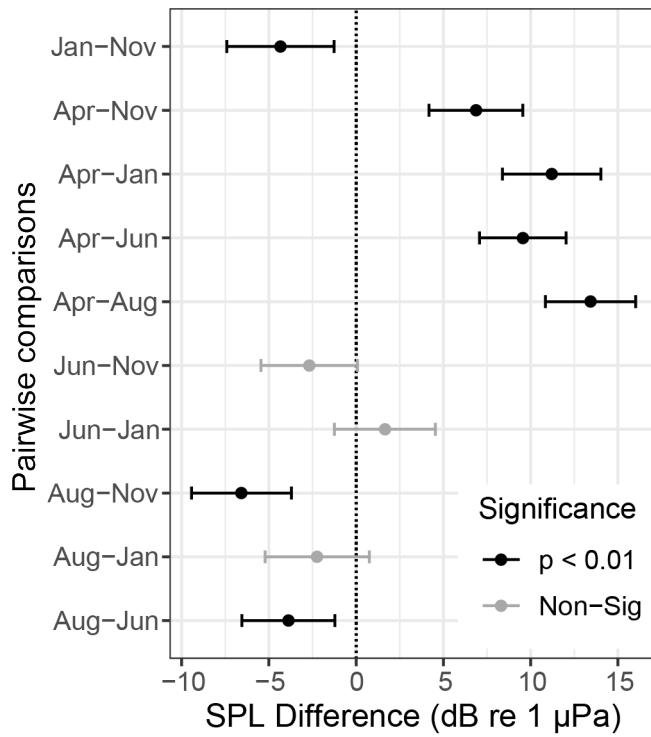


1 **Fig. 5.** Daily patterns in acoustic activity in the low-frequency band. Peak SPLs were observed at
 2 dusk on all sites and deployments, and are especially pronounced during November, April, and
 3 June when fish chorusing was observed. Each pixel represents the average SPL within one 40
 4 second subsample and each row of pixels is the SPL variation over a single 24hr period. The
 5 white rows separate the deployments. All recordings within each deployment were concurrent on
 6 each reef.
 7
 8
 9
 10

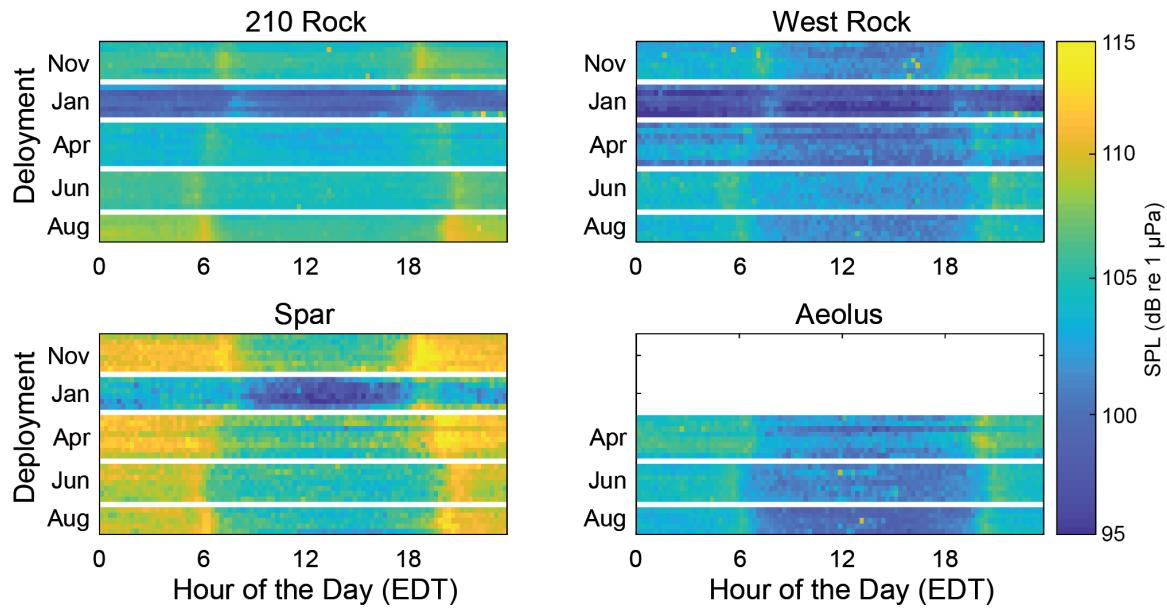


1
2 **Fig. 6.** Boxplots of average SPLs at dusk within the low-frequency band (0.1-2 kHz) highlight
3 that variations in SPL are driven by seasonal variability rather than differences among the sites.
4 The black bar is the median while the lower and upper edges of the box are the first and third
5 quartiles. The whiskers extend to either the most extreme value or to 1.5 times the interquartile
6 range and any outliers are plotted individually.

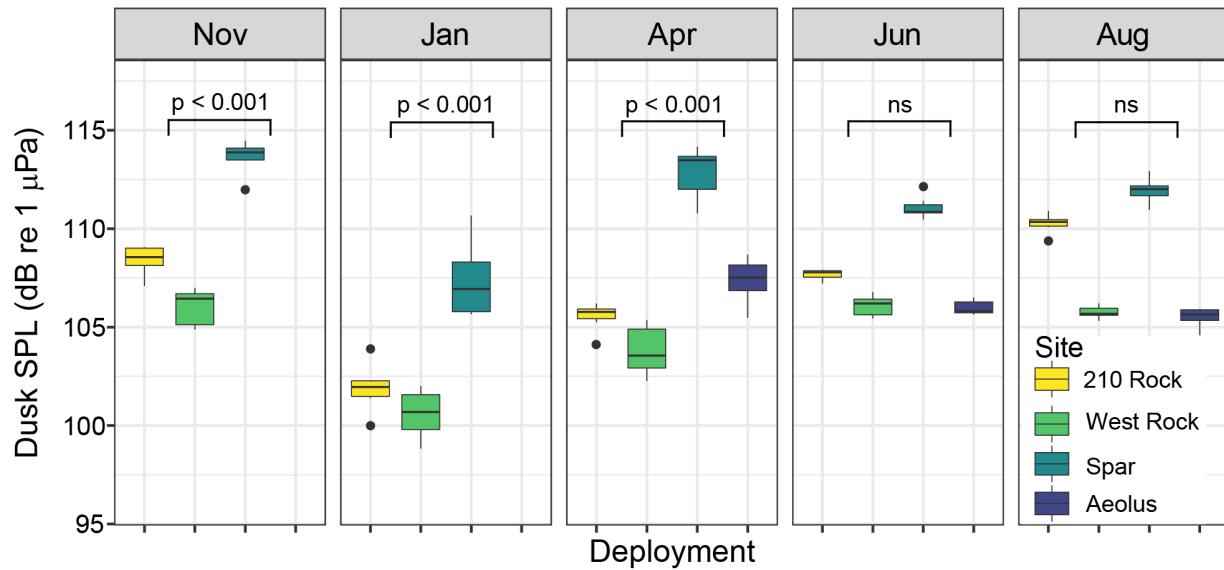
7



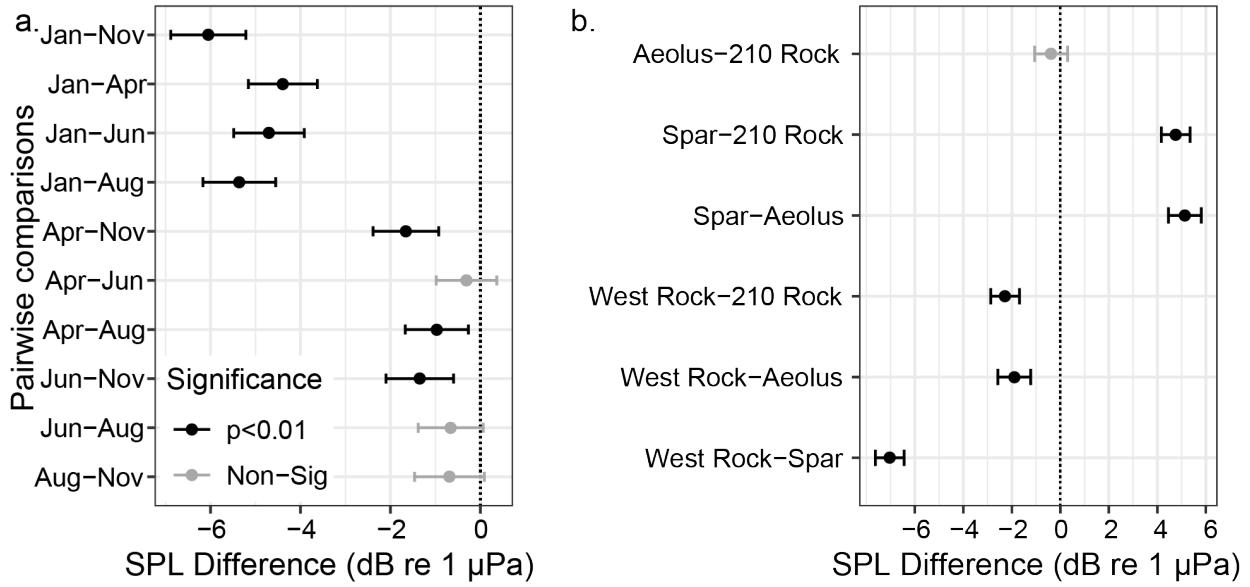
1
2 **Fig. 7.** Tukey's HSD 95% confidence intervals of pairwise comparisons between deployments for
3 low frequency (0.1 – 2 kHz) dusk SPLs. This demonstrates that dusk SPLs during April were
4 significantly higher than all other deployments and are driving the variability among the
5 deployments.



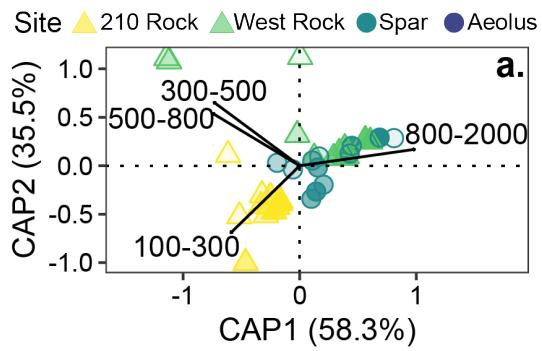
1 **Fig. 8.** Diurnal patterns in acoustic activity within the high-frequency band (7-20 kHz).
 2 Crepuscular peaks in SPL, attributed to snapping shrimp, are persistent across seasons and track
 3 the seasonal photoperiod. All sites exhibited the minimum SPLs in January. Each pixel
 4 represents the average SPL within one 40 second subsample and each row of pixels is the SPL
 5 variation over a single 24hr period. The white rows separate the deployments. All recordings
 6 within each deployment were concurrent on each reef.
 7



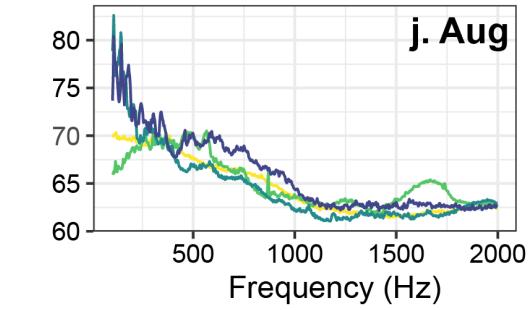
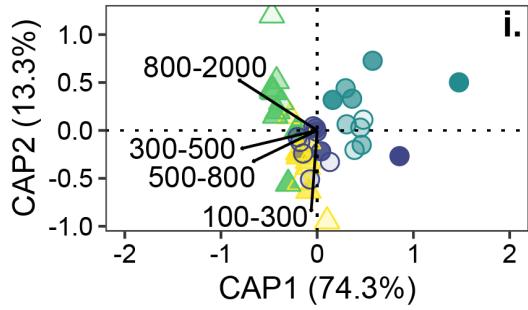
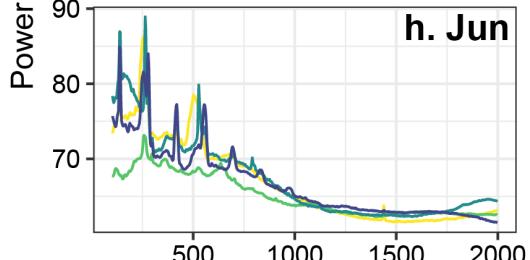
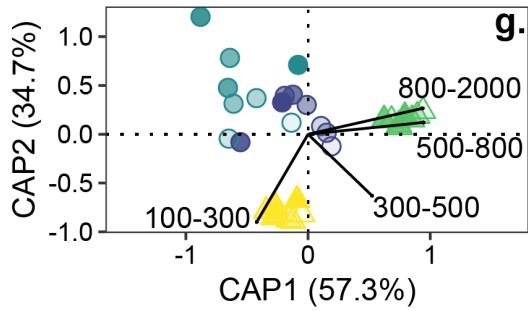
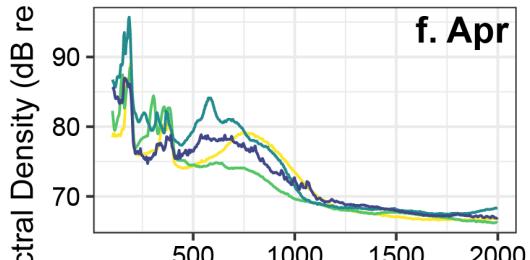
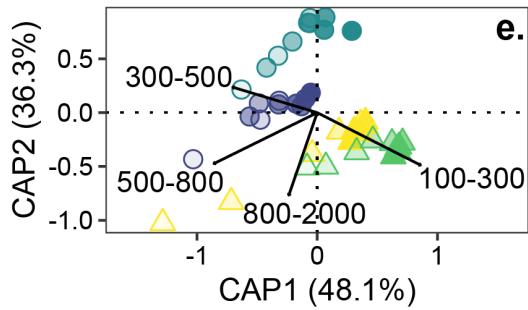
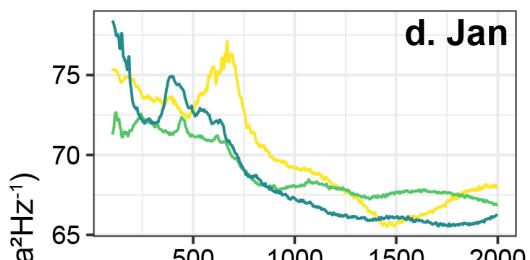
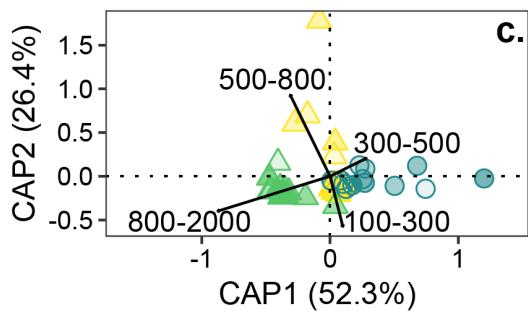
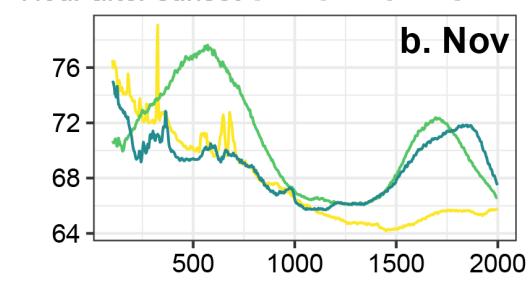
1
 2 **Fig. 9.** Boxplots of average daily SPLs at dusk in the high-frequency band (7-20 kHz). During
 3 the first three deployments dusk SPLs were significantly higher on artificial reefs than natural
 4 reefs, while variation among the sites is driven by increased SPLs on the *Spar*. The p-values are
 5 the result of Tukeys HSD test of pairwise comparisons between the reef types. For the boxplots,
 6 the black bar is the median while the lower and upper edges of the box are the first and third
 7 quartiles. The whiskers extend to either the most extreme value or to 1.5 times the interquartile
 8 range and any outliers are plotted individually.
 9
 10
 11



1 **Fig. 10.** Tukey's HSD 95% confidence interval of pairwise comparisons between (a) deployments
2 and (b) sites for the high frequency (7 – 20kHz) dusk SPLs. The results demonstrate seasonal
3 variation is driven by reduced SPLs during January (Deployment 2), while variation among the
4 sites is driven by elevated SPLs on the *Spar* and reduced SPLs on West Rock. Moreover, within
5 reef type the *Spar* and 210 Rock are louder than the *Aeolus* and West Rock, respectively.
6
7
8
9
10



Hour after sunset ● 2.5 ● 5.0 ● 7.5 ● 10.0



1 **Fig. 11.** Multivariate analyses on the spectral dissimilarity index suggest that during deployments
2 with increased acoustic activity in the low-frequency band (0.1-2 kHz) there is distinct separation
3 of the spectral content at each reef, with increased separation between the reef types. This
4 difference between natural and artificial reef was especially pronounced during April. As each
5 reef type contains the same dominant vocalizers, differences between natural and artificial reefs
6 are likely the result of less dominant vocalizations and miscellaneous acoustic activity. The left
7 column contains the dbRDA ordinations of the spectral dissimilarity index for each deployment,
8 while the right column contains the average spectra for each site within each deployment (note
9 change in PSD scale).

10

11