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13	Monitoring spatial and temporal soundscape features within
14	ecologically significant U.S. National Marine Sanctuaries
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30

31 Abstract

The U.S. National Oceanic and Atmospheric Administration's Office of National Marine 32 33 Sanctuaries manages a system of marine protected areas encompassing more than 2,000,000 km². U.S. National Marine Sanctuaries (NMS) have been designated to provide protection for 34 35 their conservation, recreational, ecological, historical, scientific, cultural, archaeological, 36 educational, or aesthetic qualities. Due to the large variability of attributes among NMS, designing coordinated system-wide monitoring to support diverse resource protection goals can 37 be challenging. Underwater sound monitoring is seeing increasing application to marine 38 39 protected area management because it is able to support this wide variety of information needs. Passive acoustics are providing invaluable autonomous information regarding habitat 40 41 associations, identifying species spatial and temporal use, and highlighting patterns in conditions that are otherwise difficult to survey. Using standardized equipment and analysis methods this 42 study collected ambient underwater sound data and derived measurements to investigate 43 temporal changes in sound pressure levels and power spectral density, identify presence of select 44 45 species of importance and support within and among site comparison of ambient underwater sound among eight sites within four U.S. NMS. Broadband sound pressure levels of ambient 46 sound (10 – 24,000 Hz) varied as much as 24 dB re 1µPa (max difference 100 to 124 dB re 47 1µPa) among the recording sites, sanctuaries, and seasons. Biotic signals, such as snapping 48 shrimp snaps and vocalizations of fishes, exhibited distinct diel and seasonal patterns and 49 50 showed variation among sites. Presence of anthropogenic signals, such as vessel passage, also varied substantially among sites, ranging from on average 1.6 h to 21.8 h per day. The study 51 identified measurements that effectively summarized baseline soundscape attributes and 52

53 prioritized future opportunities for integrating non-acoustic and acoustic variables in order to

54 inform area-specific management questions within four ecologically varying U.S. National

55 Marine Sanctuaries.

56

57 Keywords

Bioacoustics, passive acoustic monitoring, underwater soundscapes, U.S. National MarineSanctuaries.

60 1. Introduction

Over the past decade the field of 'soundscape ecology' has matured into a research discipline 61 of its own, e.g., Pijanowski et al., 2011a, 2011b, Truax & Barret, 2011, Farina, 2014, Gasc et al. 62 2016. Terrestrial ecologists initially took the lead in describing and quantifying soundscapes, 63 64 defining the term as the relationship between a landscape and the composition of its sound (Pijanowski et al. 2011b). Soundscapes are comprised of contributions from the organisms 65 66 utilizing the space (biotic sound), human activities in and around the space (anthropogenic sound) and environmental processes occurring in the space (abiotic/geophysical sound) (e.g., 67 68 Pijanowski et al. 2011a). These three components together determine the distinct sound signature at any given place, which depending on the source, can show recognizable spatio-temporal 69 70 patterns at differing time scales, reflective of changes in biotic, anthropogenic or even abiotic 71 activities (Farina et al. 2011, Pijanowski et al. 2011b, Staaterman et al. 2014). As recording effort continues to grow internationally, the field is challenged to develop analytical techniques 72 and tools that both accurately describe and characterize this variation in soundscapes. As well as 73 74 isolating relevant ecological indicators from these data that relate to targets of interest for marine science and management, such as biological diversity and ecosystem health. 75 Marine soundscape ecology is a relatively recent field, with most studies focused 76 descriptively on improving understanding of the acoustic characteristics of different marine 77 environments, and isolating contributions to their trends and status. Underwater acoustic 78 monitoring has been successful in identifying species presence/absence, habitat associations, 79 migration timing and pathways, spawning patterns and locations, environmental conditions, and 80 largescale differences among underwater habitats e.g., McCauley & Cato 2000, Parsons et al. 81 2009, Bertucci et al. 2015, Erbe et al. 2015, Davis et al. 2017, Putland et al. 2017a, Rowell et al. 82

83 2017). However, effectively deriving measurements from quantitative marine soundscape data

that can support more holistic comparisons and status and trend assessments among marine 84 soundscapes remains an area of active research focus. Due to factors including but not limited to 85 the greater efficiencies of sound propagation underwater, the transference of tools to derive 86 biodiversity indices from terrestrial soundscapes to marine settings has proven to be largely 87 unsuccessful, as they do not appear to translate consistently across the marine realm. Existing 88 analytical approaches have been further advanced and new methodologies continue to be 89 developed to better characterize marine soundscapes and their drivers (Freeman and Freeman 90 2016, Staaterman 2017, Bohnenstiehl et al. 2018, Mooney et al. 2020). 91

Deriving more comprehensive measurements from long-term passive acoustic monitoring 92 datasets is necessary to support protected area management interests (Hatch et al. 2009). Some 93 U.S. National Parks have used long-term recordings to identify thresholds to guide visitor and 94 95 wildlife noise exposure within park areas, leading to management techniques such as the use of shuttle buses to reduce car traffic and alignment of overflight patterns with roads to concentrate 96 97 peak noise conditions. In Europe, the Baltic Sea Information on the Acoustic Soundscape (BIAS project) produced seasonal soundscape maps for the demersal, pelagic and surface zones, serving 98 as a baseline for the development of monitoring and assessment of ambient noise in the Baltic 99 Sea (Nikolopoulos et al. 2016). In the USA, large-scale comparative soundscape monitoring 100 101 capacities have been steadily growing under support from multiple federal agencies (NOAA & U.S. Navy Sound Monitoring - https://sanctuaries.noaa.gov/science/monitoring/sound/, Gedamke 102 et al. 2016, Haver et al. 2018). 103

This study investigated underwater soundscapes within four U.S. National Marine Sanctuaries 104 spanning latitudes from 42° to 24° (Figure 1). The northern-most sanctuary, Stellwagen Bank 105 National Marine Sanctuary (SBNMS), has a highly seasonal ecology with spring upwelling 106 107 driving high summer productivity that attracts a variety of invertebrate schooling and predatory fish and high concentrations of feeding marine mammals. The mid-latitude site, Gray's Reef 108 National Marine Sanctuary (GRNMS), is a temperate hard-bottom reef located off the coast of 109 Georgia, with complex "live-bottom" and rocky ledges providing habitat for a wide range of 110 invertebrates, fishes and turtles, as well as transient marine mammals. The southern-most 111 112 locations, within Florida Keys National Marine Sanctuary (FKNMS), were placed within different zones of fishing and recreational use within the range of the coral reef habitat contained 113 within this protected area. Finally, the locations within western Gulf of Mexico's Flower Garden 114

Banks National Marine Sanctuary (FGBNMS) monitored two protected coral reef caps that sit 115 atop disparately placed salt domes and host diverse communities of invertebrates, fishes, and 116 turtles, as well as transient mammals. Acoustic data were collected over a two year period in a 117 standardized and coordinated manner, supporting: 1) investigation of temporal changes in sound 118 pressure levels and power spectral density, 2) identification of the presence of select species of 119 120 importance, 3) supporting within and among site comparison of ambient underwater sound among sanctuaries and 4) highlighting future opportunities for integrating non-acoustic and 121 acoustic variables in order to inform area-specific management questions of interest. 122

123 2. Methods

124 2.1 Study sites and deployment schedule

The four sanctuaries that were monitored in this study vary in size (57 to 9947 km²), are
shallow to very shallow (14.5 – 68 m recording depths) and are positioned offshore on the U.S.
continental shelf (4.8 – 185 km from shore, with the exception of FKNMS).

128 The eight passive acoustic listening stations included: Stellwagen Bank National Marine Sanctuary (SBNMS); Site 27 (Site 1) and Site 33 (Site 2), Gray's Reef National Marine 129 130 Sanctuary (GRNMS); FS15 (Site 3) and Station 20 (Site 4), Florida Keys National Marine Sanctuary (FKNMS); Western Dry Rocks (Site 5) and Eastern Sambo (Site 6), and Flower 131 132 Garden Banks National Marine Sanctuary (FGBNMS); Stetson Bank (Site 7) and East Flower 133 Garden Bank (Site 8) (Figure 1, Appendix S1: Table S1). Within each sanctuary, using available data, sites were chosen to reflect areas likely to be exposed to variable acoustic influence from 134 sound-producing species and human activities. Given the relatively localized sound propagation 135 field around shallower sites, it was understood that two recording locations would not 136 sufficiently describe soundscape conditions within site boundaries. Thus, emphasis was placed 137 on locations where other information sources were available (including past acoustic 138 information, diver surveys, other oceanographic sampling via personal communication with 139 sanctuary staff and personal research experience), where acoustic signals of interest would likely 140 be present (whether biotic or anthropogenic or both), and where overall acoustic signatures might 141 differ when compared. 142

Deployments were planned to occur concurrently at all sites for at least one lunar phase
during each season in 2016/17. However, due to the inaccessibility of some locations this was
not always achieved. Therefore, seasons were defined among sites as follows; Summer: 28th June

- 146 13th September 2016, Fall: 19th October 29th December 2016, Winter: 22 February 13th
- 147 April 2017, Spring: 26th April 15th July 2017 and recording durations ranged from 37 174
- 148 days per sanctuary (Appendix S1: Table S2).

149 **2.2 Instrumentation**

150 *2.2.1 Autonomous underwater acoustic recorders*

151 All acoustic recordings were made using SoundTrap ST300s (ST300) with external battery 152 packs (Self-noise less than sea-state 0 at 100 Hz – 2 kHz and <34 dB re 1 μ Pa above 2 kHz, 153 Ocean Instruments Inc., Auckland, New Zealand). At all recording sites, the ST300s 154 continuously sampled at a rate of 48000 Hz with a flat full-scale frequency response between 20 155 – 60 kHz (\pm 3 dB). Each individual ST300 at each site was calibrated by the manufacture directly 156 before deployment and each had unique end-to-end response sensitivity. Digitized recordings 157 (.wav files) were directly downloaded to a computer using the SoundTrap host software.

158 2.2.2 Mooring configuration

At all sites with water depths of less than 30 m (GRNMS, FKNMS and FGBNMS) the 159 acoustic recorders were deployed and retrieved by divers. In these instances, recorders were 160 dived to the benthos at each site and fixed securely to a rigid and weighted benthic stand, with no 161 surface or subsurface mooring lines or floats. The hydrophone element in these situations were 162 approximately 1 m from the seafloor. We found this depth to be the optimal balance between 163 164 reducing flow noise across the hydrophone element which can be heightened in the water column 165 and reducing the noise created from sediment moving across the hydrophone element. At sites with water depths greater than 31 m (sites at SBNMS) the acoustic recorders were suspended 166 approximately two meters off the seafloor via an acoustic release (VEMCO VR2AR) and held to 167 the substrate by two 18 kg biodegradable sandbags. Both mooring types were specifically 168 169 designed and engineered to reduce any extraneous noise from the moorings themselves.

170 2.3 Acoustic Analyses

171 2.3.1 Soundscape quantification

172 All acoustic data were analyzed using MATLAB software (version 2019b) and statistical tests

were run in SigmaPlot 13 by Systat Software, Inc. (Build 13.0.0.83) and RStudio (version

- 174 1.1.456, R version 3.5.1). The low frequency limit of the analysis was 10 Hz, to partially remove
- potential low frequency surface motion noise or low frequency interference, 10 Hz was used to

retain some portion of the energy from fin whale pulses as these signals may be of acoustic

significance to the sites. ST300s have a built-in high pass filter set at 20 Hz to reduce any

178 potential noise from mooring vibration and flow noise, therefore, there is a drop in

sensitivity/response which would cause an approximate attenuation of 13 dB at 10 Hz. All times

180 are standardized for local standard time at each site (daylight savings offset removed).

To quantify ambient sound levels at each recording site and variation with frequency and time 181 scale, power spectral densities (PSD) and broadband (10 - 24,000 Hz) RMS, median and 182 percentile sound pressure levels (BB SPLs) were obtained for all recordings. Power spectral 183 densities were calculated using a discrete Fourier transformation with a Hann window resulting 184 in 1 Hz, 60 s resolution with 50 % overlap. Spectrograms were produced with DFT length of 185 48000, using a Hanning window with 50% overlap for a 24-hour period ('sample day') at one 186 187 site within each sanctuary to illustrate and identify peak daily patterns (specifically of intense acoustic biological or anthropogenic activity) for that season and day. 188

189 To determine if season effected the broadband ambient sound recorded at each recording site, broadband RMS SPLs were averaged in 60s and 60min lengths, to determine the robustness of 190 191 the relationship at different sampling resolutions. Kruskal-Wallis or Mann-Whitney U statistical tests were subsequently used to test for differences. If such tests provided significant results, a 192 193 Dunn's pairwise multiple comparison, with Bonferroni correction, was then used to isolate further differences. Non-parametric statistical methods were used to test for differences among 194 195 seasons as the data had unequal variance among treatments and data had a non-normal distribution (Zar 1999). Broadband SPLs were also averaged, in 5 min bins, across each 196 197 recording season, within each site, to produce an average diel trend plot for each site over each season. 198

Sound pressure levels or band levels were also calculated in octave bands with a 60 s resolution for all recordings. Diel trends in select octave frequency bands, centered on 62, 125, 251, 501, 1000, and 7943 Hz were plotted for three days around each moon phase for all sites over all seasons to illustrate the variation among the selected frequency bands, to visualize the varying contributions of each and to demonstrate any periodicity within octave band levels. A period of three days was selected to depict daily patterns while recognizing anomalies such as human activities i.e., vessel passages. Wavelet scalograms were used to detect the strength and persistence of the periodicity in SPLs in the above frequency bands across the sampling periods(Grinsted et al. 2004, MATLAB wavelet toolbox).

208 2.3.2 Vessel presence and contribution to the soundscape

209 During the Summer recording period one site within each sanctuary (SBNMS – Site 1, GRNMS - Site 4, FKNMS - Site 6, FGBNMS - Site 8) was visually and aurally inspected for 210 vessel presence during the three days surrounding each lunar phase, totaling twelve days for each 211 site. Using Raven Pro 1.5, times with both audible and visible vessels were tagged so to be 212 separated from periods without the presence of vessel signals. Ninety-six 15-minute sound files 213 were loaded into Raven in 90-second pages for each day and viewed as a spectrogram using a 214 fast Fourier transform (FFT) value of 4096. After the vessel signals were identified, the hours of 215 vessel noise/day were recorded. Sound Pressure Level in 1/3 octave frequency bands were 216 217 analyzed for both times where vessel noise was present and absent at each site. The median and 90th percentile levels were plotted for each lunar phase separately as well as across the entire 218 219 month at each site. These plots were then used to compare the power spectral density levels for presence and absence of vessel signal to determine the influence of vessel presence on the 220 soundscape. 221

222 2.3.3 Detection and classification of vocalizers

223 Snapping shrimp

Data from all Sanctuaries with temperate and tropical reef conditions (GR, FK and 224 FGBNMSs), were analyzed using a snap detection algorithm to quantify the acoustic activity of 225 snapping shrimp, using methods and rationale for amplitude thresholds for detection from 226 227 Bohnenstiehl, Lillis & Eggleston, 2016. Snap rates (number of snaps per 60s) were determined for the first 60 s of each 15 min sound file for the duration of the recording period at each site. 228 The number of snaps that were detected during Dawn (site specific sunrise \pm 90 min), Noon 229 230 (noon \pm 90 min), Dusk (site specific sunset \pm 90 min) and Midnight (midnight \pm 90 min) were also compared by calculating snap rate for these periods over a standardized segment of time, for 231 each sampling day at each site during each recording season. Differences among snap rates were 232 tested for statistical significance using the Friedman Test as data had a non-normal distribution. 233 Following a significant Friedman test result, post-hoc multiple group comparisons were 234 conducted using Tukey Tests. Simple linear regression methods were used to test if snap rate 235 could be used to predict the values of SPL in the 2000 - 20,000 Hz during the different recording 236

seasons. This method was used despite the fact there was a slight deviation from a normal
distribution in the data. However, the sample size was large enough to be assumed to not impact
results, and transformations of the data may lead to more severe bias (Schmidt and Finan 2018).

240 Atlantic cod

As the distribution of Atlantic cod (*Gadus morhua*) along the North American coast is from Cape Hatteras to Ungava Bay, identification of their calls was restricted to recordings from SBNMS. Acoustic data were processed using the Atlantic cod detection algorithm (Urazghildiiev and Van Parijs 2016) and all detections were manually verified visually and aurally for true calls (Stanley et al. 2017).

246 Low frequency vocalizing whales

247 Acoustic data from all recording sites were processed using the Low Frequency Detection and Classification System (LFDCS) (Baumgartner and Mussoline 2011) using methods from Davis 248 et al., 2017; 2020, utilizing detections from fin (Balaenoptera physalus), sei (B. borealis) and 249 North Atlantic right whales (NARWs) (Eubalaena glacialis). For continuous data, a given day 250 was marked as having a species present if certain criteria were met, with each species having 251 different criteria due to the performance of the detectors and calling behavior (see Davis et al. 252 2020). These criteria were used to be conservative and confident in stating these species 253 presence. Detector evaluation/missed detection rate was quantified using the same methods as 254 Davis et al., 2017; 2020. 255

256 2.4 Wind and Wave Data

Hourly wind speed and wave height data was collected from the nearest NOAA weather

station to the recorders within each sanctuary (SBNMS: Station 44029, GRNMS: 41008,

259 FKNMS: SANF1 and FGBNMS: TABS V) (https://www.ndbc.noaa.gov/). Wave height was not

available for the sites in FKNMS and FGBNMS. Pearson Correlation tests were performed to

assess the relationship between hourly broadband (10 - 24,000 Hz) SPL and select octave bands

262 (63 Hz, 125 Hz, 251 Hz, 501 Hz center frequency) SPLs, and hourly wind speed (m/s) and wave

height (m) within all sites to assess the contribution these environmental parameters have on

these metrics.

265 **3. Results**

266 **3.1 Stellwagen Bank National Marine Sanctuary**

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267 *3.1.1 Patterns in broadband sound pressure levels and spectral composition*

Broadband (10 - 24,000 Hz) SPLs (both median and RMS values) varied by as much as 10 268 269 dB among recording sites and recording seasons within SBNMS, $(100.5 - 110 \text{ dB re } 1\mu\text{Pa}, \text{Table})$ 1), with both sites reflecting the same overall seasonal patterns. The highest median broadband 270 SPLs occurred during the Winter recording period, followed by Fall, Spring and with lowest 271 levels recorded in the Summer recording period for both sites. Season significantly affected 272 broadband SPL at both sites when using 60 sec averaging (Kruskal-Wallis; P = <0.001, Mann-273 Whitney; $P = \langle 0.001, \text{ Table 1 and Appendix S1: S3} \rangle$. Conversely, when using 60 min averaging 274 not all seasons showed significant differences. 275

Diel seasonal averages of broadband SPL (BB SPL) varied among recording sites (Figure 2).
While Site 1 tended to show an increase in BB SPL towards midday during the Summer and
Spring recordings periods, Site 2 did not show this increase during any seasonal recording
period. However, during the Winter period Site 2 exhibited an increase in BB SPL during the
nighttime hours (~6 dB increase), with the transition occurring around sunrise and sunset (Figure 2).

In relation to spectral composition, the two recording sites within SBNMS were relatively complex due to a variety of acoustic contributors, with both sites having similar overall frequency contributions with shared biotic and anthropogenic signals (Figure 3; panels 1 & 2, Figure 4a).

286 At both recording sites there was a general trend of higher SPLs at low frequencies (<200 Hz), decreasing into the higher frequencies throughout the recording seasons. This was largely 287 288 due to the presence of large commercial ships (see section below). There were also large peaks in the spectra at both Site 1 and Site 2 centered around 20 Hz due to the pulse vocalization of fin 289 290 whales (Balaenoptera physalus). This was most pronounced during the Fall and Winter recording periods, with a rise of up to 20 dB re 1 μ Pa² Hz⁻¹ (Figure 3). Particularly at Site 2, 291 there were also narrowband spikes in the spectra from 8 - 12 kHz during all seasons except 292 Winter, due to the signals from acoustic devices used on commercial fishing nets to deter 293 294 porpoises and other marine mammals. These devices increased these frequency-specific sound levels during those time periods by as much as 12 dB re 1μ Pa² Hz⁻¹ (Figure 3). 295 Sites within SBNMS showed a large amount of variation in SPLs within the select octave 296 297 bands (Appendix S1: Fig. S2a & b). During the Summer recording period, Site 1 had an increase

298 $(12-28 \text{ dB re } 1 \mu \text{Pa RMS})$ in several octave bands (centered on 62, 125, 251, and 501 Hz) which

- 299 peaked around midday, and likewise in the spring. This diel trend was not seen during other
- seasons or at Site 2. The Winter recording period was more consistent, however, episodic peaks
- 301 (≤ 17 dB re 1µPa) in SPL were still occurring, although not in consistent diel trend and largely
- due to transiting vessels (Appendix S1: Fig. S2a & b).
- 303 There was no strong linear relationship (r > -0.5 or 0.5) between BB (10 24,000 Hz) SPL or
- select octave band levels and wind speed (m/s) or wave height (m) at either site, during any
- seasonal recording period. Winter and Spring recording periods had the highest correlation
- among these variables; however, this was found to be a relatively weak to moderate relationship,
- with *r* values in the 0.257 0.425 range for wind speed, and 0.205 0.394 for wave height and
- BB SPL. The octave band levels centered on 501 Hz had the strongest linear relationship with
- these factors. However, these were only moderate, with wind speed (Pearson Test; r = 0.359 &
- 310 0.437, Site 1 & Site 2 respectively) and wave height (r = 0.263 & 0.407, Site 1 & Site 2
- respectively) during the Spring, and with wind speed (r = 0.437 & 0.422, Site 1 & Site 2
- respectively) during the Winter recording periods.

313 *3.1.2 Vessel presence and contribution to the soundscape*

At Site 1, over the three days per moon phase during the Summer Recording period manually examined for vessel presence, there was the highest vessel occurrence within this study, with 90.6 % of the hours analyzed including vessel sound (261 of 288 h), and a daily average of 21.75 ± 0.4 h of presence per day (Appendix S1: Table S4). Due to the high proportion of total hours with vessel presence, SBNMS was excluded from the further detailed vessel analysis as there were too few hours available to compare times with vs. without vessel presence.

320 *3.1.3 Detection and classification of biological vocalizers*

321 Atlantic cod

The number of cod vocalizations varied greatly between the two sites in SBNMS and among seasonal recording periods. Winter and Spring periods had the fewest numbers of detected calls, with seven and one vocalization(s) respectively. During the Spring recording period the only vocalization was detected at Site 1 (Figure 5). The Summer period had an intermediate number of vocalizations detected at both Site 1 and Site 2, with 32 and 29 vocalizations respectively. The Fall recording period had the largest number of vocalizations detected, with a substantially higher number of calls at Site 1 compared to Site 2 (4903 and 32 respectively). The peak of the
vocalizations was recorded on the 24th of November 2016, with 715 true calls, three days after
the third quarter moon phase.

331 Low frequency vocalizing whales

True detections of vocalizations from fin, sei and North Atlantic right whales were identified 332 333 in all seasonal recording periods. Fin whale vocalizations had the highest daily presence, these were present every day during the Summer, Fall and Winter recording seasons at Site 2 334 335 (Appendix S1: Fig. S1). These were also high in daily presence at Site 1 occurring in 62.2 %, 90 % and 90.2 % of days during the Summer, Fall and Winter recording periods respectively. There 336 337 were no fin whale vocalizations detected at either site during the Spring recording period (Appendix S1: Fig. S1). Sei whale vocalizations were present at both sites during all seasonal 338 recording periods. Vocalizations were present at Site 1 for 8.1 %, 20 %, 54.9 % and 44.1 % and 339 at Site 33 for 29.7 %, 24 %, 74 % and 91.2 % of days in the Summer, Fall, Winter and Spring 340 341 recording periods respectively. Vocalizations from North Atlantic right whales were present during all recording periods at Site 2 (Summer: 2.7 %, Fall: 20 %, Winter: 21.6 % and Spring: 342 29.4 %) and all with the exception of the Summer period for Site 27 (Fall: 4 %, Winter: 49 % 343 and Spring: 11.8 %). 344

345 **3.2 Gray's Reef National Marine Sanctuary**

346 *3.2.1 Patterns in broadband sound pressure levels and spectral composition*

Broadband (10 - 24,000 Hz) SPLs (both median and RMS values) varied by as much as 10 347 dB among recording sites and seasons within GRNMS (110.6 – 123.9 dB re 1µPa, Table 1). 348 Season significantly affected BB SPL at both sites when using both 60 s and 60 min averaging 349 (Kruskal-Wallis; $P = \langle 0.001, Mann-Whitney; P = \langle 0.001, Table 1 & Appendix S1: Table S3)$. 350 351 The highest median BB SPLs occurred during the Summer recording period for both sites, followed by Spring and lastly the Fall recording period for both sites. No data were available for 352 the Winter period. On average, Site 4 had consistently higher BB SPLs than Site 3 during all 353 354 seasonal recording periods. Diel seasonal averages of BB SPL were relatively consistent between recording sites (Figure 2). Both sites showed a consistent rise in BB SPL around dawn 355 356 and dusk, which followed temporal and seasonal patterns in sunrise and sunset times (length of day). Both sites tended to peak around sunrise and sunset for approximately 1.5 hours, with 357

daytime hours (defined as post-dawn peak to pre-dusk peak) being lower than nighttime hours(defined as post-dusk peak to pre-dawn peak).

360 In terms of spectral composition, both sites within GRNMS had similar overall shapes/frequency contributions with shared biotic signals such as snapping shrimp and toadfish 361 (Figure 3; panels 3 & 4). Both sites were largely dominated by the acoustic signals of snapping 362 shrimp, these snaps produced a broadband rise in the spectra at both sites between $\sim 2 - 17$ kHz. 363 which was consistent through all recording seasons sampled (Figure 3 & 4b). As the two sites 364 were relatively shallow (~ $20 \text{ m} \pm 1 \text{ m}$), there was also low frequency signal (10 - 500 Hz) 365 associated with the wind and waves acting on the water surface (Figure 3). Periods of high 366 winds, at times, caused an increase in broadband SPL, however, these factors were only mildly 367 statistically correlated at Site 4 during the Summer recording period (Pearson correlation; r (682) 368 = 0.39, P = <0.001) (see below). There were two large spectral peaks in the mid frequencies 369 (~230 Hz and again at ~460 Hz), which were most pronounced during the Spring recording 370 periods, at both sites but with Site 4 being most evident (Figure 3 and 4b). These peaks were the 371 fundamental and harmonics of the calls produced by a toadfish species, thought to be the ovster 372 373 toadfish (*Opsanus tau*), and raised the 251 Hz octave band by as much as 15 dB re 1 μ Pa during the Spring recording period (Figure 3 & 6 [panels i, j, k, 1]). Sites within GRNMS showed stable 374 375 broadband SPLs and were generally most influenced by the increase in snaps from snapping shrimp at dawn and dusk. This increased broadband SPLs by as much as 6 dB re 1µPa at both 376 sites during the Summer, and 8 dB and 6 dB re 1µPa at Site 4 and Site 3 respectively during the 377 Spring recording period. 378

379 During the Summer recording period, infrequent and close vessel passage (e.g., Figure 6; panel b ~12:20 – 13:30), SCUBA diving activity (e.g., Appendix S1: Fig. S2c; panel d, ~12:00 380 25th & 26th July), intense periods of unidentified fish chorusing around dusk (e.g, Figure 6; 381 panels a, b, c d), contact with the hydrophone stand, and times of heavy rain and/or thunder (e.g., 382 Appendix S1: Fig. S2c; panel a, ~09:00 18th July) were also found to influence octave bands (63, 383 125, 251, 501, and 1000 Hz). During the Spring recording period, octave band sound pressure 384 levels showed strong diel patterns, with Site 3 showing a distinctive peak around dusk in octave 385 386 bands centered on 251, 501 and 1000 Hz, and 125 and to a lesser extent. This resulted in a 23 and 20 dB re 1µPa increase in the 501 & 1000 Hz octave band respectively for around 4.5 hours, 387 which was more pronounced around the full moon phase (Appendix S1: Fig. S2c; panel k). At 388

- 389 Site 4, these peaks were not limited to dusk and SPLs in 251 and 501 Hz bands would peak
- around dusk and remain elevated through the dark hours and then drop off around dawn
- 391 (approximately 10 hours). This pattern was most pronounced around the first quarter and full
- moon. These two bands would also show a lesser peak around noon then drop until dusk during
- some moon phases (Figure 6).
- There were no strong linear relationships (r > -0.5 or 0.5) between BB SPL and wind speed
- 395 (m/s) or wave height (m) at sites within GRNMS. Sites had weak to moderate correlations, with
- the Summer period exhibiting the strongest of all seasons (Pearson Test; wind: r = 0.241 &
- 397 0.368, wave: r = 0.200 & 0.321 at Site 3 and Site 4 respectively).
- However, at Site 4 there were moderate relationships between the octave band levels centered on
- 501 Hz and both wind speed (r > 0.484) and wave height (r > 0.317) during the Summer
- 400 recording period, and moderate relationships (r = 0.349 0.381) between bands 63 Hz, 125 Hz,
- 401 251 Hz, and 501 Hz and wind speed at Site 3 during the Spring Recording period. Fall had the
- 402 strongest relationships (r > 0.5) between the octave band levels centered on 125 Hz and 251 Hz
- and both wind speed and wave height, and the 501 Hz band and wind speed at Site 3, as well as
 bands centered on 125 Hz, 251 Hz and 501 Hz and wind speed at Site 4.

405 *3.2.2 Vessel presence and contribution to the soundscape*

- At Site 3 within GRNMS, over the three days per moon phase in the Summer recording period manually examined for vessel presence, there was a low occurrence of vessel presence in the recordings (6.6 % or 18.9 of 288 h). This site had a daily average of 1.58 ± 0.5 h of vessel presence per day (Appendix S1: Table S4) and was the lowest of the study.
- 410 Removing times with vessel presence reduced the median SPL in the lower 1/3 octave
- frequency bands (bands centered on 251.2 Hz and below) by up to 4 dB and up to 12.5 dB re
- 412 1μ Pa in the 90th percentile (Figure 7). However, the 1/3 octave bands centered on 316 Hz and
- 413 above, the median and 90th percentile SPL slightly increased by up to 2.8 dB re 1 μ Pa \pm 0.1, as it
- removed biologically significant times of the day increase SPL in these bands.
- 415 *3.2.3 Detection and classification of biological vocalizers*
- 416 *Snapping shrimp*
- 417 Sound from the snaps of snapping shrimp were present in all recordings at both sites within
- 418 GRNMS. Snap signals were quantified in terms of both snap detection rate (per 60 seconds) and

the SPLs of the snap associated frequency band (2000 - 20,000 Hz) during all recording periods.

- 420 In general, Site 4 had higher overall snap rates than Site 3 for every recording period (Table 2,
- 421 Appendix S1: Fig. S3a). The Spring recording periods had the highest seasonal snap rates at Site
- 422 3 and Spring and Summer were equally high at Site 4.
- 423 At a 24-hour time scale, both sites exhibited typical, strong diel patterns in snap rate, with an
- 424 increase around dawn, dusk and midnight time segments compared to noon rates (Table 2,
- 425 Appendix S1: Fig. S3a). There were significant differences in snap rate among the time segments
- 426 (Dawn, Noon, Dusk & Midnight) within each site (Friedman Test; P = <0.001), with the Noon
- time segment consistently lower snap rates that the other three segments. During the Summer
- 428 both sites exhibited significantly higher snap rates during Dawn and Midnight (Table 2). During
- 429 the Fall and Spring recording periods Site 3 showed significantly higher rates during Dawn,
- 430 Dusk and Midnight, compared to Noon, whereas at Site 4 during the Fall and Spring, Dusk and
- 431 Midnight were significantly higher compared to Dawn and then Noon.
- 432 Low frequency vocalizing whales
- 433 There were no validated detections of vocalizations from any baleen whale species in any
- 434 seasonal recording periods within GRNMS.
- 435 **3.3 Florida Keys National Marine Sanctuary**

436 *3.3.1 Patterns in broadband sound pressure levels and spectral composition*

Broadband (10 - 24,000 Hz) SPL (both median and RMS values) in FKNMS had a high 437 438 degree of seasonal consistency, only varying by as much as 1.9 dB (107.8 to 109.6 dB re 1 uPa Winter and Spring respectively, Table 1). Seasonal comparisons were only available at Site 6 as 439 Site 5 only had useable data from the Summer recording period. Season statistically affected 440 broadband SPL at Site 6 when using 60 s averaging (Kruskal-Wallis; $P = \langle 0.001, \text{ Table 1} \rangle$). 441 442 However, when using 60 min averaging there was no significant difference between Summer and 443 Spring recording periods (Appendix S1: Table S3). At Site 6 the Spring recording period had the highest median broadband SPL, followed by Summer, Fall and then Winter, but these differences 444 among seasons (within 1.8 dB re 1 µPa, Table 1) were much lower than those observed at other 445 sanctuaries. Diel seasonal averages of broadband SPL were also extremely stable among seasons 446 447 and recording sites (Figure 2). Both sites exhibited a rise in SPL around dawn and dusk, which followed temporal and seasonal patterns in sunrise and sunset times. The SPL at both sites 448

peaked approximately 45 m before sunrise and post-dawn levels remained higher than pre-dawn
levels. Sound pressure levels remained constant during daylight hours.

451 In terms of spectral composition, both recording sites were again the most consistent/stable 452 among seasons (Eigure 3; panels 5 & 6). Both sites had similar overall shapes/frequency contributions, with common biotic signals such as snapping shrimp and multi-species fish 453 454 vocalizations, the low frequency abiotic signals of wind and waves at the surface, and low to medium frequency signals of small vessels (anthropogenic). The acoustic signals of snapping 455 shrimp produced a relatively broadband rise in the spectra between $\sim 2.5 - 15$ kHz, which was 456 consistent through all recording seasons. As the two sites within FKNMS were shallow (~ 15 & 457 13 m), there was also low frequency signals (10 - 500 Hz) associated with the wind and waves 458 acting on the water surface. 459

460 Additional to the ubiquitous peaks at dawn and dusk due to the patterns in snapping shrimp activity there were also additional episodic peaks in FKNMS recordings that did not appear to 461 occur in any defined pattern or time of day. During the Summer recording period at Site 6, 462 octave bands centered on 125 and 251 Hz exhibited a diel trend during the first quarter moon 463 464 phase in which SPL would rise $(5 - 8 \text{ dB re } 1 \mu \text{Pa})$ around midnight till around dusk whereby it would drop to daytime levels (Appendix S1: Fig. S2d; panel b). Octave bands centered on 501 & 465 466 1000 Hz would also peak on or after dusk (depending on moon phase) before dropping to almost daylight levels after midnight (Appendix S1: Fig. S2d; panel b, c, d). This pattern was not 467 468 observed at Site 5, except during the first and third quarter moon phases, and the increase in SPL in the dark hours were less pronounced (~3 dB) (Appendix S1: Fig. S2e; panel b & d). 469 470 Nonetheless, the dusk peaks were still present in select octave bands and would increase by as much as 15 dB around the full moon. This pattern of increase in the octave bands at dusk also 471 472 continued during the Fall, Winter and Spring recording periods at Site 6. Frequent episodic spikes in SPLs caused by vessel traffic were common in all season but most pronounced around 473 noon in the Fall and Winter periods (Appendix S1: Fig. S2d). 474

There were no strong linear relationships (r > -0.5 or 0.5) between broadband SPL and wind speed (m/s) at sites within FKNMS. Site 6 had weak correlations in the Fall, Winter and Spring recording periods (r = 0.13, 0.27, 0.01 respectively). The Fall and Winter recording periods had the strongest (weak to moderate) of relationships between octave bands centered on 251 (r =0.313 & 0.478) and 501 Hz (r = 0.331 & 0.422) and wind speed at Site 6.

480 *3.3.2 Vessel presence and contribution to the soundscape*

- At Site 5 within FKNMS, over the three days per moon phase during the Summer recording 481 482 period manually examined for vessel presence, there was a low occurrence of vessel presence in the recordings (12.6 % or 36.6 h of 288 h, and a daily average of 3.1 ± 0.37 h of vessel presence 483 per day) (Appendix S1: Fig. S4). Removing times with vessel presence reduced the median SPL 484 in the lower 1/3 octave frequency bands (bands centered on 251.2 Hz and below) by up to 5.3 dB 485 and the 90th percentile by up to 13.24 dB re 1 μ Pa, Figure 7). The 1/3 octave bands centered on 486 316 Hz and above decreased the 90th percentile by up to 5.9 dB re 1 μ Pa, however, this decrease 487 in SPL dropped to as low as 0.46 dB re 1µPa in the 5012 Hz bands and above (Figure 7). 488
- 489 *3.3.3 Detection and classification of biological vocalizers*
- 490 Snapping shrimp

491 Sound from the snaps of snapping shrimp were present in all recordings at both sites within

492 FKNMS. During the Summer recording period both sites had very similar average snap rates

493 (361 and 362 snap/60 s) (Table 2, Appendix S1: Fig. S3b). At Site 6, there were significant

494 differences in snap rate among seasons (Kruskal-Wallis; H = 6585, P = <0.001). Spring had the

- highest snap rate (614 snaps/60 s), followed by Summer (362), Fall (296), and then the Winter
- 496 recording period with the lowest (237).
- 497 Over a 24-hour time scale, both sites exhibited a diel pattern in snap rate, with an increase 498 around dawn, dusk time segments compared to noon and midnight (Table 2, Appendix S1: Fig. S3b). There were significant differences in snap rate among time segments (Dawn, Noon, Dusk 499 & Midnight) within both sites (Friedman Test; $P = \langle 0.001 \rangle$, generally with Dusk and Dawn 500 501 exhibiting consistently higher snap rates that the other time segments. At both sites during the Summer recording period, Dusk significantly had the highest snap rate, followed by Dawn and 502 Noon, and with Midnight having the lowest. During the Fall and Winter periods both Dusk and 503 504 Dawn had significantly higher snap rates to Noon and Midnight and the Spring having significantly highest rates at Dusk compared to Dawn Noon and Midnight (Table 2). 505

506 *Low frequency vocalizing whales*

507 There were no validated detections of vocalizations from any baleen whale species in any

seasonal recording periods within FKNMS.

509 3.4 Flower Garden Banks National Marine Sanctuary

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510 *3.4.1 Patterns in broadband sound pressure levels and spectral composition*

Broadband (10 - 24,000 Hz) SPL (both median and RMS values) varied by as much as 12 dB among recording sites and seasons within FGBNMS (108.8 – 121.9 dB re 1µPa, Table 1). Site 7 had significantly higher SPL than Site 8 over all season. Season significantly affected broadband SPL at both sites when using 60 sec and 60 min averaging (Kruskal-Wallis; P = <0.001) (Table 1 & S3). The Summer and Spring recording periods exhibited the highest broadband median and RMS SPL, followed by Fall and then Winter at both sites.

517 Diel seasonal averages of broadband SPLs showed similar overall patterns at both sites 518 (Figure 2). Both sites exhibited a consistent peak around dawn and dusk, which followed 519 temporal and seasonal patterns in sunrise and sunset times (length of day), for approximately 1.5 520 hours, with daytime hours (defined as post-dawn peak to pre-dusk peak) being lower than 521 nighttime hours (defined as post-dusk peak to pre-dawn peak). On average, Site 7 had 522 consistently higher broadband SPLs than Site 8 during all recording periods.

523 Both sites exhibited a broadband rise in the spectra between $\sim 2 - 15$ kHz, and this rise was consistent through all recording periods (Figure 3; panels 7 & 8). Site 7 was dominated by low 524 525 frequencies (40 – 150 Hz), due to long periods of stationary and slow-moving vessel activity close to the recording location and additional distant human activity sources (e.g., vessels and 526 527 seismic sources used in oil and gas exploration). This peak was not observed at Site 8, though did exhibit a distinctive peak in the spectra at $\sim 600 - 1500$ Hz, only present in the 90th percentile. 528 529 Extensive low frequency biological signals associated with the vocalizations from fishes were apparent at both sites in FGBNMS, however, due to the high proportion of overlap with the low 530 531 frequency anthropogenic signals, particularly at Site 7, the presence of any periodicity in these biological signals was not apparent within any of the 50 % spectral level measurements during 532 533 any of the recording seasons (Figure 3).

Broadband and octave band SPLs at Site 7 were on average 6 - 15 dB re 1µPa higher than at Site 8, depending on the season (Table 1 & Appendix S1: Fig. S2f & g). The influence of the peak of snapping shrimp signal and dawn and dusk maxima were less prominent due to low frequency dominance from other acoustic activities. At Site 7, during all recording periods, there were episodic peaks (≤ 16 dB re 1µPa RMS) in the selected octave bands, although they did not occur in a consistent diel or other pattern that could be determined. During the Fall recording period, over all moon phases, there was a substantial rise in SPLs (16 – 18 dB re 1µPa) in the 541 501 and 1000 Hz octave bands, beginning around dusk and returning to ambient levels just

542 before or around midnight depending on the moon phase (Appendix S1: Fig. S2f; panels e, f, g,

543 h). Smaller peaks also occurred at Site 8 in the Fall recording period, however, these occurred

- earlier in the day and in the 251 and 501 Hz octave bands (Appendix S1: Fig. S2g; panels e, f, g,
 h).
- There was no strong linear relationship (r > -0.5 or 0.5) between broadband (10 24,000 Hz) SPL and wind speed (m/s) at either site within FGBNMS, although, both sites showed a weak correlation in the Fall season (Pearson Test; r = 0.182 & 0.207 Sites 7 & 8 respectively). There were strong linear relationships between the octave band levels centered on 63, 125, 251 and 501 Hz and wind speed at Site 8 during the Fall recording season (r = 0.650, 0.654, 0.596, and 0.730respectively). Site 8 also had a weak to moderate relationships between octave bands 251 and 501 Hz (r = 0.237 & 0.247 respectively) and wind speed during the Spring recording period.

553 *3.4.2 Vessel presence and contribution to the soundscape*

554 At Site 8 within FGBNMS, over the three days per moon phase during the Summer recording period manually examined for vessel presence, there was a moderate occurrence of vessel 555 556 presence in the recordings (27.3 % or 78.6 h of 288 h, and a daily average of 7.1 ± 0.99 h of vessel presence per day) (Appendix S1: Table S4). Removing times with vessel presence 557 558 produced a notable reduction in median SPL in the lower 1/3 octave frequency bands (bands centered on 251.2 Hz and below) reduced by as much as 10.63 dB and the 90th percentile by up 559 to 12.58 dB re 1µPa (Figure 7). Octave bands centered on 316 Hz and above decreased the 90th 560 percentile by up to 5.2 dB re 1µPa, but above 2512 Hz the differences were negligible (< 0.28561 562 dB) (Figure 7).

563 *3.4.3 Detection and classification of biological vocalizers*

564 Snapping shrimp

Sound from the snaps of snapping shrimp were present in all files recorded at both sites within FGBNMS though Site 7 had higher overall snap rates than Site 8 for all seasonal recording periods (Table 2, Appendix S1: Fig. S3c). At Site 7, there were significant differences among seasons in snap rates (Kruskal-Wallis; H = 8861, P = < 0.001). Spring had the highest average snap rate (446 snaps/60 s), followed by Summer (377), and Fall and Winter (300 & 294 respectively). This pattern was also consistent at Site 8 (Kruskal-Wallis; H = 6339, P = < 0.001)

with Spring (335) having the highest, followed by Summer (306) and then Fall (189) with the 571 lowest. Over a 24-hour time scale both sites exhibited highly variable diel patterns in snap rate 572 573 that varied among seasons. (Table 4, Appendix S1: Fig. S3c). There were significant differences 574 in snap rate among diel time segments (dawn, noon, dusk & midnight) within both sites during all recording periods (Friedman Test; $P = \langle 0.001 \rangle$), with the exception of the Summer at Site 8. 575 576 Site 7 was the most variable of all sites in, however, Dusk most often had the highest snap rate. During the Summer recording period at Site 7, Noon and Dusk had a significantly higher snap 577 rate than Midnight and Dawn, whereas Site 8 had no significant differences. During the Fall 578 periods Site 7 and 8, had differing patterns in snap rate, with Site 7 showing significantly highest 579 rates during the Dusk time segment, followed by Midnight and Dawn, and then Noon. Whereas 580 Site 8 exhibited significantly higher rates at Dusk, Dawn and Midnight segments, compared to 581 582 Noon segments. During the Winter recording period Site 7 had significantly higher snap rates in the Dawn and Midnight time segments compared to Noon and Dusk. Again, in the Spring period 583 584 both sites had different patterns in snap rate with Site 7 showing significantly higher rate in the Midnight and Dusk segments than Noon and Dawn. At Site 8, Dusk was significantly higher 585 586 than Midnight and Dawn, with Noon being the lowest (Table 2).

587 *Low frequency vocalizing whales*

There were no validated detections of vocalizations from any baleen whale species in anyseasonal recording periods within FGBNMS.

590 **3.5 Inter-Sanctuary Comparisons**

591 *3.5.1 Patterns in broadband sound pressure and spectral composition*

Among sanctuaries, seasonal frequency power spectra varied in overall appearance, due to 592 differing biotic, abiotic and anthropogenic signals contributions. Sites within GRNMS, FKNMS 593 594 and FGBNMS exhibited a similar broadband rise in the spectra between $\sim 2 - 15$ kHz, and this 595 rise was consistent through all recording seasons. Sites within GRNMS displayed the greatest variability in the mid-range frequencies (100 - 10000) among seasons. Broadband SPLs (both 596 597 median and RMS values) also varied substantially, ranging from 100.2 - 124 dB re 1µPa (Table 1). GRNMS had the highest broadband RMS SPLs across all seasonal recording periods 598 599 sampled, with maximum levels recorded in the summer and fall and highest median levels in the summer and spring. The highest broadband RMS levels recorded in the winter were within 600

FGBNMS which also showed the highest median SPLs in the fall and winter (Table 1). FKNMS showed the highest consistency in broadband (10 - 24,000 Hz) SPL (medians and RMS), PSD and frequency distribution among sites and seasons, and SBNMS exhibited the most variation.

Diel trends in broadband SPL also varied among sanctuaries. Seasonal average diel SPL
showed similar temporal patterns among the three reef-based sanctuaries (GRNMS, FKNMS and
FGBNMS), with a rise around dawn and dusk, and following temporal and seasonal patterns in
sunrise and sunset times (length of day) due to biological signaling (Figure 2).

Wavelet analysis indicated that strength and persistence of periodicity in octave band SPLs 608 also varied among sanctuary, site, season, and octave band. The sites GRNMS and FGBNMS 609 sanctuaries exhibited strong (significant at the 95 % confidence level: wavelet scalogram) once 610 per day periodicity in the SPLs in the octave band centered on 7943 Hz during each seasonal 611 sampling period, almost entirely uninterrupted. Those interruptions largely coincided with a 612 twice per day periodicity (Site 3 Fall). Whereas Sites within FKNMS demonstrated a twice per 613 614 day periodicity in this band during all seasonal sampling periods (see biological vocalizers section below). Sites within GRNMS, which saw distinctive diel patterns in several octave band 615 616 SPLs and periods of biological chorusing around certain moon phases (Figure 6 & Appendix S1: Fig. S2c), also showed strong once per day periodicity (wavelet analysis) in SPLs in these bands. 617 618 For example, Site 4 during the Summer recording period in 63, 125, 251 Hz bands and to a lesser extent 501 Hz band, and at Site 3 and 4 in the Spring recording period all bands, with some short 619 620 interruptions (1 day) in this periodicity in the 63, 125 and 1000 Hz bands at Site 3. Similarly, sites within FKNMS exhibited a strong once per day periodicity in the 251 Hz band during the 621 622 entire Summer sampling period, and 63, 125 and 501 Hz bands during the full and third quarter 623 moons. Also, in the 251 Hz band for the entirety of the Spring sampling period. 624 SBNMS showed a dissimilar pattern in average diel SPLs from the other sanctuaries, which 625 varied among seasons, notably with SPLs peaking at approximately midday in the Summer

recording period (Figure 2). Site 1 exhibited strong once per day periodicity in the 125, 251 and
501 Hz octave band levels across the entire Summer recording period, however, nothing

628 significant during all other sampling periods. Site 2 showed no strong periodicity.

629 3.5.2 Relationship between sound levels and wind and wave data

Among all sanctuaries, there were no strong linear relationships (r > -0.5 or 0.5) between

broadband (10 - 24,000 Hz) SPL and wind speed or wave height at any site, during any

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recording period. Of all the sanctuaries, both sites within GRNMS had the strongest linear

relationship between wind speed and wave height and the octave bands centered on 125 (> r =

634 0.56), 251 Hz (> r = 0.68) and 501 Hz (r = 0.62) during the Fall recording period. Also, during

the fall period, Site 8 within FGBNMS had a strong linear relationship between wind speed and

- 636 63, 125, 251, 501 Hz octave band levels (r = 0.65, 0.65, 0.6 and 0.73). See individual Sanctuary
- 637 sections for further detail.
- 638 *3.5.3 Vessel presence and contribution to the soundscape*

639 In the three days per moon phase manually examined for vessel presence in each sanctuary, SBNMS had the highest occurrence of vessel presence in recordings (261 of 288 h) and an 640 average of 21.75 hours of vessel presence per day. The lowest vessel presence was in GRNMS 641 (18.9 of 288 h), with vessel presence in FKNMS and FGBNMS sanctuaries more moderate (36.6 642 643 h of 288 and 36.6 h of 288 h respectively). Overall, vessel presence contributed the largest amount of energy to low frequency 1/3 octave bands between 31.6 and 398.1 Hz, with 63 to 125 644 Hz bands being the most influenced at all sites. Only GR, FK and FGBNMS had enough 645 variance in vessel presence to support comparison of levels between periods without vs. with 646 647 vessels. Of these sanctuaries, FGBNMS had the greatest increase in median SPL (10.63 dB re 1µPa in band centered on 251.2 Hz) and GRNMS has the smallest increased in median SPL (4 648 649 dB re 1μ Pa) due to the contribution of vessels when times with vessels versus no vessels were 650 compared.

651 *3.5.4 Detection and classification of biological vocalizers*

Due to the geographically disparate locations of the sanctuaries, most vocalizing species were 652 sanctuary specific and their presence could not be compared among sites. However, the snaps 653 from snapping shripp were detected at sites within GRNMS, FKNMS and FGBNMS. At a 24-654 655 hour time scale all three sanctuaries exhibited strong diel patterns in snap rate, with an increase 656 around dawn and dusk, however, rates during the daytime and nighttime periods differed among sanctuary. Both sites within GRNMS and FGBNMS generally exhibited higher snap rates during 657 the Midnight time segments than Noon segments (Table 2). This was not the case at sites within 658 FKNMS, with Midnight segments being more similar to Noon time segments, and hence seeing 659 660 twice per day periodicity in SPLs in the 7943 Hz octave band.

661 Sound pressure levels in the 2000 - 20,000 Hz analysis band was a significant predictor of 662 snap rate at several sites during several seasons. At both sites within GRNMS SPL_(snap band) was a 663 significant predictor of snap rate during all seasons with the exception of Spring at Site 3. This 664 model was stronger at Site 4 than Site 3.

At sites within FKNMS, SPL did not show this same predictor strength, with both sites having a weak to no predictor value of SPL (2000 – 20,000 Hz) to snap rate during all seasons ($R^2 \le$ 0.27). FGBNMS was more similar to FKNMS than GRNMS with both sites also having a weak to no predictor value of SPL to snap rate during all seasons ($R^2 \le 0.28$), with the exception of Site 8 in the Fall ($R^2 \ge 0.62$).

670 4. Discussion

This study provided baseline acoustic characterization information, exploring the daily 671 drivers, seasonal patterns, and identified the abiotic complexities and compared these attributes 672 across geographically distributed underwater soundscapes within four ecologically varying U.S. 673 674 National Marine Sanctuaries. Studies investigating marine soundscapes have largely focused on temporal trends or variations within a single habitat (Curtis et al. 1999, Radford et al. 2008, 675 Staaterman et al. 2014). Such studies can be used to design and target continued monitoring of a 676 site or habitat to better understand changes in biological contributors, anthropogenic activities, 677 678 and/or some degree of habitat 'health' or regime shifts (Rossi et al. 2017). However, increasingly studies are exploring spatial variation within and among geographically distributed underwater 679 680 habitats (McWilliam and Hawkins 2013, Putland et al. 2017a, Haver et al. 2018).

When undertaking acoustic monitoring efforts in geographically separated and biologically 681 682 and physically dissimilar systems, a standardized approach towards both data collection and analyses is necessary for comparisons. The standardized equipment, field design and analyses in 683 this study assisted in identifying measurements that most effectively summarized soundscape 684 685 attributes at sites both within and among sanctuaries. Some factors remain difficult to standardize when recording among different systems but are important for data interpretation. Due to the 686 distinct environmental features of individual sites (e.g., differences in depth, substrate type, 687 temperature, complexity) over wide ranging monitoring projects, direct quantitative comparisons 688 among sites should note the possible influence in varying acoustic propagation characteristics. In 689 this study, the most prominent example of this is the moderately deeper (50-68 meter) locations 690 of the recording sites in SBNMS, relative to the more similar depth conditions of the remaining 691

recorders (~20 meters). Depth can play a major role in how signals propagate from the source to 692 the recorder, as the cutoff frequency increases at decreasing depths (according to normal-mode 693 694 theory). Modes near the cutoff frequency are strongly attenuated and therefore the shallower the 695 site the greater the low frequencies may be affected (Tindle et al. 1978, Tindle 1982). Due to more efficient propagation of low frequencies in deeper waters, sound levels over a larger area in 696 697 the vicinity of SBNMS recording sites may have contributed to levels at these sites, more than what was possible at the other recording locations. Therefore, care needs to be taken when 698 considering SPLs among sites, especially at low frequencies. Despite this, differences in 699 propagation characteristics cannot account for many sources of variation in soundscape 700 parameters studied here. 701

702 Spectral composition and identification of contributors

703 All four U.S. National Marine Sanctuaries were found to have differences in broadband sound pressure level (10 - 24,000), one third octave band levels and distinct spectral compositions, 704 705 each with unique characteristics due to differences in biology, human use patterns, propagation properties, and climate. Unsurprisingly, there was less variation in the measured soundscape 706 707 parameters within a sanctuary, compared to among sanctuaries. In three out of the four sanctuaries monitored, variation in sound levels over the course of the project among sampled 708 709 locations in the same sanctuary was relatively low. However, variance among sampled locations 710 in Flower Garden Bank National Marine Sanctuary (FGBNMS) was relatively high.

The difference underscores the role that 'baseline' or pilot projects can play in determining sampling needs for longer-duration efforts, as well as highlighting that even small, protected areas can still demand higher sampling levels. Small changes in physical habitat, biological and oceanographic processes and/or human use can lead to very distinct changes in the soundscape even within a relatively short distance (Radford et al. 2010, Radford et al. 2014).

In general, SBNMS soundscapes were most dissimilar to the sites within GRNMS, FKNMS and FGBNMS, with the polarizing feature being the frequency of the dominant signals within the soundscape. The frequency composition of the two sites within SBNMS were largely dominated by low frequency signals (10 – 100 Hz) with a median PSD found to be between approximately 58 - 101 dB re 1µPa² Hz⁻¹. This was largely due to the near constant presence of signal from large vessels travelling to and from Boston Harbor (Hatch et al. 2008). The more trafficked site in FGBNMS showed the most similarity to this pattern among the other sanctuaries. There was

also a substantial peak in SBNMS recordings (up to 22 dB re 1μ Pa² Hz⁻¹) in the spectra at 723 approximately 15 - 25 Hz during all seasonal recording periods, except for the Spring, due to the 724 725 presence of the fin whale (Balaenoptera physalus) pulse vocalizations, which was also consistent 726 with detection output from the Low Frequency Detection and Classification and System (LFDCS) (Baumgartner and Mussoline 2011). In the western North Atlantic, fin whales regularly 727 728 occur within Massachusetts Bay and SBNMS (Hain et al. 1992) and have been reported to sing from approximately September through June (Clark and Gagnon 2002). In a study by Moreno et 729 al, (2012) they reported fin whales vocalizations received at an acoustic listening station, very 730 close to one of the SBNMS sites in the current study, in 814 of 817 days analyzed from October 731 2007 to March 2010. This differs to some extent to the results seen here at the comparable site in 732 2016/17, where the 20 Hz vocalizations were present in everyday sampled within the Summer, 733 734 Fall and Winter, however, absent in the Spring Sampling Period. Fin whale vocalizations, and subsequently this ~ 20 Hz peak in the spectra, were not observed in any of the other sanctuaries. 735 Sound levels in higher frequencies, 2 - 24 kHz, were much lower at the sites within SBNMS 736 compared to the other sites, likely due to the absence of reef dwelling snapping shrimp. SBNMS 737 738 is thought to be beyond their northern distribution (McClure 1995). However, during all the seasonal recording periods there were narrowband impulsive signals present centered around 10 739 kHz (9 – 12 kHz) due to the presence of signals from acoustic deterrent devices. These devices 740 are attached to pelagic or bottom gillnets in attempt to reduce cetacean and pinniped bycatch 741 (Coram et al. 2014). 742

At the reef sites within GRNMS, FKNMS and Site 8 within FGBNMS, signals at low 743 frequencies (< 50 Hz) were largely due to abiotic factors such and wind and waves acting at the 744 water surface (Knudsen et al. 1948) and sporadic vessel activity. Site 7 within FGBNMS was the 745 746 exception to this, as it was dominated by the lower frequency bands (40 - 150 Hz) with a peak centered around 70 Hz during all seasons with a median and 90th percentile PSD between 747 approximately 72 – 85 dB and 87 – 91 dB re 1μ Pa² Hz⁻¹ respectively. This low frequency 748 contribution was identified to be in part due to near continuous heavy commercial shipping and 749 750 to a lesser extent, distant seismic exploration (NOAA & ONMS, 2012, BOEM & NOAA, 2018). The northwest Gulf of Mexico is one of the most active areas of oil and gas exploration and 751 development in the world, with approximately 150 oil and gas platforms located within 40 km of 752 the boundaries of FGBNM (NOAA & ONMS, 2012). These anthropogenic activities have also 753

been documented in other areas of the Gulf of Mexico, with seismic survey sources dominating 754 (Estabrook et al. 2016, Wiggins et al. 2016). Site 8 within FGBNMS had a similar spectral shape 755 756 to Site 7 from 10 - 30 and 300 - 24,000 Hz, however it lacked this low frequency peak centered on 70 Hz. These differences observed between Site 7 and Site 8 are likely due to differences in 757 anthropogenic activity and the distance between the two sites being outside of the propagation 758 limits of these signals to be present in both soundscapes. Sites within FGBNMS were the most 759 geographically separated of all sites occurring within the same sanctuary, with Site 7 located on 760 the mid-shelf and Site 8 located near the outer edge of the continental shelf, separated by 761 approximately 74 kilometers, which is over double the distance of any of the other sanctuaries. 762 Site 7 also had a heavily used shipping fareway within 10 km from the site, where as Site 8 and 763 had less used shipping fareway at a greater distance during the recording periods (Bureau of 764 Ocean Energy Management & National Oceanic and Atmospheric Administration 2018). 765 Where the low frequencies dominated in SBNMS and FGBNMS (Site 7), the shallower sites 766 within GRNMS, FKNMS and FGBNMS (Site 8), were largely dominated by the mid- to high-767 frequencies (200 – 20,000 Hz). This was consistent during all seasonal recording periods, and by 768 769 large, all driven by a common signal in the 2000 - 20,000 Hz frequency range and produced by various species of snapping shrimp (member of the Alpheus and Synalpheus genera) (Au and 770 771 Banks 1997, Versluis et al. 2000). These sites exhibited the highest median and percentile values of PSD in this 'snap band' frequency, with a median at the peak of the band found to be between 772 67 - 78 dB re 1µPa² Hz⁻¹ during the Summer and Spring recording periods. All three sanctuaries 773 that contained snapping shrimp exhibited clear spatial differences in snap rate and generally 774 775 exhibited strong seasonal and diel patterns, increasing around dawn and/or dusk, a pattern which has been previously observed in many different locations and habitats around the world (Radford 776 777 et al. 2010, Ricci et al. 2016, Lillis and Mooney 2018). However, in support of the observed trend by Lillis & Mooney, 2018, not all sites showed the typically reported increased rate during 778 dark periods compared with light periods. For example, sites within the FKNMS showed a peak 779 780 in snaps at dusk and exhibited higher snap rates during the day compared to night (light and dark periods respectively). Understanding of these spatial variations in snap rate and pattern is not 781 782 well understood and could be at times be due to small bathymetric and depth differences in the sound propagation and reflections. However, these inter-sanctuary variations could also be 783

indicative of ecological differences such as species composition and the diversity of hosts (Lillisand Mooney 2018).

786 There were also various distinct spectral peaks in the mid-frequencies (101 - 1000 Hz)787 depending on the season and site. At times, these peaks were due to the vocalizations of a large number of various fish species. Most perceptible was the pulse repetition rate or fundamental 788 frequency and 1st harmonic (peak 231 & 462 Hz respectively) of the boat-whistle calls produced 789 by the toadfish, thought to be Opsanus tau (Fine 1978), seen within GRNMS during the Spring. 790 As water temperatures rise in the spring sexually mature males of the species establish nests and 791 produce advertisement signals or boat-whistles for the females (Maruska and Mensinger 2009, 792 Van Wert and Mensinger 2019). These signals also presented a peak in the spectra during the 793 Summer recording period, however to a much lesser degree due to diminishing mating season, 794 and the fundamental frequency and 1st harmonic were higher in frequency (270 & 540 Hz 795 respectively) due to the increase in water temperature (Fine 1978). During certain seasons, fish 796 797 vocalizations would constitute a traditionally defined chorus, whereby the sound from many individuals is continuously above ambient background levels for an extended period using an 798 799 averaging time of 1 sec., and several distinct types of choruses were present together. However, during this time they largely held their own aural or temporal niche within the soundscape. For 800 801 example, during the spring at Sites 3 & 4 within GRNMS, there were up to four distinctive fish choruses occupying the same time but residing in different frequency bands (See Figure 4b). 802 803 These choruses would often peak together around dusk with two choruses subsequently dropping to just above ambient levels, one staying elevated during the night and dropping sharply after 804 805 dawn, and one chorus exhibiting a peak around dusk and again a smaller peak approximately 2 hours later before dropping again (See Figure 6). Interestingly, these choruses during the dark 806 807 hours were most often frequency partitioned (peak frequency), although they also exhibited some 808 temporal partitioning in the peak of the energy. In contrast, the vocalizations occurring during light hours were often overlapping and would not usually constitute a 'chorus' by the traditional 809 definition. This observation gives evidence for environmental constraints (dark vs. light) and the 810 use of different acoustic strategies to avoid masking or misinterpretation by the targeted receiver 811 812 during these time periods, supporting the acoustic niche hypothesis (Krause 1993). Partitioning of the acoustic environment with temporal or frequency separation has been demonstrated in a 813 wide variety of animal groups, including insects, birds and mammals (Wilkins et al. 2013), 814

however, partitioning of the acoustic space in the marine environment, and especially in fishes, is
not well documented (Ruppe et al. 2015, Desiderà et al. 2019).

817 In this study, broadband SPLs, the diel plots, and wavelet analyses were used to illustrate the diel patterns and differences among sanctuaries and seasons. The use of broadband sound 818 pressure levels illustrates the ability of certain identifiable signals to raise ambient background 819 820 levels irrespective of their frequency. Various other unidentified fish, invertebrate, and marine mammal species were also regularly contributing to the monitored soundscapes, however, their 821 acoustic intensity was either not high enough or they were not calling in significant numbers to 822 raise the ambient background levels for detection when examining the PSD (seasonal) or 823 averaged SPLs (60 s). For example, Atlantic cod and haddock are present and producing low 824 frequency spawning vocalizations (40 - 400 Hz peak in energy) within SBNMS during the 825 826 spring and winter seasons. However, these signals are not raising the ambient background levels over any extended duration as they are completely dominated by the higher amplitude signals of 827 828 large vessels. This overlap could also be resulting in periods of acoustic masking and a reduction in the communication spaces of these animals during critical life history periods (Putland et al. 829 830 2017b, Stanley et al. 2017).

831 Anthropogenic contributions

832 In the last decade it has become apparent that the signals produced by large vessels are 833 increasing rapidly in many ocean regions (Hildebrand 2009). Scientists and policy makers are viewing it as a major concern as it has many implications for the populations of acoustic 834 signalers, from behavioral changes to reduction in communication spaces during critical 835 836 biological periods (Erbe 2002, Halliday et al. 2017, Putland et al. 2017b). In the current study, 837 the signals from various vessel types raised the ambient sound level by up to 13.2 dB in the 251.2 Hz 1/3 octave band and below when comparing times with and without vessels 838 839 (subsampled in the summer, one site per sanctuary, three days surrounding each lunar phase). However, at Site 1 within SBNMS where analyses were unable to be run due to the lack of 840 841 time samples where the ambient soundscape did not include vessel signal, it was noted that an individual vessel transit past the hydrophone could raise ambient sound pressure levels by as 842 much as 60 dB re 1 µPa between 50–2500 Hz. This frequency bandwidth overlaps with a large 843 majority of biological sources in these sanctuaries' soundscapes, potentially causing energetic 844 845 masking in species who use acoustic communication during vital life history events e.g., Atlantic

cod and haddock (Clark et al. 2009, Putland et al. 2017b, Stanley et al. 2017). Site 1 within 846 SBNMS had the highest amount of vessel presence in the recordings analyzed (90.6 % of h per 847 day), corresponding to close proximity to the Boston Traffic Separation Scheme which is utilized 848 by large oceangoing cargo ships, tankers and cruise ships (Hatch et al. 2008). Despite this, sites 849 within SBNMS did not exhibit the greatest median broadband sound pressure levels during any 850 851 seasonal recording period and was found to be between 10.3 - 18.7 dB re 1µPa lower than the greatest site. This is likely due to the relative absence of biological contributions in the higher 852 frequency range (> 1000 Hz), especially seasons relevant to onshore fishes spawning cycles and 853 snapping shrimp peaks. Care must be taken when using and reporting broadband SPL metrics, as 854 it does not reflect the frequency contributions that make up the level and is not necessarily an 855 appropriate metric when referring to comparable levels encountered by biological receivers at 856 different locations. 857

Within the shallower sanctuaries, occurrence of vessels in the recordings was much reduced 858 (< 7.09 h/day average) and was composed of smaller vessel types. Despite this, during the times 859 when vessels were present, they could significantly raise the SPL within the 50 - 10,000 Hz 860 861 frequency band. When comparing periods of time with and without vessels, and its modification of the frequency spectra, care needs to be taken especially when removing times with vessels 862 863 present. For example, this study highlighted that often high energy biological contributions can be greatly time dependent, therefore if the duration of a vessel presence spans a long enough 864 865 time window, particularly at a biologically significant time of day, removing it could be also be removing time that is greatly influenced by peaks biological activity. 866

867 Teasing apart the contribution of human activities vs. abiotic sources (e.g., wind & waves) to the ambient soundscape can also be difficult, especially if there is no reliable wind speed or wave 868 869 height data available in close proximity to the recording site. The current study found Site 8 870 (FGBNMS) exhibited strong linear relationships between wind speed and low frequency octave bands during seasons of high wind. However, at the other site within the sanctuary, where there 871 were higher occurrences and levels of low frequency anthropogenic sound, these relationships 872 873 were not present. Site 2 (SBNMS) had the highest broadband SPLs of any site during the Winter 874 recording period, it also had significantly higher winds speeds during this time when compared to the other seasonal recording periods. However, there was only a moderate association between 875

factors, again another site with significant vessel activity and consequently low frequencyambient sound.

878 **Future directions**

879 As passive acoustic monitoring capacity has increased, a variety of challenges arise from 880 these progressively longer-term and larger-scale programs. They are producing terabytes of data over multiple years and consequently demanding storage and analysis methods that can 881 efficiently ingest high volumes of data, identify signals of interest and effectively summarize 882 883 attributes of descriptive value. Techniques such as signal recognition software or computer learning techniques and automated and semi-automated acoustic detectors seek to enable the 884 885 eventual unsupervised detection, and in some cases, classification of vessels, impulsive signals, baleen whale, fishes, and invertebrates (Baumgartner and Mussoline 2011, Bohnenstiehl et al. 886 2016, Urazghildiiev and Van Parijs 2016, Ranjard et al. 2017, Ricci et al. 2017, Lin et al. 2018). 887 While output from detectors designed to identify specific sounds of interest remain important, 888 889 peak performance is often constrained to a relatively small number of target sounds (biological and anthropogenic) and specific contexts or geographic regions. Methods that necessitate 890 891 significant human oversight are less feasible to apply to such large and wide-ranging datasets, and transitions to more automation often require significant training and ground-truthing with 892 893 additional information sources. For example, the current study utilized a time intensive method 894 of vessel identification by hand browsing subsampled data. While this method was accurate and sufficient for the current use, it is not sustainable for application to the entire data set. This 895 confirmed data set, however, is useful for ground-truthing more automated approaches. 896 897 There has been significant interest by both scientists and managers in metrics that can

898 summarize the full range of acoustical energy a soundscape of interest and extract information on the local habitats biodiversity, state and/or health (Sueur et al. 2014). However, several marine 899 900 based studies and research working groups have identified the challenges and complexities in applying terrestrially derived metrics (e.g., Acoustic Richness, Acoustic Entropy Index, Acoustic 901 Complexity Index, Acoustic Diversity Index) to marine acoustic environments. For example, a 902 903 few loud or omnipresent but varying sound sources (e.g., snapping shrimp, seismic air guns, large vessels) can strongly modulate these metrics, masking other biologically important 904 characteristics. Unlike terrestrial environments in which species are often partitioned in acoustic 905 906 space, marine species tend to overlap in both frequency and temporal space (Parks et al. 2014,

Bohnenstiehl et al. 2018). However, it's also important to note that the characteristics of
biological signals and the health/biodiversity of a habitat may not always be directly related,
therefore, applying a single metric or method is not going to necessarily represent the multitude
of factors that determine this (Mooney et al. 2020). It is important that we understand these
dynamic and address the biases and limitations they can potentially produce when conducting
soundscape measurements.

Answering questions of management interest often requires the ability to compare both 913 914 contemporary and time-series soundscape measurements among wide-ranging (regional, international) projects. Such comparisons must be able to account for or at least acknowledge the 915 variation introduced by differences in recording location and habitat, recording hardware and/or 916 analytics and a standardized approach towards both data collection and analysis is necessary for 917 918 valuable results (Erbe et al. 2016). Increased standardization both within and among projects is therefore a subject of keen interest within the soundscape monitoring community (see 919 International Quiet Ocean Experiment - Standardization and Marine Bioacoustical 920 Standardization, ISO-terminology, Consortium for Ocean Leadership report-921 922 https://adeon.unh.edu/standards, https://www.iso.org/standard/62406.html, http://oceanleadership.org/understanding/u-s-quiet-ocean-project/). Within U.S. National Marine 923 924 Sanctuaries, levels of anthropogenic input of sound are not directly managed, but instead are the subject of interagency dialog and recommendations as part of NOAA's mandate to reduce or 925 926 eliminate likely injury to resources within these sites (Hatch and Fristrup 2009). Understanding the relative contributions of noise from proposed new activities in relation to previous baseline 927 928 conditions can be essential to site assessments of potential impacts, as well as supporting the 929 design of mitigating recommendations. A standardized system-wide passive acoustic monitoring 930 network, allows for the extraction of several measures of condition "state", both contemporarily 931 and showing trends over time, including the presence of sound producing marine wildlife, the presence of human activities, and, as developed, metrics that correspond with biological diversity 932 (e.g., Freeman and Freeman 2016). In addition, metrics can be further developed to address 933 reported conditions on "pressure" to the "states", including impacts associated with levels of 934 935 noise produced by human activities, further defined within sanctuaries to frequencies, time periods and areas within particular biological importance. This study indicated a need for more 936

937 continuous sampling early in site evaluations to quantify base sampling needs required to capture938 indicators of interest.

939 Ongoing work is also focusing on integration of acoustic measures used together with complimentary data types and sources (e.g., environmental information, Automatic Identification 940 System (AIS) vessel tracking, acoustic telemetry, and underwater visual surveying, as well as 941 additional development of automated techniques) can provide more complete measures and 942 wider understanding of ecosystem health and species interactions and potential impacts of 943 specific sound-producing human activities (Kaplan et al. 2015, Putland et al. 2017c, Staaterman 944 et al. 2017, Solsona Berga 2018, Zemeckis et al. 2019). With this data integration and ground-945 truthing, such metrics have been used to rapidly assess large areas of coral reef habitat and assist 946 in detection and characterization of ecological changes (Freeman and Freeman 2016). Further 947 identification of vocalizing and chorusing species will also continue to inform studies of 948 biological acoustic partitioning and aid in long-term monitoring of visitation patterns and 949 acoustic ecology within these protected areas (Erbe et al. 2015). 950

951 Conclusions

952 Each sanctuary revealed a complex soundscape that was composed of some relatively rare 953 events, such as seasonal fish chorusing or thunderstorms, and relatively common events, such as 954 large vessel transits and shimming shrimp snaps. The variability in geographic location, physical habitat and biological inhabitants found among sanctuaries led to distinct sound signatures that 955 956 varied in time, e.g., day, moon phase and season. It was found that there were different acoustic 957 dominants among the sanctuaries, ranging from a more anthropogenically driven SBNMS to 958 more biologically driven GRNMS and FKNMS, and with FGBNMS including a combination of 959 both more anthropogenically and more biologically driven locations. These dominant drivers were the foremost cause of the observed seasonal fluctuations in the acoustic measurements 960 961 recorded, except for strong weather events in some sanctuaries during some seasons. Among all the acoustic signals occurring, the signals from both small and large vessels stood out as the most 962 ubiquitous and chronic soundscape influencers. The collected data begins to report on conditions 963 in ambient sound levels and associated drivers at each sanctuary and support the generation of 964 capacity in sanctuaries for longer-term temporal comparisons to better understand and monitor 965 changes across the systems. The current study identified challenges to monitoring and comparing 966 967 acoustic conditions in geographically and biologically dissimilar systems. It is hoped that

identifying a common framework in terms of field design, equipment, and simple acoustic
measurements, will encourage further compatibility and comparisons among future monitoring
and management effort.

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982 Supporting Information

Additional supporting information may be found online at: [link to be added in production]

985 Open Research

- All acoustic data collected for this study is permanently stored at National Oceanic and
- 987 Atmospheric Administration National Centers for Environmental Information Archive. This can
- 988 be viewed and requested via the Passive Acoustic Data Map Viewer
- 989 <u>https://maps.ngdc.noaa.gov/viewers/passive_acoustic/</u>
- 990

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1209

1210 6. Tables

- 1211 Table 1. Broadband (BB) sound pressure level (dB re 1 µPa) statistics, using 60 s bins, for
- 1212 each recording site during each recording season. Stellwagen Bank National Marine
- 1213 Sanctuary (SBNMS), Gray's Reef National Marine Sanctuary (GRNMS), Florida Keys National
- 1214 Marine Sanctuary (FKNMS), Flower Garden Banks National Marine Sanctuary (FGBNMS).
- 1215 NB. Bolded numbers signify the highest median and italicized numbers signify highest Root
- 1216 Mean Squared (RMS) broadband SPL (per site) per sampling season.
- 1217

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Sanctuary	SBNMS		GRNMS		FKNMS		FGBNMS	
Site ID	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
Summer: June								
– September								
BB Median	100.5	100.2	117.0	119.8	109.4	109.5	118.9	110.5
10 th percentile	93.6	94.8	114.0	117.9	108.3	108.3	116.6	108.8
90 th percentile	108.0	107.6	119.6	121.9	111.1	111.0	121.9	113.7
BB RMS	107.8	114.3	123.9	121.5	112.0	109.5	120.3	118.0
Fall: October –								
December								
BB Median	104.5	106.3	113.3	116.4		108.9	116.8	110.4
10 th percentile	98.8	100.9	110.6	114.5		107.7	114.4	110.4
90 th percentile	110.4	112.3	115.2	118.1		110.4	119.4	112.1
BB RMS	109.4	111.5	117.8	117.5		113.4	117.5	111.5
Winter:								
February –								
April								
BB Median	105.2	110				107.8	116.5	
10 th percentile	100.35	103.6				106.6	114.3	
90 th percentile	110.3	113.9				109.5	118.8	
BB RMS	108.6	111.6				111	117.2	
Spring: April –								
July								
BB Median	100.9	101.4	115.6	118.6		109.6	117.3	111.2

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1218	10 th percentile	94.6	96.4	113.0	116.9	108.4	115.6	109.7
1219	90 th percentile	108.1	96.4	118.5	120.3	111.0	119.2	113.7
1215	BB RMS	107.8	106.8	118.2	121.1	111.6	117.9	113.9

Table 2. Median snap rate per season and within Dawn, Noon, Dusk and Midnight time 1220 segments in the seasonal recording periods. * indicate significant difference detected among 1221 snap rates for the different time segments within a site and season, and lower-case letters indicate 1222 differences among seasons or time periods within a site (Friedman Test & Tukey Test). Gray's 1223 Reef National Marine Sanctuary (GRNMS), Florida Keys National Marine Sanctuary (FKNMS), 1224 Flower Garden Banks National Marine Sanctuary (FGBNMS). NB. Bolded numbers signify 1225 sampling season and italicized numbers signify time segment with statically highest snap rate per 1226 1227 site.

Sanctuary	Sanctuary GRNMS		Fŀ	KNMS	FGBNMS		
Site	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	
Summer	675 b	864 a	361	362 b	377 b	306 b	
Dawn	904 a	1006 a	340 b	341 b	318 b	253	
Noon	461 c	665 c	335 b	340 b	384 a	284	
Dusk	670 b	838 b	378 а	356 a	381 a	307	
Midnight	855 a	997 a	297 с	292 с	355 b	296	
Sig	*	*	*	*	*		
χ^2	68	68	61	58	22	5	
Р	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.2	
Fall	452 c	639 b		296 c	300 c	189 c	
Dawn	457 a	672 b		285 a	274 b	205 a	
Noon	231 b	398 c		275 b	244 c	139 b	
Dusk	495 a	725 a		311 а	317 а	228 a	
Midnight	479 a	703 a		252 b	280 b	196 a	
Sig	*	*		*	*	*	
χ^2	78	62		66	50	76	
Р	<0.001	< 0.001		< 0.001	< 0.001	< 0.001	
Winter				237 d	294 c		
Dawn				258 a	286 a		
Noon				201 b	232 b		
Dusk		-		263 а	226 b		
Midnight		-		208 b	291 а		
Sig				*	*		

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χ ²			22	45	
Р			 < 0.001	< 0.001	
Spring	685 a	869 a	614 a	446 a	335 a
Dawn	783 a	970 a	555 b	373 b	308 b
Noon	491 b	622 c	546 b	384 b	291 c
Dusk	722 a	885 b	638 a	410 a	327 a
Midnight	726 a	916 a	533 b	423 a	314 b
Sig	*	*	*	*	*
χ ²	72	64	53	37	51
Р	<0.001	< 0.001	< 0.001	< 0.001	< 0.001

1228

1229 7. Figure Legends

1230 Figure 1. Map showing recording sites within each sampled National Marine Sanctuary.

1231 Stellwagen Bank National Marine Sanctuary (SBNMS); Site 27 – Site 1, Site 33 – Site 2, Gray's

1232 Reef National Marine Sanctuary (GRNMS); FS15 – Site 3, Station 20 – Site 4, Florida Keys

1233 National Marine Sanctuary (FKNMS); Western Dry Rocks – Site 5, Eastern Sambo – Site 6,

1234 Flower Garden Banks National Marine Sanctuary (FGBNMS); Stetson Bank – Site 7, East

1235 Flower Garden Bank – Site 8.

1236 Figure 2. Diel time series plots showing seasonal averages of broadband sound pressure

1237 level at each site. Shaded grey bars indicate seasonal range of sunrise and sunset times (in local

1238 standard time). Stellwagen Bank National Marine Sanctuary (SBNMS), Gray's Reef National

1239 Marine Sanctuary (GRNMS), Florida Keys National Marine Sanctuary (FKNMS), Flower

1240 Garden Banks National Marine Sanctuary (FGBNMS). Seasons: Summer (June - Sept), Fall (Oct

1241 – Dec), Winter (Feb – April), Spring (May – July). NB. Varying x-axis used to better illustrate

1242 fluctuations and all times of day are standardized for local standard time at each site.

1243 Figure 3. Seasonal power spectral density levels for each recording site, including median,

1244 10th and 90th percentiles. Solid line represents seasonal average and dotted line represents

1245 percentiles (DFT length = 48000, Hanning window, and 50% overlap). Stellwagen Bank

1246 National Marine Sanctuary (Sites 1 & 2), Gray's Reef National Marine Sanctuary (Sites 3 & 4),

1247 Florida Keys National Marine Sanctuary (Sites 5 & 6), Flower Garden Banks National Marine

1248 Sanctuary (Sites 7 & 8). Seasons: Summer (June - Sept), Fall (Oct – Dec), Winter (Feb – April),

Spring (May – July). NB. data for Fall, Winter and Spring was excluded from Site 5 due to
damage to the hydrophone element.

1251 Figure 4. Spectrogram showing 24 h sample and labels indicating signal origin. a) Site 2 in

1252 SBNMS (Fall, full moon), b) Site 4 GRNMS (Spring, full moon), c) Site 6 in FKNMS (Spring,

1253 Full Moon), & d) Site 7 in FGBNMS (Fall, Full Moon). DFT length = 48000, Hanning window,

and 50% overlap and color scale identical in each spectrogram.

1255 Figure 5. Daily number of true cod grunts detected during the seasonal recording periods

1256 at Site 1 & 2 in Stellwagen Bank National Marine Sanctuary (SBNMS). Open, right half

1257 open, closed, and left half open circles denote new, first quarter, full and third quarter moons

1258 respectively. Seasons: Summer (June 26 – Aug 3), Fall (Oct 17 – Dec 7), Winter (Feb 20 – April

1259 13), Spring (May 6 – June 10). NB. Asterisk indicates number of grunts detected on November
23 which exceeded axis scale.

1261 Figure 6. Sound pressure levels in octave bands centered on 63, 125, 251, 501, 1000, and

1262 7943 Hz during three days over all moon phases during the Summer, Fall and Spring

1263 recording periods at Site 4 in Gray's Reef National Marine Sanctuary. Open, right half

open, closed, and left half open circles indicate new, first quarter, full and third quarter moonsrespectively.

1266 Figure 7. Median and 90th percentile in 1/3 octave sound pressure levels for periods of time

1267 with (red) and without (blue) vessels present during the sub-sampled Summer recording

1268 period (June – September) (left panels) and median and 90th percentile change in 1/3 octave

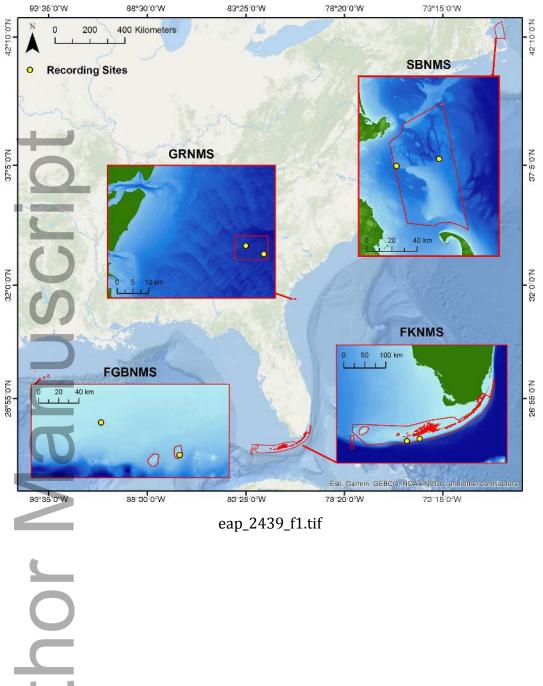
1269 band sound pressure levels between all time and time without vessels present (dB re 1µPa)

1270 (right panels). Gray's Reef National Marine Sanctuary (GRNMS), Florida Keys National

1271 Marine Sanctuary (FKNMS), Flower Garden Banks National Marine Sanctuary (FGBNMS).

1272 NB. SBNMS site not included in graphical representation due to the lack of hours without vessel

1273 presence for comparison.



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