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**Acoustic comparison of a patchy Mediterranean shallow water seascape: *Posidonia oceanica* meadow and sandy bottom habitats**

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1 **Acoustic comparison of a patchy Mediterranean shallow water**  
2 **seascape: *Posidonia oceanica* meadow and sandy bottom habitats**

3 Ceraulo M.<sup>1,3</sup>, Papale E.<sup>1</sup>, Caruso F.<sup>1,4</sup>, Filiciotto F.<sup>2</sup>, Grammauta R.<sup>1</sup>, Parisi I.<sup>5</sup>, Mazzola S.<sup>1</sup>,  
4 Farina A.<sup>3</sup> and Buscaino G.<sup>1</sup>

5 <sup>1</sup>National Research Council – Institute for Coastal Marine Environment – Bioacousticslab Capo  
6 Granitola, Via del Mare, 6 – 91021 Torretta Granitola, Campobello di Mazara (TP), Italy

7 <sup>2</sup>National Research Council – Institute for Coastal Marine Environment – Spianata S. Ranieri, 86 -  
8 98122 Messina Italy

9 <sup>3</sup>Department of Pure and Applied Sciences (DiSPeA) – University of Urbino– Campus Scientifico  
10 "Enrico Mattei"– 61029 Urbino, Italy

11 <sup>4</sup>Woods Hole Oceanographic Institution, Biology Department, Woods Hole, MA 02543, USA

12 <sup>5</sup>eConscience, no-profit organization, via Provinciale 610 , 90046, Monreale (PA), Italy

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### 28 **Abstract**

29 Soundscapes are strongly linked with the physical structure and biological features of the habitats  
30 and their study can reveal ecological processes of the underwater environment. Objective of this  
31 study is to characterize two Mediterranean habitats, the *Posidonia oceanica* meadow and the sandy  
32 bottom, and demonstrate their acoustic diversification basing on their soundscapes. Firstly, the  
33 habitats have been compared using two different acoustic metrics, the Power Spectral Density  
34 (PSD) and the Acoustic Complexity Index (ACI), measured in different frequency band. Then, the  
35 acoustic biological component of the habitats has been identified and characterized: five biological  
36 signals were described and their acoustic properties and temporal patterns were defined. Finally, the  
37 geophonical and anthropogenic components of the two habitats have been compared. In the low  
38 frequency ( $< 0.5$  kHz) the sandy habitat showed higher values of PSD and lower values of ACI.  
39 From 0.5 to 24 kHz the greatest values of both parameters were recorded in the *Posidonia* habitat  
40 due to the acoustic activity of snapping shrimps and fishes. The wind speed resulted significantly  
41 correlated with PSD from 0.1 to 2 kHz for both habitats, but the correlation is less intense in  
42 *Posidonia* habitat suggesting a noise attenuation phenomenon. The two habitats present biophonical  
43 component belonged to different fish species and invertebrates; they showed alternated temporal  
44 pattern and different frequency allocation. The *Posidonia* habitat resulted acoustically richer than  
45 sandy habitat, confirming the importance of ecoacoustic method to study ecological processes.  
46 Finally, a strong acoustic impact from the anthropogenic component was revealed: it achieves 60%  
47 of daytime during the summer, especially in sandy habitat. Results demonstrated not only the  
48 possibility to discriminate habitats through the sound information but also the need to protect  
49 marine ecosystems from the human noise.

50

51

52 **Key words: Soundscape ecology, Posidonia meadow, sandy habitat, fish signals, ACI, noise,**  
53 **Mediterranean Sea**

### 54 **1. Introduction**

55 The sound characterizing aquatic and marine environment, or soundscape, is produced by the  
56 combination of geophonies, biophonies and antropophonies (Pijanowski et al. 2011). The  
57 geophonies are the result of sounds produced by physical agents as wind, waves and rain; the  
58 biophonies are produced by mammals, fishes and crustaceans vocalization; finally the  
59 antropophonies are originated during mechanical human activities, as ship noise, seismic  
60 prospection (air-gun), seabed drilling, etc..(Farina 2014).

61 In marine environment, seagrass meadows, rocky and coral reefs, are complex habitats  
62 characterized by an higher number of shelters and food opportunities (La Mesa et al. 2011), which  
63 lead the colonization of an high number of species (Giakoumi and Kokkoris 2013).

64 The complexity of the habitats, in terms of structure of the animal community, is connected with the  
65 complexity of its biophonical component (Kennedy et al. 2010). The food and shelters availability  
66 of the habitats can determine a different biophony, since the acoustic activity of fishes and  
67 crustaceans is related to feeding (Radford et al. 2008b), territorial and feeding competition  
68 (Myrberg 1997, Amorim & Hawkins 2000) and spawning behavior (Lugli et al. 1995, Aalbers &  
69 Drawbridge 2008). These differences can be better observed during specific periods of the day and  
70 year. The circadian cycles of the acoustic activity in marine coastal environment are regulated by  
71 the light: the acoustic emission of marine vertebrates and invertebrates increases during the night  
72 and mostly during the new moon periods (Lammers et al. 2008, Radford et al. 2008a, b, Lillis et al.  
73 2014, Staaterman et al. 2014, Buscaino et al. 2016, Caruso et al. 2017). The acoustic activity of  
74 many fish species shows seasonal pattern following spawning and breeding periods (Amorim 2006,  
75 McCauley 2012, Buscaino et al. 2016), and androgenic factors regulate the tropic state of the sonic  
76 muscles following a strong seasonal cycle (Connaughton et al. 1997).

77 The physical structure of the habitats determine not only variation in biaphonic components, but  
78 also in the geophonical components. In terrestrial ecosystem, it was demonstrated that wind and rain

79 noise depends on the openness of the vegetation, the leaf area density and width and the breadth of  
80 canopy (Aylor 1972), making the spectral profile of ambient noise habitat specific (Slabbekoorn  
81 2004). In our knowledge, specific studies of geophony propagation in marine coastal habitats have  
82 not been published yet, but the physics parameters of different marine environments determine  
83 phenomena of scattering and absorption (Hermand 2004, Knobles et al. 2008) that are commonly  
84 used by side scan sonar to differentiate the sea bottom.

85 The study of aquatic and marine soundscape was dealt recently using eco-acoustic indices (Lillis et  
86 al. 2014, Staaterman et al. 2014, Kaplan et al. 2015, Bertucci et al. 2015, 2016 Buscaino et al.  
87 2016). Their use highlights the presence of biological sounds also during high background noise  
88 condition, making faster and easier the analysis and interpretation of huge amount of data (Sueur et  
89 al. 2008, Farina 2014). Harris et al. (2016), testing the correspondence between fish biodiversity  
90 and three different indices, founded that the Acoustic Complexity Index (ACI) (see Pieretti et al.  
91 2011 for details on computation) is a good descriptor of the acoustic community in temperate  
92 marine environment.

93 The Mediterranean system comprises a plurality of ecosystems that allow high degree of biological  
94 diversity (Bianchi & Morri 2000). However, the coastal environment are area at high risk for sound  
95 pollution due to the increase of human pressure (Samuel et al. 2005) and different coastal habitats  
96 are exposed to different levels of human pressure, due to e.g. fishery activity, commercial shipping  
97 and recreational interests (Halpern et al. 2008). Noise increases concerns about health and fitness  
98 for all marine species from invertebrates to vertebrates (Celi et al. 2015, Filiciotto et al. 2014, 2016,  
99 Papale et al. 2015, Everley et al. 2016, Simpson et al. 2016), with influence species survival.  
100 Human disturbance of habitats soundscape reduces the orientation capacity of different species  
101 (Holles et al. 2013) because the recognition of a distinct acoustic signature for each habitat is a key  
102 mechanisms for their viability (Simpson 2005, Radford et al. 2010). The Mediterranean soundscape

103 has been investigated only recently (Buscaino et al. 2016), and no studies describe both spatial and  
104 temporal acoustic patterns.

105 The Mediterranean shallow waters are characterized by an alternation of *Posidonia oceanica*  
106 meadow, sandy and rocky bottoms. *Posidonia oceanica* (a protected species for Habitat Directive  
107 92/43/EU) is the dominant seagrass species in the Mediterranean Sea and it is the base for a crucial  
108 habitat that provides refuge, nursery and food sources for fish and invertebrate species. Instead, the  
109 sandy bottom habitats mostly provide refuge for infauna (Thiel & Ullrich 2002), and it determines  
110 peculiar speciation of organisms that become exclusive (Tunesi et al. 2006).

111 The purpose of this study is to distinguish two different Mediterranean habitats, the *Posidonia*  
112 *oceanica* meadow and the sandy bottom, basing on their soundscapes. In particular, this study aims  
113 at: 1) comparing the soundscape of the *Posidonia oceanica* meadow and sandy bottom habitats  
114 considering two different metrics, the Power Spectral Density (PSD) and the Acoustical Complexity  
115 Index (ACI) and their daily and seasonal trend; 2) evaluating if the physical agents influence in  
116 different way the background noise of the two habitats 3) identifying, describing and comparing the  
117 biotic sonic component of the two habitats; 4) analyzing the impact of anthropogenic noise on the  
118 two habitats

119

## 120 **2. Material and Methods**

### 121 2.1. Study area and data collection

122 Data collection was carried out along the south-western coast of Sicily, in an area comprised  
123 between Capo Granitola and Tre Fontane villages (Fig. 1). Here, the seascape is distinguished by an  
124 alternation of patches of sandy, rock and *Posidonia oceanica* meadows. This area is characterized  
125 by upwelling phenomena (Bonanno et al. 2014) promoting the primary and secondary production

126 Along the Sicilian coasts, *Posidonia* meadow covers about 76000 ha (Calvo et al. 2010). Thanks to  
127 the favorable ecological conditions and pristine natural state, the western side is one of the most  
128 dense and extensive beds of all the entire Mediterranean Sea (Calvo et al. 2009, 2010).

129 For this study, we selected three patches of sandy bottom and three patches of *Posidonia* beds on  
130 rocky bottom, alternatively distributed along the coast. Six sites were chosen (and named for  
131 *Posidonia* meadows P01, P02, P03; for sandy bottom S01, S02, and S03; Fig.1) inside the patches:  
132 the sites P01 and S01 were located to the south-western side of the coast, the sites P03 and S03 to  
133 the south-eastern and the sites P02 and S02 in the southern. The patches were selected using *Google*  
134 satellite imagines, ecosounder and visual observation.

135 An autonomous recorder was located for each site. They were selected considering a minimum  
136 distance from the patch boundary of 30 m. The recorders were deployed between 10 and 12 m depth  
137 at about 3 m from the bottom and 9 m from the surface, using a ballast and a buoy to maintain a  
138 vertical assessment of the hydrophone (Fig. 2). For details about site locations see Tab.1.

139 The autonomous recorder consisted on an omnidirectional calibrated hydrophone with a flat  
140 sensitivity response of  $-174.5 (\pm 2)$  dB re  $V/\mu\text{Pa}$  from 0.1 to 100 kHz (model Benthowave Low  
141 Noise Broadband Hydrophone BII 7016 T6) and a Digital Signal Processor (model C5535 DSP-  
142 TMS320C5535) coupled with an AIC3204 audio codec (Texas Instruments).

143 In order to balance the limits of the data storage and the battery operating time, the instruments  
144 were set to record for 10 minutes continuously followed by 20 minutes of pause (33% of duty  
145 cycle), using a sample rate of 48 kHz at 16 bit. This configuration allowed to record for about 7  
146 consecutive days during each deployment. The recordings took place during winter (January and  
147 February) and during summer (June, July and September). Recordings were carried out during the  
148 new moon week, to be sure to record the maximum sound activity of crustaceans and fishes (as  
149 found by Lillis et al. 2014 and by Staaterman et al. 2014). All recorders were synchronized before  
150 the deployment and data were acquired for a total of 1487 hours.

151 Data of speed and wind direction were collected during the recording sessions by SIAS (Regione  
152 Siciliana - SIAS – Servizio Informativo Agrometeorologico Siciliano). The meteorological station is  
153 located at about 7 km far from the recording sites.

154

### 155 2.2. Data analysis

156 The dataset was aurally and visually inspected through spectrogram survey in order to obtain the  
157 preliminary identification of biological sources and to evaluate the presence/absence of ship noise  
158 in each 10-minute file. In order to obtain a good representation of the soundscape of the areas, data  
159 analysis was carried out by considering three bands:

160 - Low Frequency (LF): from 0.1 kHz to 0.5 kHz

161 -Medium Frequency (MF): from 0.5 kHz to 2 kHz

162 - High Frequency (HF): from 2 kHz to 20 kHz

163 The choice of these bands allowed us to improve the description of biological components of the  
164 marine soundscape. Generally, fish emission range up to 2 kHz (Ladich & Fine 2006, Picciulin et  
165 al. 2013) but they are extremely variable between different groups. For this reason, we considered  
166 two bands of frequency (LF and MF) as potentially used by different fish species. The third band  
167 (HF) could be occupied by invertebrates broadband pulses that extend from 2 kHz up to 120 kHz  
168 (Au & Banks 1998, Buscaino et al. 2011, Di Iorio et al. 2012) or signals (both impulsive and tonal)  
169 of *delphinidae* species (Papale et al. 2014, Buscaino et al. 2015, Caruso et al. 2017). The day time  
170 (night and day) was established basing on the solar time of each recording session using ephemeris  
171 tables (Night and Day software- Benvegnù M. and Menichelli M.)

172

#### 173 2.2.1. Power Spectral Density Analysis

174 Using MATLAB code, the Power Spectral Density (PSD - dB re 1  $\mu\text{Pa}^2/\text{Hz}$ ) of each recording was  
175 calculated through the *welch* function (Welch 1967) (24000 points FFT,  $2^{15}$  points Hamming



176 Window, 50% Overlap).The PSD values have been summarized on the three bands (LF, MF, HF  
177 bands).

178

### 179 2.2.2. Acoustic Complexity Index Analysis

180 In order to compare the biological acoustic community of the two habitats, filtering out the non-  
181 animal produced sounds, we computed the Acoustic Complexity Index (ACI) (Pieretti et al. 2011,  
182 Harris et al. 2016, Buscaino et al. 2016) on the three frequency bands.

183 ACI was computed through the *SoundscapeMeter* plug in of WaveSurfer platform (for details on  
184 algorithm see Pieretti & Farina 2013). In order to obtain a temporal resolution adapted to amplify  
185 the most representative fish sound emissions (temporal step of 0.064 sec), all data were resampling  
186 at 32 kHz, and a FFT of 2048 points (frequency resolution of 15.6 Hz) was used.

187 Moreover, the *SoundscapeMeter* permits to apply an amplitude filter (named *noise filter*) on the  
188 data before computing the calculation of the index (Farina et al. 2016). As found by Buscaino et al  
189 2016, during the choruses of snapping shrimps and fishes, the number of signals emitted is so high  
190 that the energy between one temporal step and the subsequent is comparable. It determines lower  
191 values of the index than expected. For this reason, we decided to process the data twice each time  
192 using a different filter setting: one without using amplitude filter (ACI no flt), and one using an  
193 amplitude filter of 2000  $\mu\text{V}^2/\text{Hz}$  (ACI flt).

194 Successively, the ACI values obtained for each frequency were summed on the LF, MF and HF  
195 bands.

### 196 2.2.3. Identification and description of the biophonic component

197 Biological sounds of the soundscape of the habitat considered were identified. Since the certain  
198 attribution of some sounds to specific biological source was not possible, we first acoustically  
199 characterized them. Subsequently, their daily patterns were described through the count of the  
200 signals.

201 To characterize the sounds, a subsample, with a good signal to noise ratio, was randomly selected  
202 for each sites. Using AvisoftSASlab Pro, the data selected were processed applying different filters  
203 in order to isolate the different sounds from the other biophonic components, without interfere with  
204 the signals characterized. In detail:

205 - Signals within the LF band: undersampling from 48 kHz to 11.025 kHz; high pass filter of  
206 0.1 kHz; low pass filter of 0.5 or 0.8 kHz depending from the signals.

207 - Signals within the MF band: undersampling from 48 kHz to 11.025 kHz; high pass filter of  
208 0.35 kHz; low pass filter of 1.5 kHz.

209 - Signals within the HF band: high pass filter of 1 kHz

210 The characterization was carried out using the pulse train analysis of AvisoftSASlab Pro, changing  
211 the hysteresis, the threshold, the time constant and the group time according of each signal  
212 analyzed. For each signal and train of signals we measured: duration (s), peak of frequency (Hz)  
213 and bandwidth (Hz) (for single signal); number of pulses (n) and pulse rate (n/s) (for train of  
214 signals).

215 To describe the daily trend of the principal acoustical components, we processed files of 2 min/hour  
216 collected during the three days over the new moon day. All the signals within this subsample were  
217 counted. The count process was carried out through both visual and acoustic inspection of files and  
218 by using the pulse train analysis of AvisoftSASlab Pro for frequent signals.

219

### 220 2.3. Statistical Data Analysis

221 The two acoustic habitats were compared using two metrics:

222 1. PSD values measured at the three frequency bands and averaged for each recording;

223 2. ACI values measured at the three frequency bands and averaged for each recording;

224 In detail, considering PSD values as dependent variables, linear mixed models (LMEM) (Bates et  
225 al. 2012) were applied to determine if the factors "habitat" (Posidonia, Sand), "daytime" (Day,

226 Night), and "season" (Winter, Summer) affected the acoustic levels at the three frequency bands.  
227 The factors "sites" and "month of recording" were included as random factors. We excluded the  
228 recordings with the presence of boats to consider this factor separately. The best-fit model was  
229 selected by means of model averaging based on the information criterion (AIC). Validation graphs  
230 (e.g. residuals versus fitted values, Q-Q plots, and residuals versus the original explanatory  
231 variables) were analyzed in order to control possible model misspecification and the presence of  
232 outliers.

233 For ACI values, we compared only the data from summer recordings, when biological emissions of  
234 fish are present in both habitats. It was not possible to apply any linear mixed models because the  
235 low variability of the index in sandy habitat compared to the variability in the Posidonia habitat  
236 violates the homogeneity of variance criterion. As a consequence, the non-parametrical Kruskal-  
237 Wallis analysis, and post-hoc multiple comparisons test were carried out to compare the values of  
238 ACI of the two habitats (for each frequency band), in relation to the daytime.

239 The influence of wind speed on the PSD values, measured on the three frequency bands, was  
240 investigated using the linear regression model (LRM). In order to reduce the variability produced by  
241 the different exposure of the sites along the coast, firstly we carried out a LRM - using wind  
242 velocity as independent variable and PSD measured at LF band as dependent variable - splitting  
243 data for each site and for each wind direction. Basing on these results, we carried out the LRM on  
244 PSD values of all frequency bands considering only cases when the wind direction affects all sites.

245 The characterization of the biophonic components of the two habitats was carried out considering  
246 the mean value of the different parameters for each signals. We statistically compared the  
247 parameters of sounds present both in sandy and Posidonia habitats. We tested their acoustic  
248 variables for normally distribution (Shapiro - Wilk test); since data was not normally distributed,  
249 the U-Mann Whitney test was performed.

250 The percentage of files with the presence of vessel noise was compared between the habitats and  
251 between seasons on each site. These data were tested for normally distribution (Shapiro-Wilks test  
252 for each group of data). We applied T-test for independent data between two habitats and T-test for  
253 paired data to compare the data from each site between the seasons.

254

### 255 **3. Results**

256 In total, we recorded 1487 hours (450.7 during the winter and 1036.5 during the summer): 765.8  
257 hours were collected in Posidonia habitat (254 during the winter and 515 during the summer) and  
258 717.3 hours in sandy habitat (196 during the winter and 521 during the summer). In the Fig. 3 we  
259 showed the spectrogram of three days of recording (2 min/hour) collected during the three days  
260 over the new moon day. The different components are marked.

#### 261 3.1. Acoustical habitat comparison through PSD and ACI

262 The best model (LMEM) selected using the information criterion (AIC), included habitat, daytime,  
263 season and their interaction as independent variables. In Tab. 2 the results of the models for each  
264 frequency band are shown. Significant differences resulted between the two habitats for each  
265 frequency band, considering the season and the daytime (Fig.4). In detail, during the winter (both  
266 night and day) sandy habitat was noisier (higher level of PSD) than Posidonia, considering LF and  
267 MF bands. During the summer, sandy habitat was still noisier than Posidonia in LF band, but  
268 Posidonia habitat had higher level of PSD in MF band. The HF band was noisier in Posidonia than  
269 in sandy habitat both during winter and summer, during the day and the night time.

270 Considering the ACI values, the efficiency of the index in term of biophonic amplification, resulted  
271 different using or not using the filter for each band considered. The daily pattern of ACI (with and  
272 without filter) was plotted for each band (Fig. 5). At the low frequency (LF), where the continuous  
273 noise of boats, wind and wave is predominant, the use of the filter reduced the power of attenuation  
274 of these sounds. At medium and high frequency bands, where the effect of geo- anthropophonic

275 noise is not so strong and the biophonic choruses are more intense, the use of the filter was essential  
276 to amplify the biophonic component of the soundscape. Basing on these results, we carried out the  
277 subsequent analysis using different settings of ACI in relation to the band involved. Comparing ACI  
278 values, the two habitats presented significant differences for all frequency bands both during day  
279 and night. In particular, Posidonia habitat showed higher biophonic activity than sandy habitat  
280 (Kruskal-wallis test: LF band  $\chi^2=1673.5$ ,  $df=3$ ,  $p<0.001$ ; MF band  $\chi^2=958.71$ ,  $df=3$ ,  $p<0.001$ ; HF  
281 band  $\chi^2=444.2$ ,  $df=3$ ,  $p<0.001$ ) (Fig. 6).

282 Considering the effect of wind speed on PSD values, we reported the results of LRM using data  
283 split for each site and for each wind direction (Tab.3). Only significant correlations have been  
284 reported in Fig.7. The highest significant angular coefficients ( $\beta$ ) resulted for the south wind  
285 direction within each site. In Tab.4 the results of the models using the cases when the wind  
286 direction was from south, are presented. In the LF and MF bands, the PSD resulted positively  
287 correlated with the wind speed in both habitats, but the angular coefficient ( $\beta$ ) of the regression line  
288 resulted higher in sandy habitat than in Posidonia meadow. In the HF band, the PSD was not  
289 correlated with the wind speed in both habitats.

### 290 3.2. Identification and description of the biophonic component

291 Through the visual and acoustic analysis of the spectrograms, different biological elements of the  
292 soundscape were identified as characterizing the two habitats. In Table 5, the acoustic features (both  
293 spectral and temporal) of signals are shown.

294 Considering the three bands:

295 - Low Frequency band (Fig. 8 D-E):

296 In this band, we found fish sounds (probably emitted by one or two similar species) (LF fish)  
297 made up of train of pulses. These signals were recorded both in Posidonia and in sandy habitats,  
298 but they showed some different spectral and temporal characteristics (respectively Fig. 8-D and  
299 8-E; Tab. 5). In particular, the duration of pulses ( $Z=-7.85$   $p<0.001$ ) and the peak of frequency

300 (Z=-10.11  $p<0.001$ ) were significantly different, with longer duration and higher frequency  
301 peak in sandy bottoms. Other parameters did not show any differences among the habitats  
302 (Bandwidth of pulse: Z=-1.0  $p>0.05$ ; Number of pulse: Z= -1.01  $p>0.05$ ; Pulse Rate: Z=-0.6  
303  $p>0.05$ ).

304 - Medium frequency band (Fig. 8 B-C):

305 Biological sounds dominated only in Posidonia habitat also in this case. Two different types of  
306 sounds were identified: tonal (MF fish Fig. 8 B) and impulsive sounds (MF pulse Fig. 8 C).

307 - High frequency bands (Fig. 8 A):

308 Snapping shrimp pulses dominated the high frequency band, only in Posidonia habitat.

309 In Fig. 9 the temporal distribution of biological sounds counted along the day is showed. The LF  
310 fish sounds were recorded only during the summer in both habitats, and they showed a circadian  
311 pattern, with pitches during sunrise and sunset. The MF fish sounds were recorded mostly in  
312 Posidonia habitat during the night in summer period, with the presence of chorus at sunset. During  
313 the winter, they were sporadic and no choruses were recorded. In sandy habitat, we found only few  
314 tonal signals during summer, but no evidence of chorusing either pattern was present. The MF pulse  
315 sounds were recorded only in Posidonia habitat during both winter and summer but only during the  
316 daytime. Finally, the snapping shrimp pulses were recorded only in Posidonia habitat in both winter  
317 and summer, showing pitches at sunrise and sunset. An increase during the summer was present.

318

### 319 3.3. Habitat comparison through number of boats

320 Focusing on the anthropogenic noise, the percentage of presence of boats (number of 10 minutes  
321 files with the presence on vessel noise on the total number of recordings) for each site during both  
322 winter and summer is shown in Fig.10. The percentage of boats counted visualizing the recordings  
323 spectrograms was lower in Posidonia than in sandy habitat (T=-2.7;  $p<0.05$ ) and increased during  
324 the summer (T=-3.2;  $p<0.05$ ).

325

326 **4. Discussions**

327 The main goal of this study is to analyze the differences in the soundscape of two of the most  
328 typical habitats of the Mediterranean Sea: the Posidonia and the sandy habitats. For the passive  
329 acoustic comparison, we decided to consider three different frequency bands. The two environments  
330 showed different characteristics both using PSD and ACI values and by analyzing the biological  
331 sonic component.

332 Focusing on the results of acoustic energy data, the comparison between the two habitats showed  
333 differences in all the frequency bands considered.

334 The sandy habitat presented higher values of power spectral density at the low frequency band (LF),  
335 both in summer and in winter compared to Posidonia habitat. Since this result was obtained  
336 excluding the files with the presence of boats, it could be due to both a different biotic sound  
337 activity, or to a different response to the geophysical component. The results of the Acoustic  
338 Complexity Index indicated that the biophonic component is not responsible for higher values of  
339 PSD in sandy habitat. Indeed, we found that Posidonia habitat, during the summer daytime, showed  
340 higher values of index. The analysis of geophysical component, instead, revealed a higher angular  
341 coefficient of the linear relation among PSD and wind velocity, showing a stronger noise increase at  
342 the low and medium frequency bands in sandy habitat. Therefore, our data suggested that the  
343 geophysics component have different effects on the soundscape, due to the physical structure of the  
344 two habitats. In terrestrial environment, the presence of vegetation is recognized to be an important  
345 factor to reduce noise energy (Embleton 1963, Aylor 1972, Kragh 1981). In marine environment,  
346 the seagrass photosynthetic activity produces free gas contained within the aerenchyma and bubbles  
347 on the surface of the plant tissue. This phenomenon affects the local sound propagation and  
348 backscattering (Clay & Medwin 1977, Hermand et al. 1998, Wilson & Dunton 2009, Wilson et al.  
349 2013), determining a unique acoustic footprint, commonly used to map and characterize the  
350 submerged macrophyte (Wilson et al. 2013). As consequence the physical structure of Posidonia

351 meadow, could attenuate also the noise produced by geophysical factors, making Posidonia habitat  
352 a potential acoustic refuge for marine species.

353 The power spectral density measured at MF band showed higher values of energy in sandy habitat  
354 compared to Posidonia only during the winter. During the winter, these results could be generated  
355 by geophonic components that strongly affects sandy habitat. Instead, during the summer, Posidonia  
356 soundscape is more affected by biophonic components. This result is confirmed by ACI data  
357 measured during summer: the index captures the acoustic activity of fishes during the night and the  
358 presence of impulsive signals (at low and medium frequency) during the daylight hours.

359 Considering all frequency bands results, the application of the Acoustic Complexity Index in this  
360 paper has demonstrated to be a useful proxy for the biotic acoustic activity. The choice to split the  
361 analysis basing on the frequency bands of the principal biologic components recorded, helped the  
362 results interpretation. Harris et al. (2016) compared different acoustic indices relating these to reef  
363 fish abundance and diversity. They found a strong correlation between the ACI and species richness  
364 and evenness. Also, Stateerman et al. (2014) and Bertucci et al. (2016) used successfully this index  
365 to study and compare different soundscapes. We found that the use of this index should take into  
366 account different settings that can strongly affect the results. The application of an intensity filter to  
367 the data or not, can help to discriminate far and fore acoustic fields, leading to amplify the strongest  
368 ecoacoustic events (Farina et al. 2016) or to attenuate the non biotic component of the soundscape.  
369 Harris et al. (2016) do not consider this parameter and the index applied on those conditions does  
370 not show these problems on his study. It could be because Harris et al. (2016) correlated the index  
371 values with other species assemblage diversity indices, not with the number of signals. Moreover,  
372 the reason of different results could be found on different habitats analyzed and in temporal  
373 resolutions used. Kaplan et al (2015) and Buscaino (2016) found that ACI values result to be lower  
374 than expected when the density of calling activity is too high. Through a differential frequency band  
375 approach, we decided to adapt the computation of the index using the filter only in those bands not  
376 strongly affected by geophysical component. This method allowed to amplify the chorus of MF



377 fishes and HF snapping, but determined the attenuation of the less frequent and less intense pulses  
378 in the MF band. Until now, in marine environments single metrics were separately considered to  
379 describe habitat complexity. In this study, more methodologies have been carried out and developed  
380 together for the first time, to unroll the acoustic complexity of different habitats.

381 Focusing on the sonic biotic component, different sounds of marine animals have been identified as  
382 principal elements of soundscape of these habitats. They occupy differently the acoustic spectrum,  
383 reducing the overlap of signals along time and/or frequency dimensions.

384 At LF band, two types of signals have been recorded and characterized. They showed typical  
385 acoustic properties and daily patterns belonging to fishes of the *Ophidiidae* family, in particular  
386 *Ophidion rochei* (Parmentier et al. 2010). Within the vocalizations of this species, the acoustic  
387 features (in amplitude and frequency band), the pulse rate and the peaking during dusk and dawn  
388 are distinctive. *Ophidion rochei* is a Mediterranean species that typically lives in the sand, but its  
389 presence in Posidonia habitat has been also recorded (Keskin 2007). In this study, we obtained that  
390 the frequency characteristics of these fish sounds are different between the two habitats: even if the  
391 bandwidth is comparable, the peak of frequency in sandy habitat is higher of about 20 Hz. The MF  
392 band (over 0.5 kHz) is not used by other species in sandy habitat, while in Posidonia is totally  
393 saturated by the presence of other fish sounds. Therefore, we can hypothesize that the differences  
394 found in the frequency characteristics could be due to an acoustic adaptation to the two habitats.  
395 However, since this variation, a certain attribution to one or more species needs further studies.

396 The MF band was occupied by biological signals only in the Posidonia environment. We obtained  
397 acoustic presence of pulses along all daytime and of fish tonal sounds during summer night (with  
398 peak at the dawn). The short pulses were present irregularly during the daytime in winter and  
399 summer. They totally disappeared during night. The correct attribution of these sounds is still not  
400 clear. In our knowledge, a fish species that emit sounds following this seasonal and daily pattern  
401 have not been described before. Also, we can exclude invertebrates as source of these signals, since

402 generally, their acoustic activity, even if present along all the year, shows increase during the night  
403 and peaks during dusk and dawn (Radford et al. 2008b, 2010, Buscaino et al. 2011, 2016). The  
404 daily pattern of these pulses suggests an association with a phenomenon linked to presence of light.  
405 Therefore, one possible explanation could be the connection with photosynthetic or decomposition  
406 phenomena within *Posidonia* meadows. Hermand (2004) suggests the production of photosynthesis  
407 bubbles from *Posidonia* leaves, and their collapse could produce impulsive sounds (Versluis et al.  
408 2000, Pettit et al. 2015). Further studies are necessary to prove this correlation.

409 Regarding fish tonal sounds in MF band, as far as we are aware, these signals have still not been  
410 described. They show frequency characteristics and daily pattern similar to *Therapon theraps* as  
411 recorded in the Great Barrier Reef in Australia and Arabian Sea (Mahanty et al. 2015, McCauley &  
412 Cato 2000). *Therapon theraps* is typical from Indo-West Pacific and Australian waters, but  
413 individuals of this species and of *Therapon jarbua*, have been reported in the Mediterranean Sea  
414 along the Aegean (Minos et al. 2012), Adriatic (Lipej et al. 2008) and Israeli coasts (Golani &  
415 Appelbaum-Golani 2010). They are considered invasive species, probably arrived in Mediterranean  
416 through a Lessepsian migration. The intense and regular occurrence of these broadband sounds,  
417 along summer days, suggests a stable presence of this species along the Mediterranean coasts.

418 Invasive species could determine changing in the acoustic community acting as selective pressures  
419 on the native species (Farina et al. 2013). This could lead to variation in communication features of  
420 local species in order to improve the transmission of information (Acoustic Adaptation Hypothesis,  
421 Morton 1975). The absence of visual census monitoring in this area does not allow confirming the  
422 presence of this specie in this part of the Mediterranean Sea. However, our results draw attention to  
423 the importance of the acoustic method to reveal any rapid changes within an ecosystem.

424 The HF band is occupied by impulsive signals of snapping shrimps only in *Posidonia* habitat. They  
425 follow circadian and seasonal rhythms as already described along all temperate habitats (Radford et  
426 al. 2008b, Bohnenstiehl et al. 2016, Buscaino et al. 2016). Their acoustic activity is connected to  
427 different abiotic factors, such as dissolved oxygen concentration (Watanabe et al. 2002) and water

428 temperature (Bohnenstiehl et al. 2016). Regarding the seasonal activity, the summer increase in the  
429 number of snaps occurs during the night and both dawn and dusk chorus, but not during the rest of  
430 the day. Comparing this result with the number of snapping shrimps counted in Lampedusa area  
431 (Buscaino et al. 2016), a different trend of the snapping activity during the winter season is evident.  
432 Buscaino et al. (2016) in Lampedusa area, showed that the number of snapping signals recorded  
433 during January and February did not present a strong circadian trend as shown here. This could be  
434 due to the presence of the upwelling wind, typical of the northern sector of Sicilian Channel (Patti et  
435 al. 2010), that determines less variation of water temperature along the year. However, we cannot  
436 exclude that the differences found among these two areas of the Sicily Channel could also be due to  
437 a different species composition, since the habitat monitored around Lampedusa Island is more  
438 heterogeneous due to the presence of a mix of *Posidonia oceanica* patches, sand and rocks.

439 Mostly in *Posidonia* habitat where different fish sounds share the same temporal dimension, all the  
440 biological components recorded partition different frequency spaces, supporting the idea of the  
441 evolutionary adaptive function of the acoustic niche (Krause 2012, Farina 2014).

442 Finally, we found that the anthropogenic activity affects strongly both these coastal habitats but in a  
443 different way. The percentage of boats recorded in sandy habitat is higher than in *Posidonia* and it  
444 increases during the summer. The explanation could be found in the use of the area by recreational  
445 boats. Clear and bright waters with sandy bottom result to be more attractive than the dark waters of  
446 *Posidonia* bottom. This is supported by the fact that both habitats located near the more touristic  
447 area present the highest percentage of boats. Anyway, considering all the daytime, in summer  
448 period, the presence of boats was identified in the 30% of the recordings. It means that, considering  
449 the general diurnal vessel activity, it can reach the 60% during the daylight summer days.

450 Noise pollution can affect marine organisms' acoustic communication through auditory masking (in  
451 which the perception of one sound is affected by the presence of another sound) (Clark et al. 2009).  
452 In particular, the noise generated mainly by boats is able to determine this occurrence. The sounds  
453 produced by engines and related vibrating accessories elements of the boats, in fact, occupy the

454 acoustic space reserved for the acoustic communication among fish (Brumm & Slabbekoorn 2005,  
455 Wysocki & Ladich 2005, Vasconcelos et al. 2007, Codarin et al. 2009) and, probably, crustaceans.  
456 Our results evidence how the presence of boats is able to 'camouflage' the transmission systems of  
457 intra- and inter-specific information between these organisms, limiting or preventing some  
458 fundamental bio-ecological processes during the animal life.

459

### 460 **5. Conclusion**

461 The soundscape analysis confirmed to be a key approach to understand ecological processes and  
462 habitat discrimination. The acoustic information transmitted by different communities can be  
463 received by species helping them on orientation processes.

464 Our work demonstrated that the Posidonia habitat is not only richer than sandy one in term of  
465 number of species, but it is also acoustically richer in term of biophonic components. The presence  
466 of shelters and food opportunities makes this habitat crucial for species survival and the acoustic  
467 activities of sonic species revealed the importance of this environment. The bare sandy bottom  
468 creates a particular habitat where only few vocalizing species are adapted to live and reproduce. The  
469 human pressure, in term of noise, in both coastal habitats is very alarming mostly during summer  
470 period when the recreational boats traffic increases and the resulting noise pollution determines an  
471 almost constant disturb along all days. Anthropogenic noise impact negatively on the marine  
472 organisms in different way (Clark et al. 2009, Slabbekoorn et al. 2010, Filiciotto et al. 2016) and  
473 further studies should be conducted in posidonia and sand shallow water Mediterranean ecosystems  
474 to quantify and manage this negatively effects on marine organisms.

475 Therefore, the acoustic monitoring method could represent a useful and not-invasive tool for the  
476 evaluation of the human pressure on the bio-ecological and conservation factors in the marine  
477 ecosystems, in order to achieve a Good Environmental Status (GES) defined by the Marine Strategy  
478 Framework Directive 2008/56 CE. Posidonia soundscape can be considered as a cue for the

479 conservation status of this habitat, and must be monitored and evaluated for management purposes,  
480 especially when new anthropogenic activities are planned in the area.

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488 study.

489

## 490 **8. Author contributions statement**

491 M.C. conceived the study, collected data, performed analysis and paper writing; F.C., F. F., E. P.,  
492 G.B. and R.G., took part to the data collection, participating to the paper writing and PSD analysis.  
493 I. P. performed acoustic processing to the counting processes; G.B. and A.F. conceived, founded  
494 and guided the study, the results interpretation and participating to the paper reader.

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696 **10. Figures**

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698 Figure 1 Study area. In green Posidonia patches, in light yellow sandy patches

699

700 Figure 2 Schematic representation of the deployment system

701

702 Figure 3 Three continuous days of recordings (2 minutes/ hour) during both season, in Posidonia  
703 habitat (left side) and sandy habitat (right side). The dashed white lines indicate the night time. The  
704 letters indicate the different components of the soundscapes. In detail, SS: Snapping shrimps  
705 impulses; MF: MF fish choruses; LF: LF fish choruses; I: MF impulses; W: Waves; R: Rain; B:  
706 Boat noise. N.B. The lower intensity band between 3500 and 5500 Hz is due to non-linear response  
707 of Digital Acquisition Card.

708

709 Figure 4 Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and  
710 maximum) of PSD measured on two habitats, during daytime of the different seasons. The star  
711 represents the significant differences ( $p < 0.05$ ) between habitats.

712

713 Figure 5 Mean  $\pm$  Standard Error of ACI values, measured without using an amplitude filter (dark  
714 green - posidonia habitat; dark yellow sandy habitat) and using an amplitude filter (light green -  
715 posidonia habitat; light yellow sandy habitat)

716

717 Figure 6 Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and  
718 maximum) of ACI (without amplitude filter at LF band, and with amplitude filter in MF and HF  
719 bands) measured on two habitats, during summer daytime. The star represents the significant  
720 differences ( $p < 0.05$ ) between habitats.

721

722 Figure 7 Scatterplot of PSD values and wind speed, when the direction of wind was from south  
723 (dark green - posidonia habitat; dark yellow sandy habitat) and from south-western (light green -  
724 posidonia habitat; light yellow sandy habitat)

725

726 Figure 8 Spectrogram, waveform and amplitude spectrum of the principal biological signals  
727 recorded. HF Band: A. Impulsive signals of snapping shrimps; MF band: B. Tonal fish sound C.  
728 Impulses; LF band: D. train of fish impulses recorded in Posidonia Habitat E. train of fish impulses  
729 recorded in Sandy Habitat. N.B. The waveform and the amplitude spectrum are computed on  
730 isolated sounds, see the MS for details of filters applied.

731

732 Figure 9 Mean and standard error of number of signals counted during three days of recording in all  
733 sites for both season. HF Band: averaged number of impulsive signals of snapping shrimps; MF  
734 band: averaged number of tonal fish sound (barr) and impulses (point with connection line); LF  
735 band: averaged number of train of fish impulses

736

737 Figure 10 Percentage of boats measured on each site of recordings during both season. The star  
738 represents the significant differences ( $p < 0.05$ ) between seasons (for each site) and habitats.

739

740

741

742

## 743 **11. Tables**

744

745 Table 1 Characteristics of the recording sites.

746

747 Table 2 Results of selected LMEM models using PSD values as dependent variables and including  
748 habitat, daytime, season and their interaction as independent variables. Sites and moths of  
749 recordings were included as random factors.

750

751 Table 3 Results of regression linear model using wind velocity as independent variable and PSD  
752 measured at LF band as dependent variable, splitting data for each site and for each wind direction.  
753 In bold the significant models.

754

755 Table 4 Results of regression linear model using southern wind velocity as independent variable and  
756 PSD measured at LF, MF and HF bands as dependent variables.

757

758 Table 5 Mean  $\pm$  SE of signals features measured for the principal biological sounds individuated on  
759 each frequency band

Figure 01

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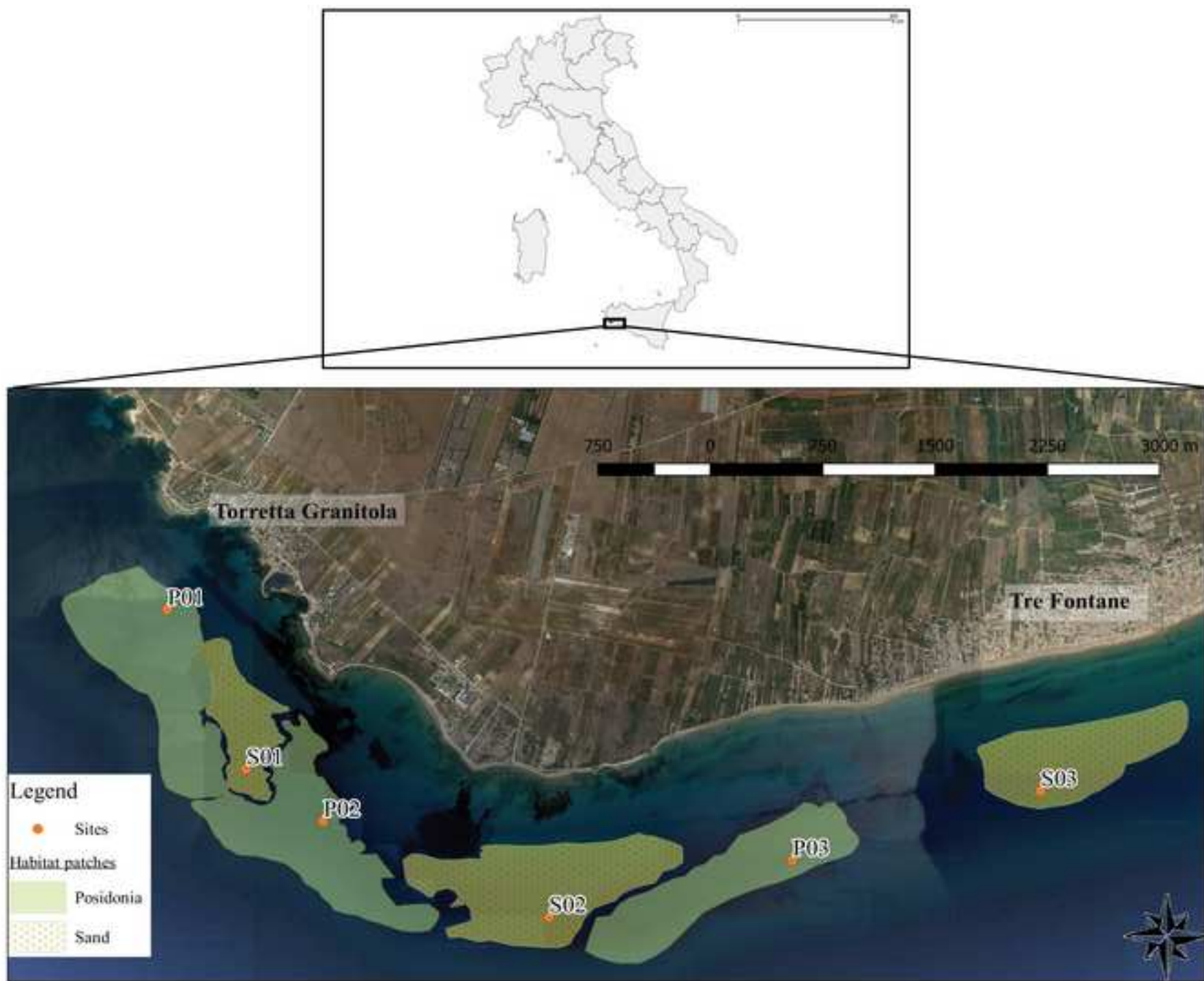


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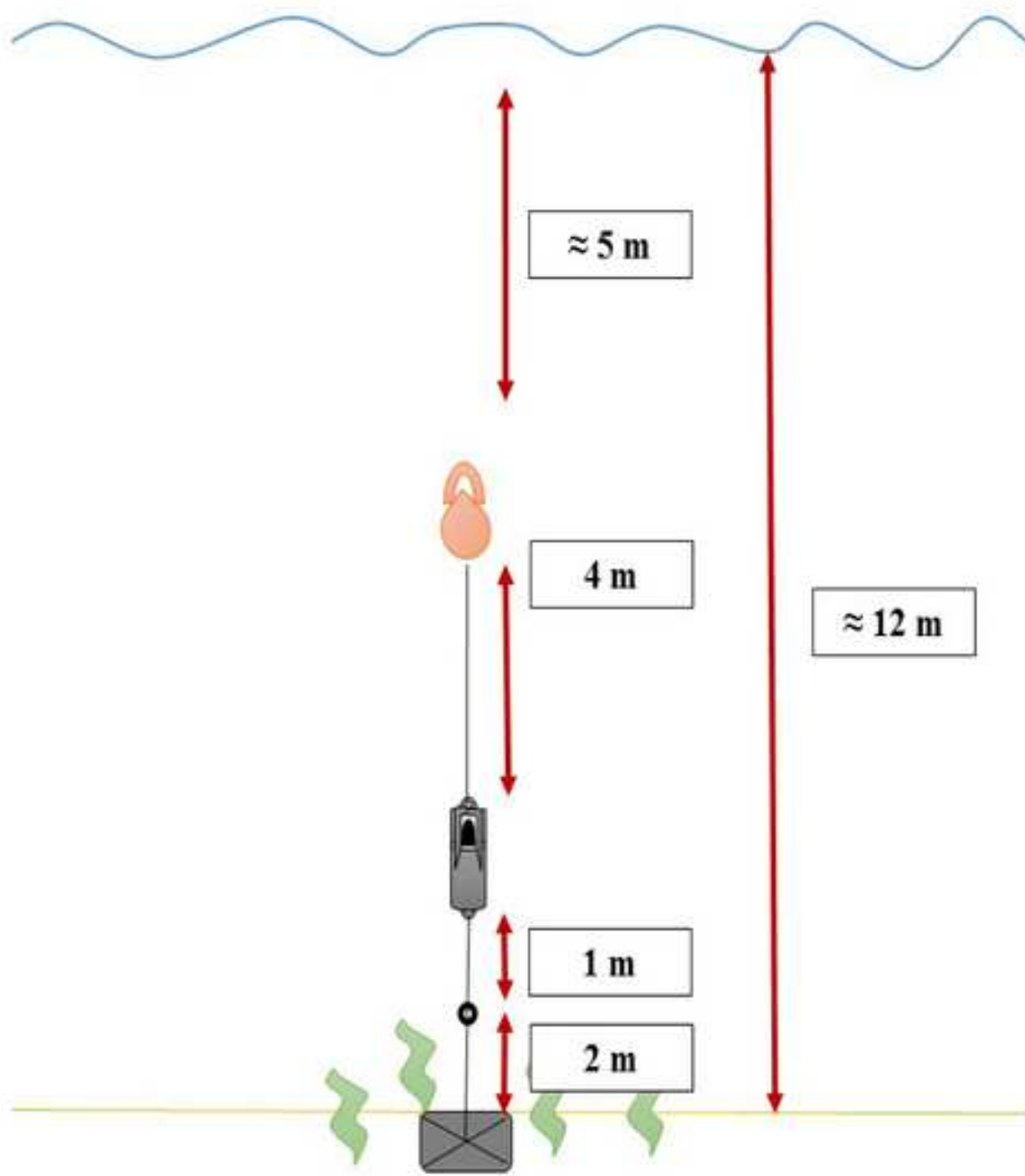


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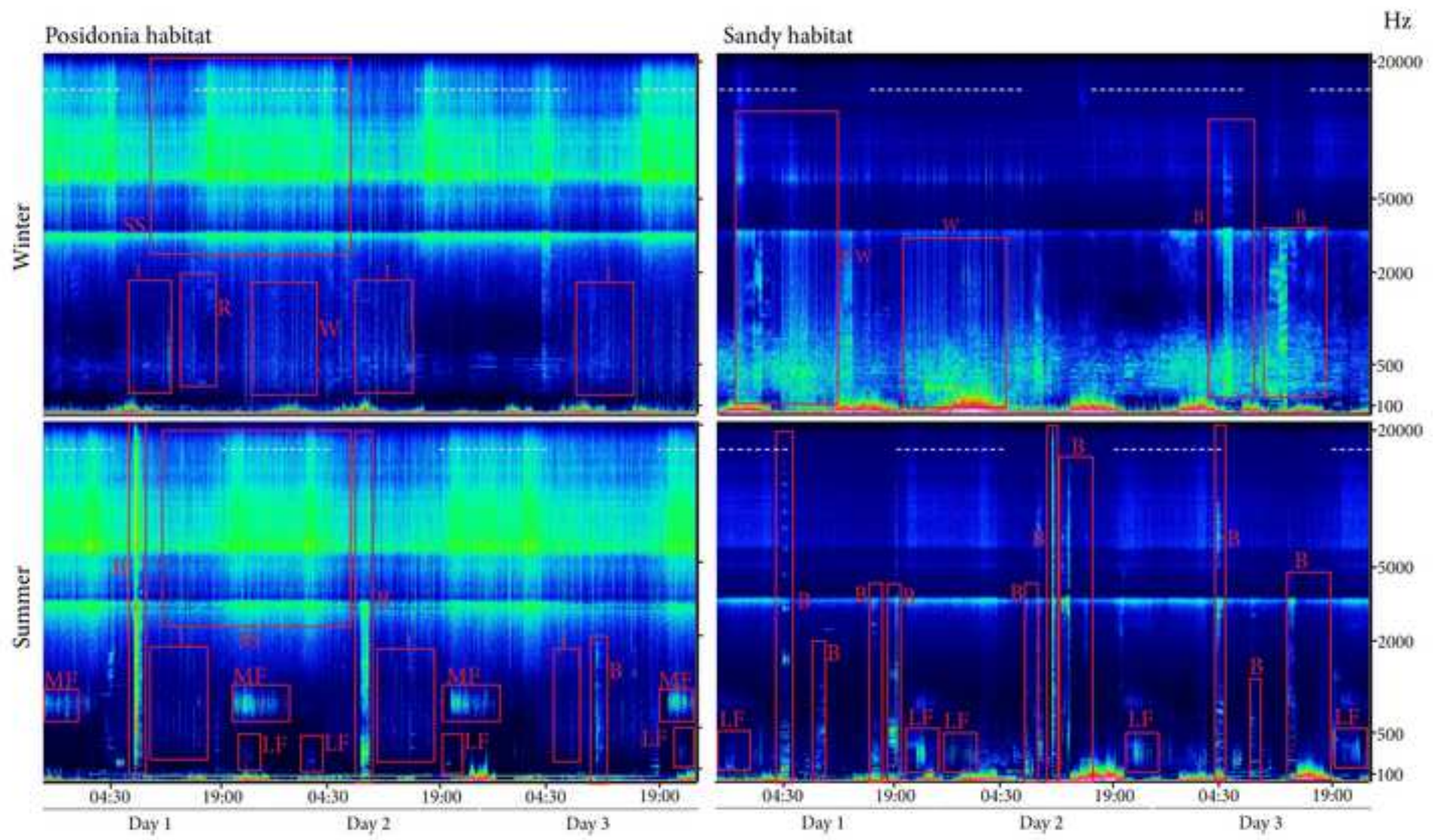




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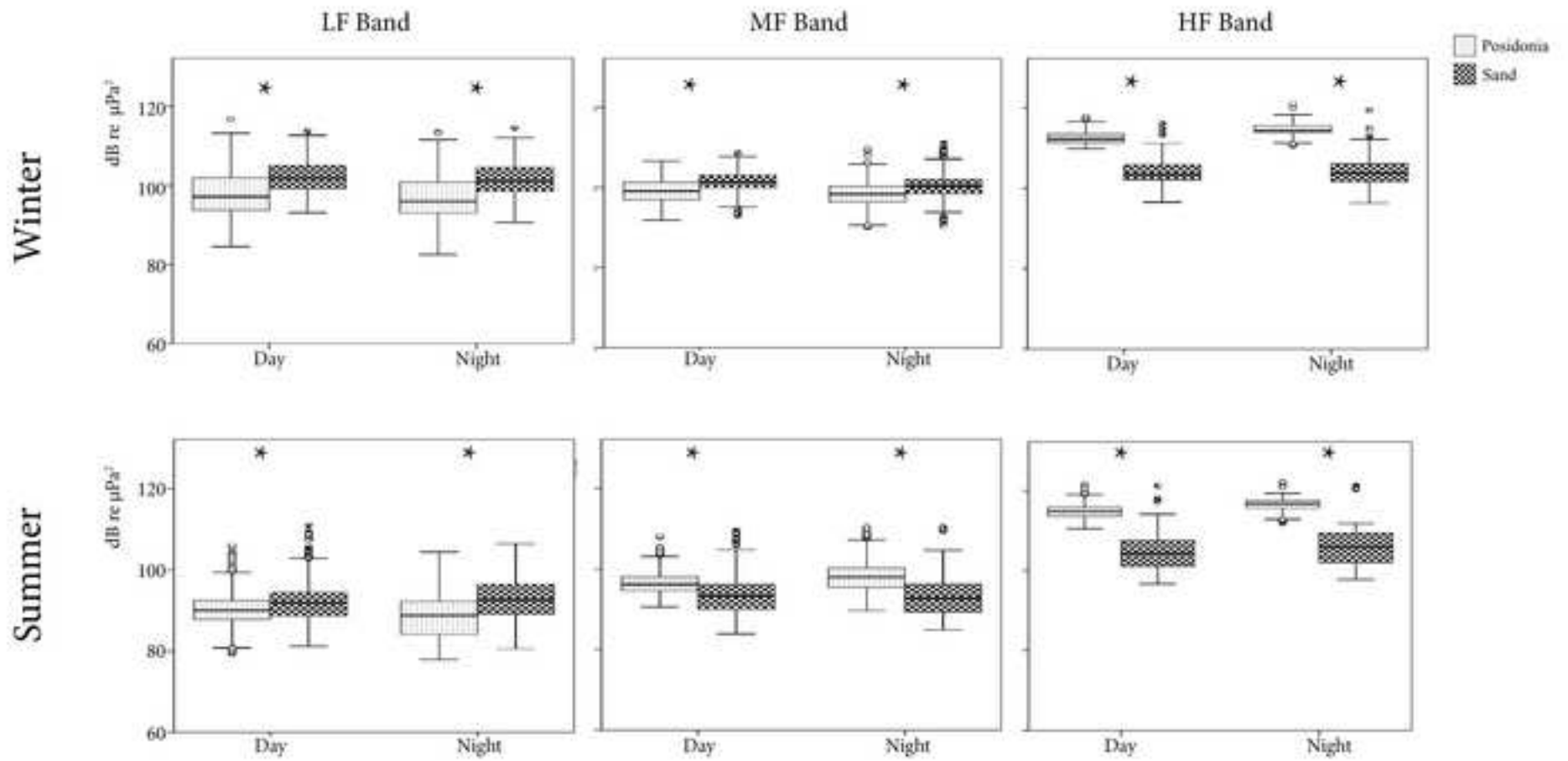


Figure 05

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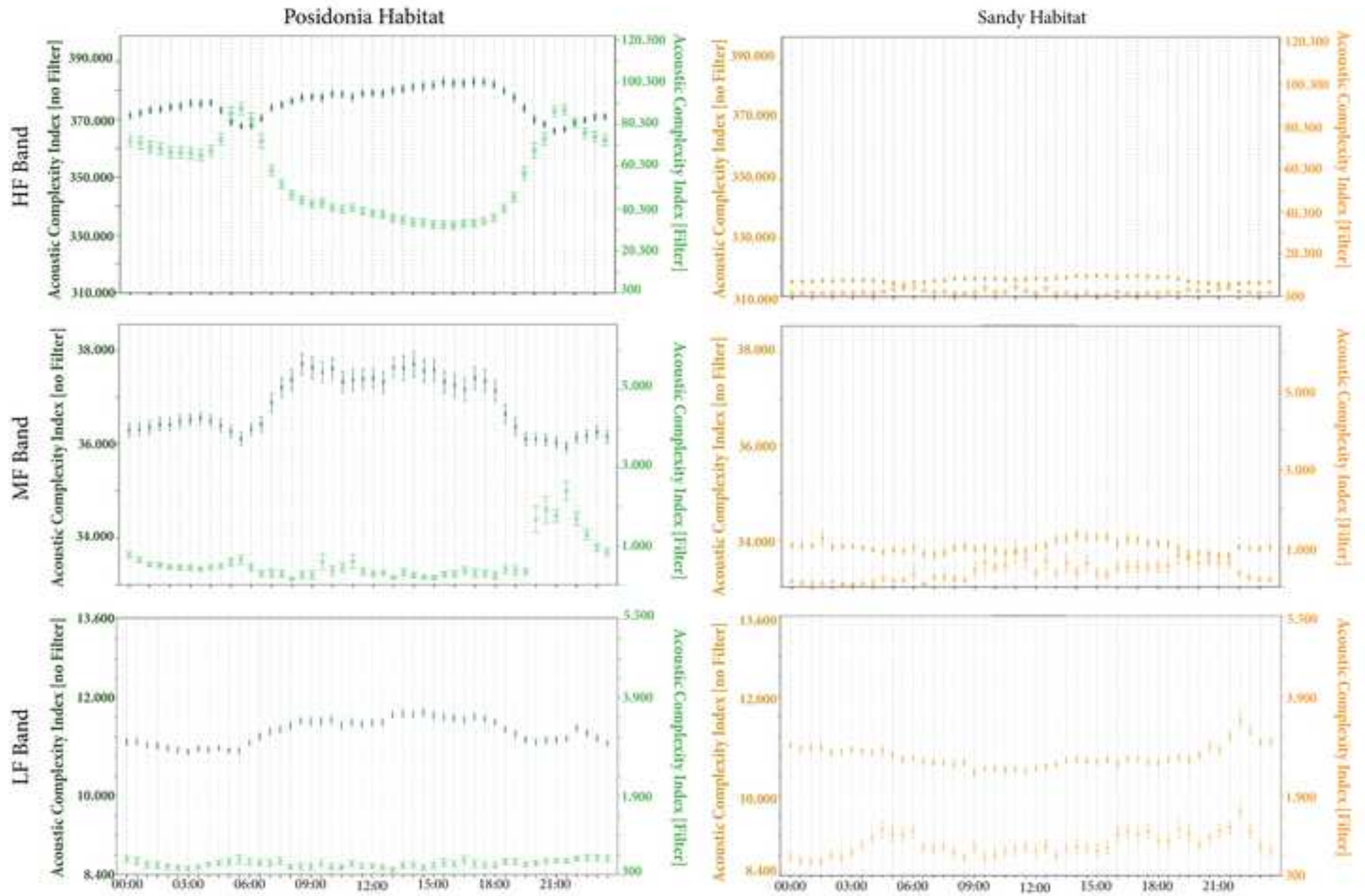


Figure 06

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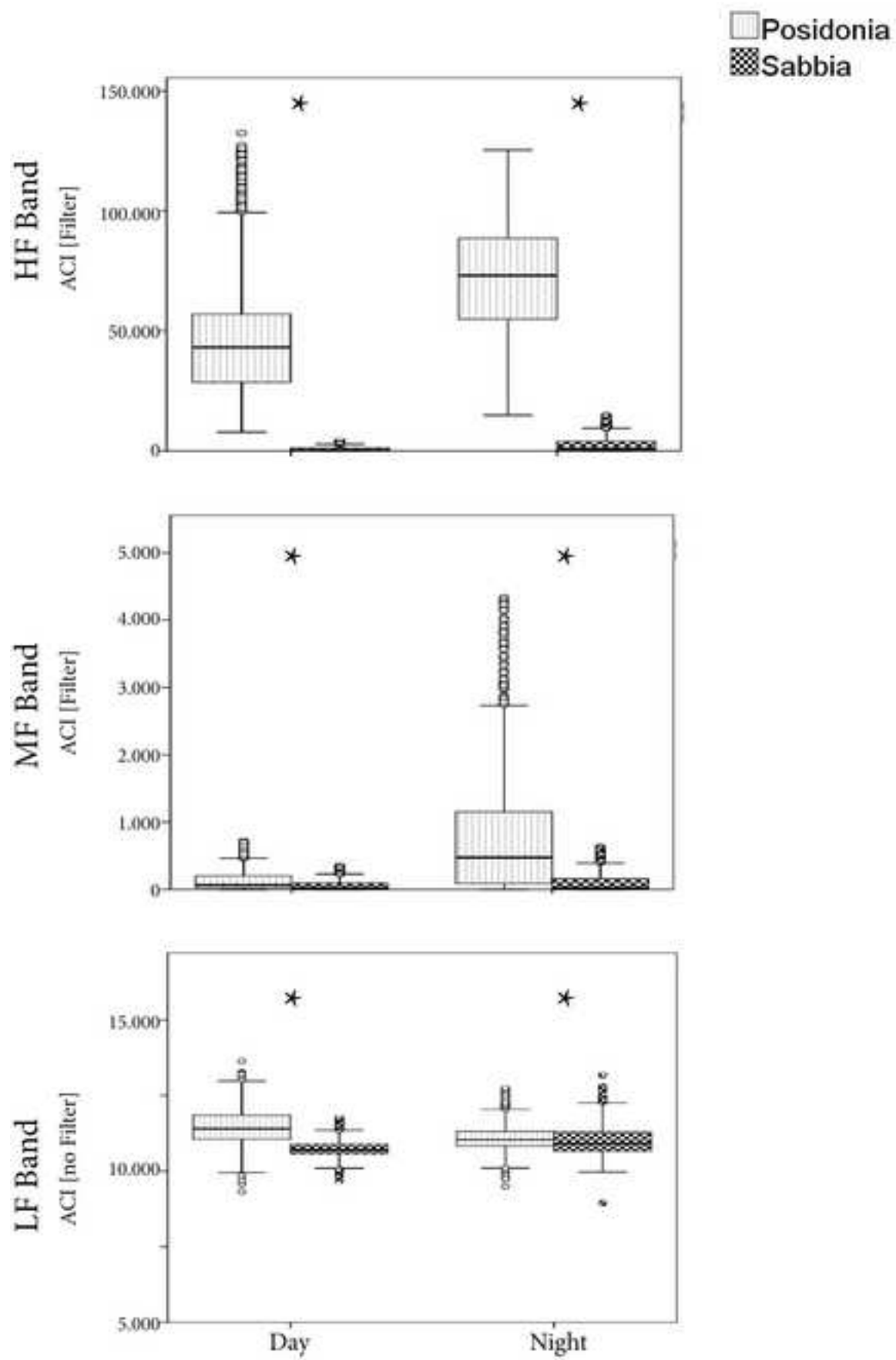


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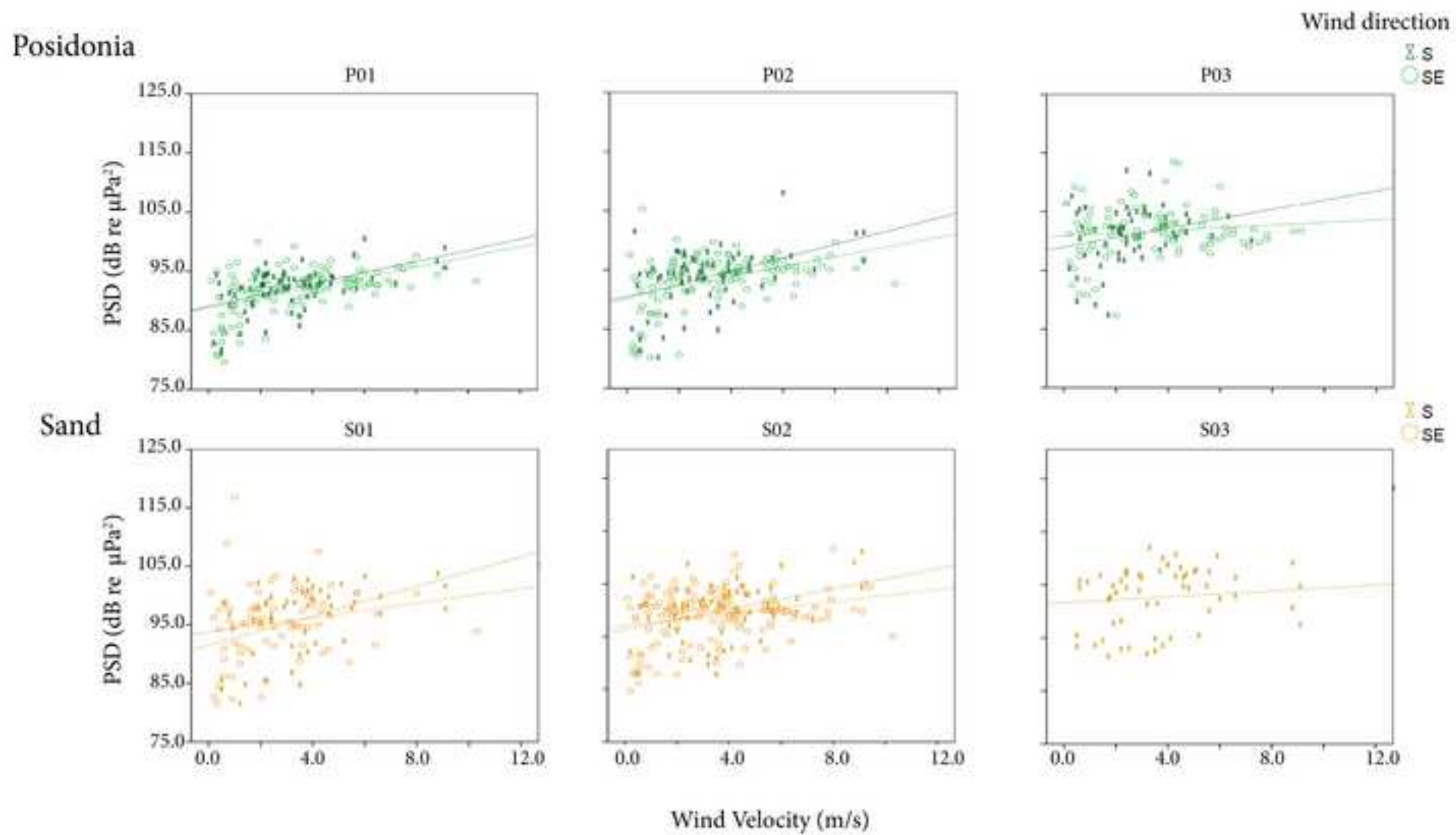


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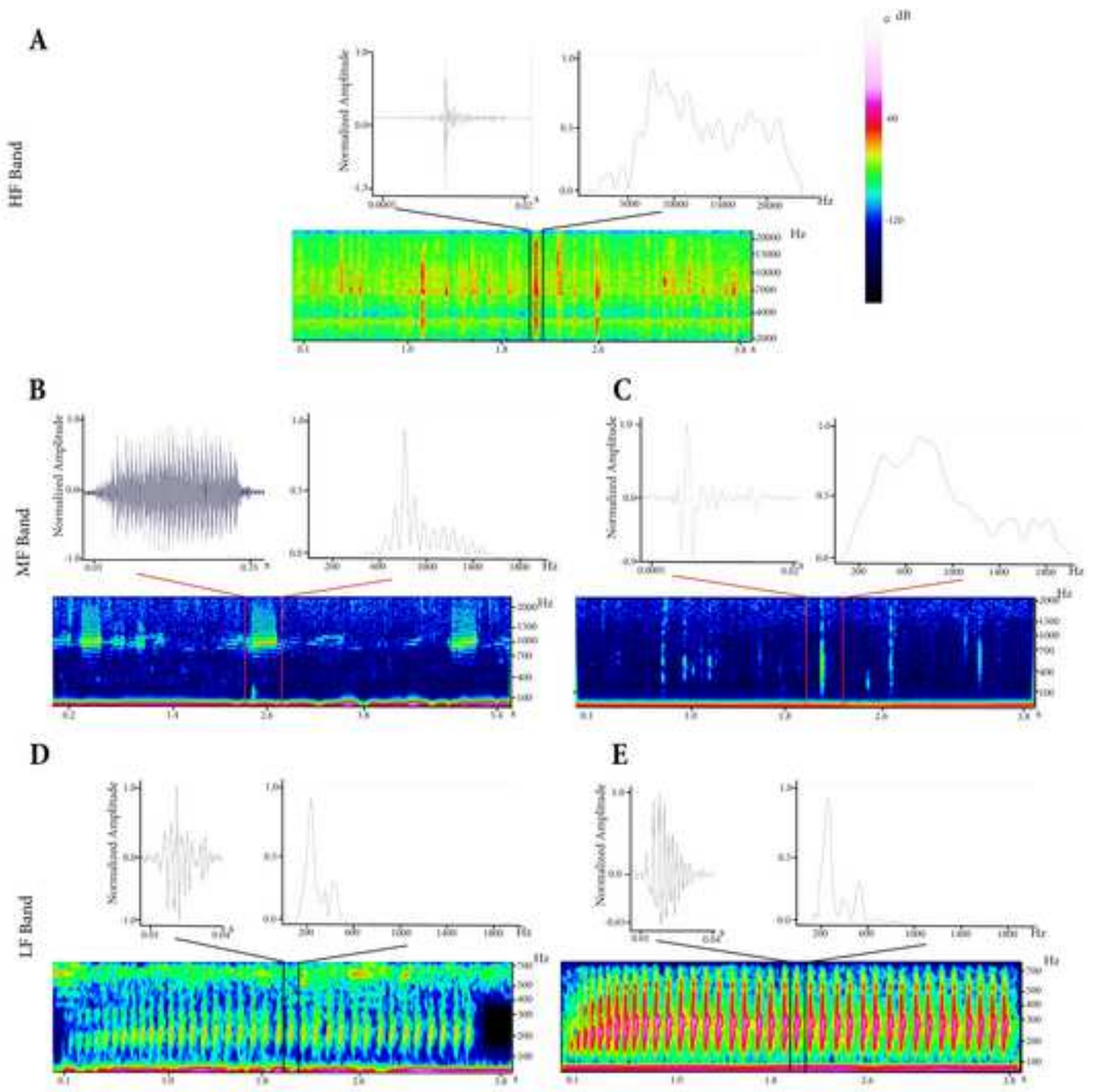


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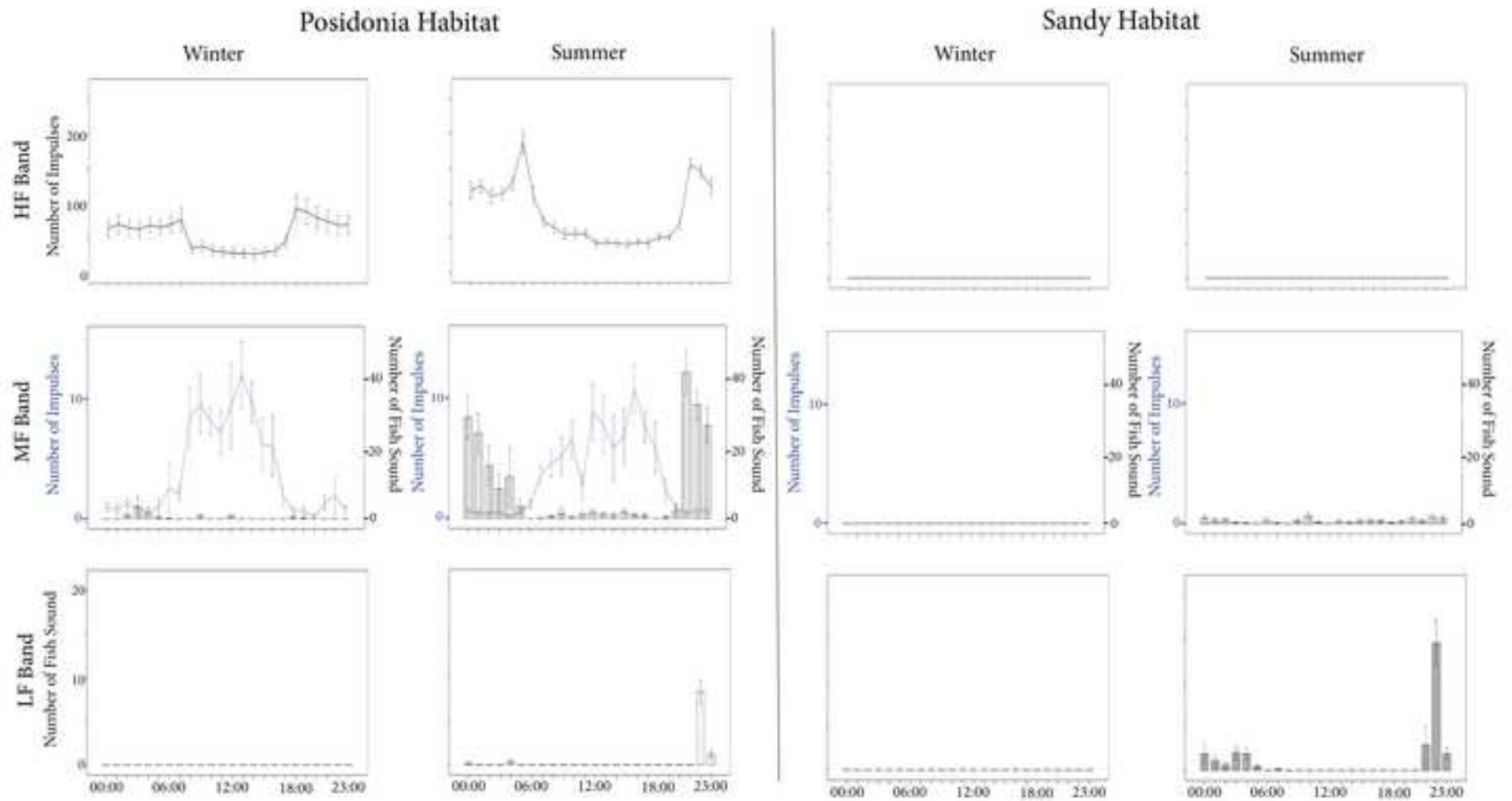


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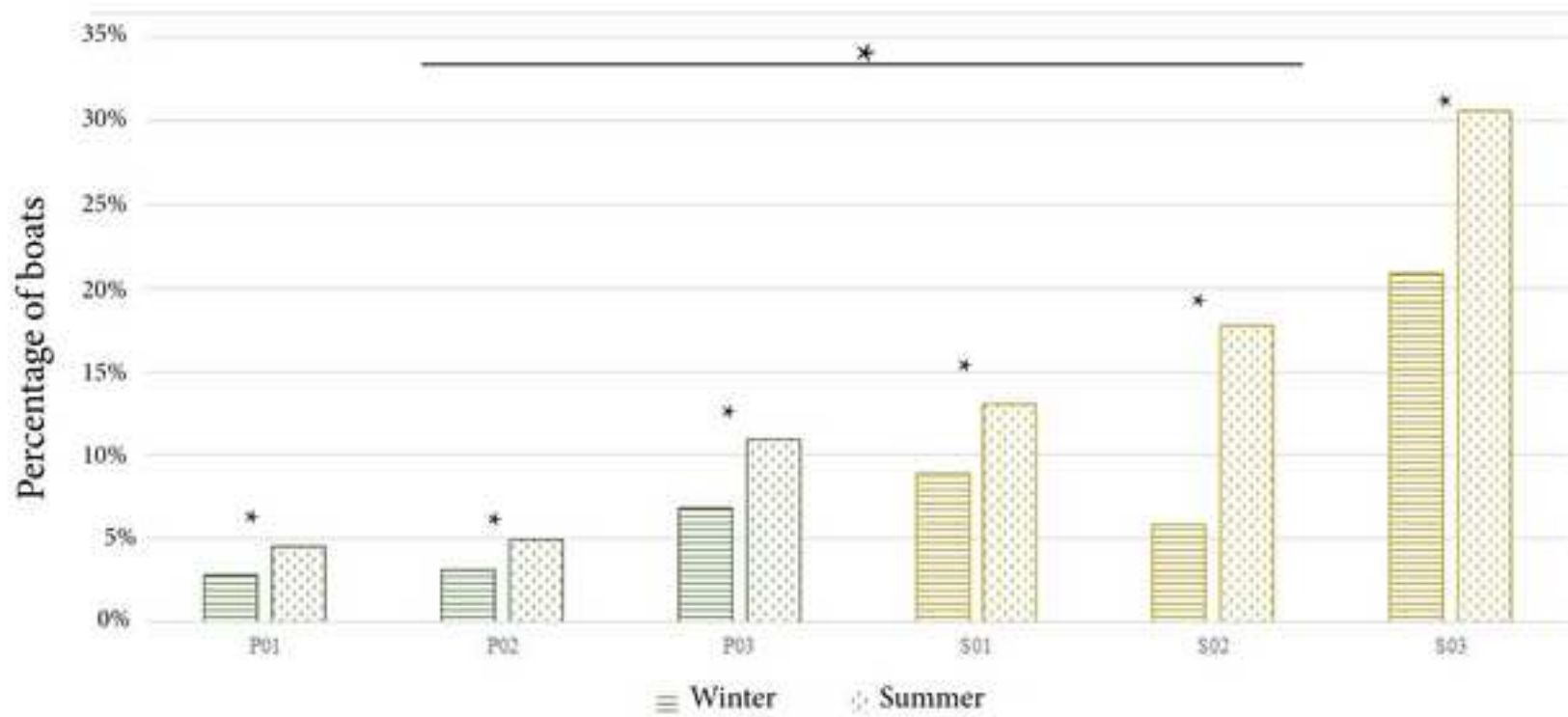


Table 01

Habitat	Site	Latitude	Longitude	Bathymetry (m)	Distance from the coast (m)
Posidonia	P01	37°34'218 N	12°38'976 E	12,9	530
Sand	S01	37°33'611 N	12°39'357 E	12,4	450
Posidonia	P02	37°33'400 N	12°39'672 E	12,5	973
Sand	S02	37°33'058 N	12°40'723 E	11,9	930
Posidonia	P03	37°33'259 N	12°41'839 E	12,9	725
Sand	S03	37°33'542 N	12°42'967 E	12,1	722



Table 02

		Estimate	Std.Error	df	t value	p
<b>PSD - HF band</b>	Intercept	114.68	1.67	4.0	68.79	<0.001
	Habitat (Sand Vs Posidonia)	-10.64	2.36	4.0	-4.52	<0.05
	Daytime (Night VS Day)	1.77	0.06	7298.0	27.39	<0.001
	Season (Winter VS Summer)	-2.24	0.12	7.0	-17.95	<0.001
	Habitat* Daytime	-0.44	0.10	7303.0	-4.44	<0.001
	Habitat*Season	2.23	0.15	93.0	15.13	<0.001
	Season*Daytime	0.40	0.12	7302.0	3.41	<0.001
	Habitat*Season*Daytime	-1.59	0.18	7302.0	-9.02	<0.001
<b>PSD - MF band</b>	Intercept	96.39	0.89	5.0	108.63	<0.001
	Habitat (Sand Vs Posidonia)	-3.59	0.65	4.0	-5.56	<0.01
	Daytime (Night VS Day)	1.71	0.12	7300.0	13.72	<0.001
	Season (Winter VS Summer)	3.37	1.22	3.0	2.77	0.06
	Habitat* Daytime	-1.55	0.19	7300.0	-8.14	<0.001
	Habitat*Season	4.87	0.32	6486.0	15.30	<0.001
	Season*Daytime	-2.57	0.22	7300.0	-11.52	<0.001
	Habitat*Season*Daytime	1.27	0.34	7300.0	3.75	<0.001
<b>PSD - LF band</b>	Intercept	90.97	1.96	6.0	46.34	<0.001
	Habitat (Sand Vs Posidonia)	0.76	2.39	4.0	0.32	0.77
	Daytime (Night VS Day)	-1.86	0.15	7300.0	-12.27	<0.001
	Season (Winter VS Summer)	7.60	1.60	3.0	4.75	<0.05
	Habitat* Daytime	3.12	0.23	7300.0	13.42	<0.001
	Habitat*Season	1.63	0.39	6681.0	4.20	<0.001
	Season*Daytime	0.87	0.27	7300.0	3.19	<0.01
	Habitat*Season*Daytime	-2.94	0.41	7300.0	-7.15	<0.001

Table 03

	Site	Wind Direction	$\beta$	$R^2$	t	p		Site	Wind Direction	$\beta$	$R^2$	t	p
<i>Posidonia</i>	P01	N	.238	.021	1.62	.109	<i>Sand</i>	S01	N	.243	.011	.72	.475
		NE	.093	.001	.19	.850			NE	-1.373	.026	-1.01	.318
		E	-.232	.004	-.39	.702			E	.320	.003	.30	.767
		SE	.204	.043	2.30	.023			SE	.572	.090	2.65	.010
		S	.529	.214	4.30	<0.001			S	.894	.237	3.86	<0.001
		SW	.623	.067	1.22	.235			SW	.613	.021	.50	.624
		W	-.302	.014	-.73	.468			W	-.700	.018	-.63	.535
		NW	-.097	.003	-.56	.575			NW	-.654	.018	-.66	.514
	P02	N	.301	.032	1.87	.064		S02	N	.524	.038	1.82	.072
		NE	-.172	.002	-.32	.748			NE	-1.820	.050	-1.54	.130
		E	-.284	.005	-.44	.660			E	-.606	.007	-.52	.609
		SE	.250	.060	2.73	.007			SE	.885	.231	6.49	<0.001
		S	.665	.233	4.51	<0.001			S	.993	.227	4.09	<0.001
		SW	.369	.018	.61	.547			SW	-2.742	.170	-1.87	.079
		W	.176	.006	.47	.639			W	-.190	.001	-.12	.907
		NW	-.165	.006	-.79	.432			NW	.063	.000	.17	.863
	P03	N	.140	.004	.50	.616		S03	N	.738	.046	1.88	.063
		NE	-.602	.018	-.95	.348			NE	-2.646	.026	-.94	.355
		E	.427	.003	.35	.731			E	-.771	.010	-.58	.564
		SE	.497	.184	4.49	<0.001			SE	.441	.036	1.80	.076
		S	.817	.121	2.75	.008			S	1.033	.130	2.89	.005
		SW	-.130	.001	-.13	.901			SW	.000	.000	.00	1.000
		W	1.779	.098	1.68	.106			W	-1.046	.016	-.55	.592
		NW	.402	.022	1.21	.229			NW	.383	.016	.94	.349

Table 04

	Habitat	$\beta$	$R^2$	t	p
<b>HF band</b>	<i>Posidonia</i>	-0.09	0.06	-1.12	0.26
	<i>Sand</i>	0.11	0.05	0.92	0.35
<b>MF band</b>	<i>Posidonia</i>	0.55	0.11	5.00	< 0.001
	<i>Sand</i>	0.99	0.18	5.90	< 0.001
<b>LF band</b>	<i>Posidonia</i>	0.71	0.05	3.24	< 0.001
	<i>Sand</i>	0.96	0.10	4.36	< 0.001

Table 05

		HF Band	MF Band		LF Band
		<i>HF Snapping Impulses</i>	<i>MF Fishes</i>	<i>MF impulses</i>	<i>LF Fishes</i>
<u>Posidonia</u>	<i>Duration of pulse (sec.)</i>	0.0025 ( $\pm$ 0.00009)	0.15 ( $\pm$ 0.08)	0.0034 ( $\pm$ 0.0004)	0.0054 ( $\pm$ 0.0016)
	<i>Peak frequency (Hz)</i>	8971.81 ( $\pm$ 4347.48)	840 ( $\pm$ 75.37)	724.14 ( $\pm$ 315.64)	210.50 ( $\pm$ 36.41)
	<i>Bandwidth of pulse (Hz)</i>	21788.87 ( $\pm$ 2253.97)	762.85 ( $\pm$ 266.2)	2088.92 ( $\pm$ 313.96)	400.14 ( $\pm$ 112.04)
	<i>Number of pulse of train (N)</i>	-	-	-	30.0 ( $\pm$ 8.33)
	<i>Pulse rate (N/sec)</i>	-	-	-	9.74 ( $\pm$ 1.53)
<u>Sand</u>	<i>Duration of pulse (sec.)</i>	-	-	-	0.0061 ( $\pm$ 0.0016)
	<i>Peak frequency (Hz)</i>	-	-	-	231.63 ( $\pm$ 28.93)
	<i>Bandwidth of pulse (Hz)</i>	-	-	-	402.60 ( $\pm$ 140.56)
	<i>Number of pulse of train (N)</i>	-	-	-	26.08 ( $\pm$ 7.0)
	<i>Pulse rate (N/sec)</i>	-	-	-	9.74 ( $\pm$ 1.80)