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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
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28 Abstract

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

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

54 **1. Introduction**

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

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

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

The physical structure of the habitats determine not only variation in biophonic components, butalso in the geophonical components. In terrestrial ecosystem, it was demonstrated that wind and rain

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

The study of aquatic and marine soundscape was dealt recently using eco-acoustic indices (Lillis et 85 al. 2014, Staaterman et al. 2014, Kaplan et al. 2015, Bertucci et al. 2015, 2016 Buscaino et al. 86 2016). Their use highlights the presence of biological sounds also during high background noise 87 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 and three different indices, founded that the Acoustic Complexity Index (ACI) (see Pieretti et al. 90 91 2011 for details on computation) is a good descriptor of the acoustic community in temperate marine environment. 92

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

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

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

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

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120 **2.** Material and Methods

121 2.1. <u>Study area and data collection</u>

Data collection was carried out along the south-western coast of Sicily, in an area comprised between Capo Granitola and Tre Fontane villages (Fig. 1). Here, the seascape is distinguished by an alternation of patches of sandy, rock and *Posidonia oceanica* meadows. This area is characterized by upwelling phenomena (Bonanno et al. 2014) promoting the primary and secondary production Along the Sicilian coasts, Posidonia meadow covers about 76000 ha (Calvo et al. 2010). Thanks to the favorable ecological conditions and pristine natural state, the western side is one of the most dense and extensive beds of all the entire Mediterranean Sea (Calvo et al. 2009, 2010).

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

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

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

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

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

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155 2.2. <u>Data analysis</u>

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

- 160 Low Frequency (LF): from 0.1 kHz to 0.5 kHz
- -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 marine soundscape. Generally, fish emission range up to 2 kHz (Ladich & Fine 2006, Picciulin et 164 165 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 (HF) could be occupied by invertebrates broadband pulses that extend from 2 kHz up to 120 kHz 167 (Au & Banks 1998, Buscaino et al. 2011, Di Iorio et al. 2012) or signals (both impulsive and tonal) 168 of *delphinidae* species (Papale et al. 2014, Buscaino et al. 2015, Caruso et al. 2017). The day time 169 (night and day) was established basing on the solar time of each recording session using ephemeris 170 tables (Night and Day software- Benvegnù M. and Menichelli M.) 171

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173 2.2.1. Power Spectral Density Analysis

Using MATLAB code, the Power Spectral Density (PSD - dB re 1 μ Pa²/Hz) of each recording was calculated through the *welch* function (Welch 1967) (24000 points FFT, 2¹⁵ points Hamming Window, 50% Overlap). The PSD values have been summarized on the three bands (LF, MF, HFbands).

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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-

animal produced sounds, we computed the Acoustic Complexity Index (ACI) (Pieretti et al. 2011,

Harris et al. 2016, Buscaino et al. 2016) on the three frequency bands.

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

Moreover, the *SoundscapeMeter* permits to apply an amplitude filter (named *noise filter*) on the data before computing the calculation of the index (Farina et al. 2016). As found by Buscaino et al 2016, during the choruses of snapping shrimps and fishes, the number of signals emitted is so high that the energy between one temporal step and the subsequent is comparable. It determines lower values of the index than expected. For this reason, we decided to process the data twice each time using a different filter setting: one without using amplitude filter (ACI no flt), and one using an amplitude filter of 2000 μ V2/Hz (ACI flt).

Successively, the ACI values obtained for each frequency were summed on the LF, MF and HFbands.

196 2.2.3. Identification and description of the biophonic component

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

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

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220 2.3. <u>Statistical Data Analysis</u>

221 The two acoustic habitats were compared using two metrics:

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;

In detail, considering PSD values as dependent variables, linear mixed models (LMEM) (Bates et

al. 2012) were applied to determine if the factors "habitat" (Posidonia, Sand), "daytime" (Day,

Coastal mediterranean soundscape

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

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

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

The characterization of the biophonic components of the two habitats was carried out considering the mean value of the different parameters for each signals. We statistically compared the parameters of sounds present both in sandy and Posidonia habitats. We tested their acoustic variables for normally distribution (Shapiro - Wilk test); since data was not normally distributed, the U-Mann Whitney test was performed. The percentage of files with the presence of vessel noise was compared between the habitats and between seasons on each site. These data were tested for normally distribution (Shapiro-Wilks test for each group of data). We applied T-test for independent data between two habitats and T-test for paired data to compare the data from each site between the seasons.

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255 **3. Results**

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

261 3.1. <u>Acoustical habitat comparison through PSD and ACI</u>

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

Considering the ACI values, the efficiency of the index in term of biophonic amplification, resulted different using or not using the filter for each band considered. The daily pattern of ACI (with and without filter) was plotted for each band (Fig. 5). At the low frequency (LF), where the continuous noise of boats, wind and wave is predominant, the use of the filter reduced the power of attenuation of these sounds. At medium and high frequency bands, where the effect of geo- anthropophonic noise is not so strong and the biophonic choruses are more intense, the use of the filter was essential to amplify the biophonic component of the soundscape. Basing on these results, we carried out the subsequent analysis using different settings of ACI in relation to the band involved. Comparing ACI values, the two habitats presented significant differences for all frequency bands both during day and night. In particular, Posidonia habitat showed higher biophonic activity than sandy habitat (Kruskal-wallis test: LF band χ^2 =1673.5, df=3, p<0.001; MF band χ^2 =958.71, df=3, p<0.001; HF band χ^2 =444.2, df=3, p<0.001) (Fig. 6).

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

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3.2. Identification and description of the biophonic component

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

294 Considering the three bands:

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

In this band, we found fish sounds (probably emitted by one or two similar species) (LF fish) made up of train of pulses. These signals were recorded both in Posidonia and in sandy habitats, but they showed some different spectral and temporal characteristics (respectively Fig. 8-D and 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).

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

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

- 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 pattern, with pitches during sunrise and sunset. The MF fish sounds were recorded mostly in 311 Posidonia habitat during the night in summer period, with the presence of chorus at sunset. During 312 the winter, they were sporadic and no choruses were recorded. In sandy habitat, we found only few 313 tonal signals during summer, but no evidence of chorusing either pattern was present. The MF pulse 314 sounds were recorded only in Posidonia habitat during both winter and summer but only during the 315 daytime. Finally, the snapping shrimp pulses were recorded only in Posidonia habitat in both winter 316 317 and summer, showing pitches at sunrise and sunset. An increase during the summer was present.

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3.3. Habitat comparison through number of boats

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

326 **4. Discussions**

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

Focusing on the results of acoustic energy data, the comparison between the two habitats showeddifferences in all the frequency bands considered.

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

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meadow, could attenuate also the noise produced by geophonical factors, making Posidonia habitata potential acoustic refuge for marine species.

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

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

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

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

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

The MF band was occupied by biological signals only in the Posidonia environment. We obtained acoustic presence of pulses along all daytime and of fish tonal sounds during summer night (with peak at the dawn). The short pulses were present irregularly during the daytime in winter and summer. They totally disappeared during night. The correct attribution of these sounds is still not clear. In our knowledge, a fish species that emit sounds following this seasonal and daily pattern have not been described before. Also, we can exclude invertebrates as source of these signals, since generally, their acoustic activity, even if present along all the year, shows increase during the night
and peaks during dusk and dawn (Radford et al. 2008b, 2010, Buscaino et al. 2011, 2016). The
daily pattern of these pulses suggests an association with a phenomenon linked to presence of light.
Therefore, one possible explanation could be the connection with photosynthetic or decomposition
phenomena within Posidonia meadows. Hermand (2004) suggests the production of photosynthesis
bubbles from Posidonia leaves, and their collapse could produce impulsive sounds (Versluis et al.
2000, Pettit et al. 2015). Further studies are necessary to prove this correlation.

Regarding fish tonal sounds in MF band, as far as we are aware, these signals have still not been 409 described. They show frequency characteristics and daily pattern similar to Therapon theraps as 410 411 recorded in the Great Barrier Reef in Australia and Arabian Sea (Mahanty et al. 2015, McCauley & Cato 2000). Therapon theraps is typical from Indo-West Pacific and Australian waters, but 412 individuals of this species and of *Therapon jarbua*, have been reported in the Mediterranean Sea 413 414 along the Aegean (Minos et al. 2012), Adriatic (Lipej et al. 2008) and Israeli coasts (Golani & Appelbaum-Golani 2010). They are considered invasive species, probably arrived in Mediterranean 415 416 through a Lessepsian migration. The intense and regular occurrence of these broadband sounds, along summer days, suggests a stable presence of this species along the Mediterranean coasts. 417 Invasive species could determine changing in the acoustic community acting as selective pressures 418 on the native species (Farina et al. 2013). This could lead to variation in communication features of 419 local species in order to improve the transmission of information (Acoustic Adaptation Hypotesis, 420 Morton 1975). The absence of visual census monitoring in this area does not allow confirming the 421 presence of this specie in this part of the Mediterranean Sea. However, our results draw attention to 422 the importance of the acoustic method to reveal any rapid changes within an ecosystem. 423

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

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

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

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

Noise pollution can affect marine organisms' acoustic communication through auditory masking (in
which the perception of one sound is affected by the presence of another sound) (Clark et al. 2009).
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

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

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

Therefore, the acoustic monitoring method could represent a useful and not-invasive tool for the evaluation of the human pressure on the bio-ecological and conservation factors in the marine ecosystems, in order to achieve a Good Environmental Status (GES) defined by the Marine Strategy 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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484 **7. Foundings**

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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

and guided the study, the results interpretation and participating to the paper reader.

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695 696	10. Figures
697	
698	Figure 1 Study area. In green Posidonia patches, in light yellow sandy patches
699	
700	Figure 2 Schematic representation of the deployment system
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702 703 704 705 706 707	Figure 3 Three continuous days of recordings (2 minutes/ hour) during both season, in Posidonia habitat (left side) and sandy habitat (right side). The dashed white lines indicate the night time. The letters indicate the different components of the soundscapes. In detail, SS: Snapping shrimps impulses; MF: MF fish choruses; LF: LF fish choruses; I: MF impulses; W: Waves; R: Rain; B: Boat noise. N.B. The lower intensity band between 3500 and 5500 Hz is due to non-linear response of Digital Acquisition Card.
708	
709 710 711	Figure 4 Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and maximum) of PSD measured on two habitats, during daytime of the different seasons. The star represents the significant differences (p <0.05) between habitats.
712	
713 714 715	Figure 5 Mean \pm Standard Error of ACI values, measured without using an amplitude filter (dark green - posidonia habitat; dark yellow sandy habitat) and using an amplitude filter (light green - posidonia habitat; light yellow sandy habitat)
716	
717 718 719 720	Figure 6 Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and maximum) of ACI (without amplitude filter at LF band, and with amplitude filter in MF and HF bands) measured on two habitats, during summer daytime. The star represents the significant differences (p<0.05) between habitats.
721	
722 723 724	Figure 7 Scatterplot of PSD values and wind speed, when the direction of wind was from south (dark green - posidonia habitat; dark yellow sandy habitat) and from south-western (light green - posidonia habitat; light yellow sandy habitat)
725	
726 727 728 729 730	Figure 8 Spectrogram, waveform and amplitude spectrum of the principal biological signals recorded. HF Band: A. Impulsive signals of snapping shrimps; MF band: B. Tonal fish sound C. Impulses; LF band: D. train of fish impulses recorded in Posidonia Habitat E. train of fish impulses recorded in Sandy Habitat. N.B. The waveform and the amplitude spectrum are computated on isolated sounds, see the MS for details of filters applied.

731

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

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- Figure 10 Percentage of boats measured on each site of recordings during both season. The star represents the significant differences (p<0.05) between seasons (for each site) and habitats.
- 739
- 740
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- 743 **11. Tables**
- 744
- 745 Table 1 Characteristics of the recording sites.

746

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

750

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

754

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

757

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

Figure 01 Click here to download high resolution image







Hz









Wind Velocity (m/s)







Habitat	Site	Latitude	Longitudine	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 01

		Estimate	Std.Error	df	t value	р
	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
PSD - HF band PSD - MF band	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
	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
PSD ME hand	Season (Winter VS Summer)	3.37	1.22	3.0	2.77	0.06
rsb - Mr balld	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
	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
DCD IEband	Season (Winter VS Summer)	7.60	1.60	3.0	4.75	<0.05
PSD - LF band	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

	Site	Wind Direction	β	R ²	t	P		Site	Wind Direction	β	\mathbb{R}^2
_		N	.238	.021	1.62	.109		1	N	243	.011
		NE	.093	.001	.19	.\$50			NE	-1:373	.026
	P01	E	- 232	.004	- 39	.702	S01	E	.320	.003	
		SE	.204	.043	2.30	.023		SE	.572	.090	
		S	.529	.214	4.30	<0.001		5	.894	.237	
		SW	.623	.067	1.22	.235		SW	.613	.021	
		W	- 302	.014	73	.468		W	700	.018	
		NW	097	.003	56	.575			NW	654	.019
		N	.301	.032	1.87	.064			N	.524	.038
-		NE	172	.002	32	.748		1	NE	-1.820	.050
1 ia		E	284	.005	44	.660	1		E	606	.007
10	742	SE	.250	.060	2.73	.007		803	SE	.885	.231
Sile	202	s	.665	.233	4.51	< 0.001	Sa	502	s	.993	.227
00		SW	.369	.018	,61	.547			SW	-2.742	.170
-		W	176	.006	.47	.639		1	W	- 190	.001

-,79

.50

.95

.35

4.49

2.75

~13

1.68

1.21

.432

.616

.348

.731

.008

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.106

229

<0.001

NW

N

NE

E SE S

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W

NW

S03

р

.475

318

.767

.010

.624

.535

.514

.072

.130

.609

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1.000

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349

< 0.001

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t

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6.49

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Table 03

NW

N

NE

E

SE

s

SW

W

NW

P03

- 165

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-.602

.427

.497

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-.130

1.779

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.018

.003

.184

.121

.001

.098

.022

	Habitat	β	R ²	t	p
	Posidonia	-0.09	0.06	-1.12	0.26
HF band	Sand	0.11	0.05	0.92	0.35
MF band	Posidonia	0.55	0.11	5.00	< 0.001
	Sand	0.99	0.18	5.90	< 0.001
LF band	Posidonia	0.71	0.05	3.24	< 0.001
	Sand	0.96	0.10	4.36	< 0.001

Table (05
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		HF Band	MF Band		LF Band	
		HF Snapping Impulses	MF Fishes	MF impulses	LF Fishes	
	Duration of pulse (sec.)	0.0025 (± 0.00009)	0.15 (± 0.08)	0.0034 (± 0.0004)	0.0054 (± 0.0016)	
	Peak frequency (Hz)	8971.81 (± 4347.48)	840 (± 75.37)	724.14 (± 315.64)	210.50 (± 36.41)	
Posidonia	Bandwidth of pulse (Hz)	21788.87 (± 2253.97)	762.85 (± 266.2)	2088.92 (± 313.96)	400.14 (± 112.04)	
	Number of pulse of train (N)				30.0 (± 8.33)	
	Pulse rate (N/sec)	*			9.74 (± 1.53)	
	Duration of pulse (sec.)		14		0.0061 (± 0.0016)	
	Peak frequency (Hz)		1.5		231.63 (± 28.93)	
Sand	Bandwidth of pulse (Hz)	÷			402.60 (± 140.56)	
	Number of pulse of train (N)		2	5 <u>-</u> 2	26.08 (± 7.0)	
	Pulse rate (N/sec)	-		-	9.74 (±1.80)	