

# Report of Acoustic Complexity and Diversity Indices (Lošinj case study)

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## 1. Introduction

Nowadays, the study of the soundscape represents a field of growing interest because of the implications it has for the assessment of human–landscape interactions. Soundscape can be defined as the aggregation of sounds from physical (wind, waves, etc.), biological (sounds produced by animals), and anthropogenic (vessels, ports, roads, etc.) sources. In marine and terrestrial habitats, sounds can fluctuate over daily and seasonal time scales creating peculiar soundscape signatures, defined as the main acoustic features of the soundscapes.

In aquatic coastal areas, the abiotic sounds are determined by winds and waves, including breaking surface waves, rainfall and waves beating against cliffs. The wind contribution dominates from a few Hz to 30 kHz, and surface waves cause mostly infrasonic noise at frequencies from 10 to 100 Hz. Rainfall also produces energy peaks from 15–20 kHz, while thunder and lightning generate sounds at lower frequencies, which contribute to background noise even if the storm is distant.

The abiotic sources are different. Snapping shrimp produce wideband pulses from 3 to 100 kHz, with an irregular pulse repetition rate, which results from the rapid closing of their enlarged claws and the consequent collapsing cavitation bubbles. Vocal fishes produce impulsive or frequency-modulated sounds at low frequencies and low amplitudes, with differences in the duration and number of pulse trains for each species. Marine mammals in Mediterranean coastal habitats, such as bottlenose dolphins, use two types of sound: broadband impulsive signals (echolocation clicks and burst pulse sounds), ranging from a few kHz up to 120 kHz and narrowband frequency-modulated sounds as whistles, chirps or low frequency narrow-band calls.

At the end, anthropogenic noise in coastal areas is mainly due to vessel traffic particularly at low frequencies (<1 kHz). Vessel traffic noise is primarily due to the cavitation and rotation of boat propellers, as well as the operation of winches and other shipboard equipment. As a consequence, boat noise change in relation to the type and size of the vessel and its speed.

### 1.1 The Acoustic Complexity Index (ACI)

The acoustic data are traditionally analysed through the manual and aural quantification of sound occurrences, which is an extremely time-consuming procedure. Two automated processing methods allow for a significant reduction in the time required to process large acoustic datasets: (i) approaches based on the automatic detection of calls, including Gaussian mixture models, artificial neural networks, or hidden Markov models among others or (ii) the application of acoustic indices such as acoustic richness

(AR), the Acoustic Diversity Index (ADI) and the Acoustic Complexity Index (ACI). Compared to the automatic detection of calls, the use of acoustic indices to unravel complex biophonic patterns does not require prior knowledge of the targeted signals and is of straightforward application.

The hypothesis on which the ACI formula is based lays on the observation that many biotic sounds are characterized by an intrinsic variability of intensities, while some types of human generated noise present very constant intensity values. Accordingly, the long-term objective of the ACI is to develop an acoustic information extraction procedure of the natural soundscape, representing a tool to determine changes in behaviour and composition of a vocalizing community and, consequently, to monitor acoustic dynamics in a quick way.

Following Pieretti et al (2011), the ACI is calculated according to a formula which involves only a few steps (see Fig. 1). On the basis of a matrix of the intensities extrapolated from the spectrogram (divided into temporal steps and frequency bins), the ACI calculates the absolute difference ( $d_k$ ) between two adjacent values of intensity ( $I_k$  and  $I_{(k+1)}$ ) in a single frequency bin (fl):

$$d_k = |I_k - I_{(k+1)}|$$

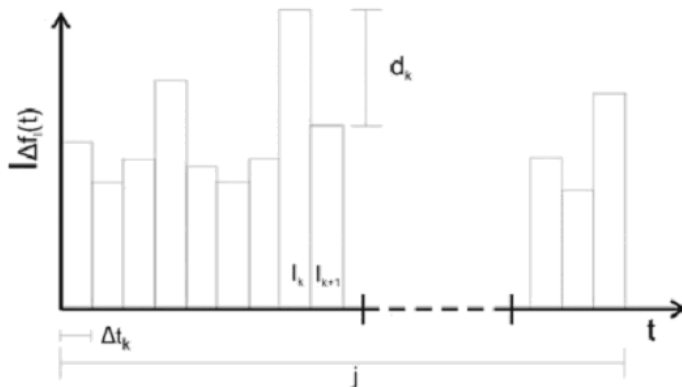
and then adds together all of the  $d_k$  encompassed in the first temporal step of the recording ( $j$ , e.g. 5 s, 30 s, 60 s, etc.):

$$D = \sum_{k=1}^n d_k \quad \text{for:} \quad j = \sum_{k=1}^n \Delta t_k; \quad n = \text{number of } \Delta t_k \text{ in } j$$

where  $D$  is the sum of all the  $d_k$  contained in  $j$ . This result is then divided by the total sum of the

intensity values registered in  $j$

$$ACI = \frac{D}{\sum_{k=1}^n I_k}$$



**Fig. 1.** Explanatory graph of the Acoustic Complexity Index ( $t$ : time;  $\Delta t_k$ : a single time fraction;  $\Delta f_l$ : a single frequency bin;  $I_{\Delta f_l}(t)$ : intensity registered in a  $\Delta f_l$  frequency bin). For example, when the FFT size is set at 512 points, the output matrix will be composed by 310078  $\Delta t_k$  ( $\Delta t_k = 0.02321$  s) and 256 frequency bins ( $\Delta f_l = c43$  Hz), with:  $j = c215$ ;  $\Delta t_k$ ;  $m = 310,078/215 = c1442$ ;  $q = 256$ ;  $n = 215$ .

Thereafter, the ACI, which was worked out on all of the temporal steps encompassed in the recording, is calculated

$$ACI_{(\Delta f_l)} = \sum_{j=1}^m ACI \quad \text{for : } m = \text{number of } j \text{ in the entire recording}$$

where the  $ACI(f_l)$  corresponds to the ACI of an entire frequency bin. Finally, the total ACI for all of the frequency bins is calculated

$$ACI_{tot} = \sum_{l=1}^q ACI_{(\Delta f_l)} \quad \text{for : } \Delta f = \sum_{l=1}^q \Delta f_l; \quad q = \text{number of } \Delta f_l$$

where the  $ACI(tot)$  is the total value of the index for the entire recording.

As result higher ACI values are generated by greater variability in intensity (e.g. from multiple sound sources), whereas sounds generated by anthrophony or geophony, which tend to be more constant in intensity, produce low ACI values

## 1.2 Applications of the Acoustic Complexity Index (ACI) on aquatic soundscapes

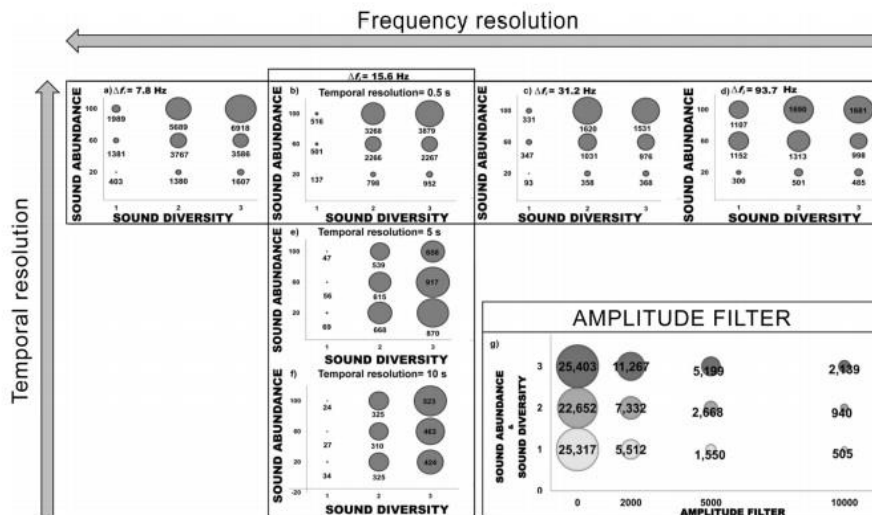
An increasing number of studies have applied the ACI to aquatic environments in order to gain information about diversity or ecological state (Staaterman et al. 2014, Buscaino et al. 2016, Harris et al. 2015). One of the principal challenges in using acoustic indices to explore large data sets is the ability to discern how specific acoustic events contribute to a particular index value over a given period of time. Previous literature has used the ACI as a metric to track fish vocalizations (Staaterman et al., 2014), shrimp-produced sounds (McWilliam and Hawkins, 2013), species diversity in temperate reefs (Harris et al., 2015) and sounds produced by animal communities in noise-polluted environments (Buscaino et al., 2016; Duarte et al., 2015). Also Pieretti et al. (2017) and Ceraulo (2018) used the Acoustic Complexity Index (ACI) as a proxy for marine sounds of biological origin.

According to Buscaino et al (2016), the ACI values were strongly correlated with the pulsed biotic elements in the relative frequency bands (250 and 500 Hz 1/3 octave bands for pulsed fish sounds and > 4000 Hz 1/3 octave bands for snapping shrimps) but the non-impulsive biophonies with frequency modulation were not well detected by ACI, probably due to the frequency and temporal resolution used for the ACI calculations. In this study ACI levels were not correlated with the geophonic or anthropophonic elements, the latter being rather depicted by octave band sound pressure levels. The ACI was therefore used as an indicator of biological sounds, in order to separate biological from anthropogenic inputs into the soundscape.

Other studies have tried to apply the ACI and correlate it to biodiversity measurements with contrasting results. For instance, while Kaplan et al. (2015) could not find a clear correlation among ACI and fish assemblages of tropical reefs at Virgin Islands (US), Harris et al. (2015) found ACI to be a reliable metric for describing biodiversity in reef fish communities of temperate sites in north-eastern New Zealand. More recently Davies et al (2020) showed significant correlations between number of species and ACI values; however, the sign of these correlations changed almost yearly along a 5 year period, showing that more in-depth analyses are needed.

The Acoustic Complexity Index has been shown to have a number of drawbacks (Kaplan et al., 2015; McWilliam and Hawkins, 2013). These drawbacks can arise from interference by the biophony: the ACI has shown to be increased heavily by snapping shrimp, which produce a high intensity broadband 'snap', meaning an increased ACI when diversity has only marginally increased (McWilliam and Hawkins, 2013). In contrast, chorusing behaviour can heavily decrease ACI (Kaplan et al., 2015). Buxton et al. (2018) also found that acoustic indices did not reliably predict bioacoustic activity in marine habitats. Suggesting such a result was due to the overlap of many biological signals with both the snapping shrimp and anthropogenic sounds. Staatermann et al. (2014) found a similar effect with index values being dominated by the presence and intensity of Bocon toadfish (*Amphichthys cryptocentrus*) or snapping shrimp.

Studying the fish vocal communities, Bolgan et al. (2018) demonstrated that ACI does not discriminate between sound abundance and sound diversity and that in the presence of boat noise, no correlation was found between the ACI and the number of emitted sounds. The same authors highlight how ACI is strongly influenced by all settings that must be chosen prior to its calculation and by the choice of amplitude filter (Figure 2).



**Figure 2.** The effect of the settings on the ACI, where the size of the bubbles and their labels represent the value of the ACIsum (controlled experiment CE). (a–d) Show the influence of the frequency resolution ( $\Delta f_i$  on  $CE_i$ ); (b,e and f) show the influence of the temporal resolution of the ACI algorithm ( $CE_i$ ); and (g) shows the effect of the amplitude filter ( $\mu V^2/Hz$ ) in the presence of fish choruses ( $CE_e$ ), where (1) is the chorus of *S. umbra*, (2) is the chorus of *S. umbra* with 20 sounds  $\cdot \text{min}^{-1}$  of *O. rochei* and 20 *Kwa* sounds  $\cdot \text{min}^{-1}$ , and (3) is the chorus of *S. umbra* with 30 sounds  $\cdot \text{min}^{-1}$  of *O. rochei* and 30 *Kwa* sounds  $\cdot \text{min}^{-1}$ .

In their case of study, for example, the best representation of the vocal fish community occurred when the filter was not applied. The authors indicate that each specific situation requires *ad hoc* settings for the ACI to be representative of variation in fish sound abundance and diversity.

Similarly, Bohnenstiehl et al (2018) show that ACI can be modulated strongly by variations in the activity of a single sound-producing species, with additional sensitivity to call type and the resolution of the analysis. Variations in ACI, therefore, cannot be assumed to track call diversity, and the utility of these metrics as ecological indicators in marine environments may be limited. Similarly, Lyon et al (2019) indicate that ACI values for both high and low frequency bands showed no associations with habitat complexity or fish community structure in a tropical, back-reef system.



### 1.3 Aims

In the context of the Soundscape study, we could test the response of ACI to our recordings, with particular attention for the response of the ACI index to the boat passages, the dolphins and the fish vocalizations. Nowadays no study has addressed the response of the ACI index to dolphins vocalizations. On the other side, an intuitive and immediate concern is the degree to which ship noise or other environmental/anthropogenic sounds contribute to index values. In order to achieve this goal, particular care should be given to the choice of the settings needed for running the index.

### 1.4 Summary of the ACI settings in aquatic soundscape studies

By running the ACI there are different settings that need to be defined: it is requested to define the length of the recording segment, with values commonly ranging from 1 to 60 s, and the frequency range, that is often related to the target biological sounds. The number of points used in calculating the Fast Fourier Transform (that converts the audio signal from the time domain to a representation in the frequency domain; NFFT) and sampling rate of the data (fs) determine both the frequency ( $\Delta f = fs/NFFT$ ) and temporal resolution ( $\Delta T = NFFT/fs$ ) of the analysis. Further a noise filter can be applied.

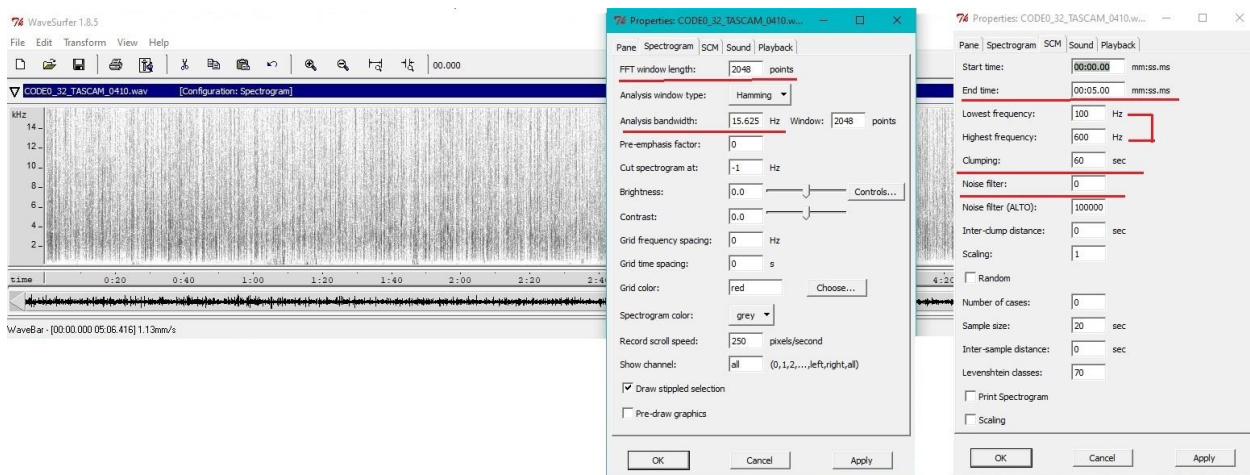


Figure 3 – Settings for the *plug-in* SoundscapeMeter run by WaveSurfer in order to calculate the ACI index

A literature review has been run in order to define the settings used by different authors and the context on which the ACI has been used in the listed studies. Data are summarized by Table 1. Unfortunately, it is

still unclear how differences in sampling rate, bit depth, and spectral resolution impact the performance of the indices. Additionally, some settings, ie. the frequency resolutions are commonly applied but their choice is rarely justified in the literature and often appears to be a consequence of selecting a default value.

Table 1 - Summary of ACI settings in aquatic soundscape studies

Frequency resolution ( $\Delta f_i$ )	Temporal resolution (s)	Noise filter	Scopes of the paper	Reference
Not indicated	Not indicated	Not indicated	Compare soundscapes between and within several benthic habitat on the 2–4 kHz frequency band.	McWilliam, J.N., Hawkins, A.D., 2013. A comparison of inshore marine soundscapes. <i>J. Exp. Mar. Biol. Ecol.</i> 446, 166–176
25 Hz FFT = 160	1 s	Not indicated	To examine the acoustic composition on the 'low frequency band' (25-2000 Hz), including fish vocalizations - it was not used on the high frequency band (2000 to 10 000 Hz) dominated by snapping shrimp and odontocete activity	Staaterman, E., Paris, C., DeFerrari, H., Mann, D., Rice, A., D'Alessandro, E., 2014. Celestial patterns in marine soundscapes. <i>Mar. Ecol. Prog. Ser.</i> 508, 17–32
86 Hz FFT=512,	30 s	Not indicated	To explore the acoustic diversity of three temperate ponds in three different habitats.	Desjonquères C., Rybak F., Depraetere M., Gasc A., Le Viol I., et al., 2015. First description of underwater acoustic diversity in three temperate ponds. <i>PeerJ.</i> 3: e1393-16.
50 Hz	12 s	Not indicated	To determine how species assemblages link to	Kaplan, M., Mooney, T., Partan, J., Solow, A., 2015. Coral reef species

Recorded at 16 kHz; FFT=880			biological sound production.	assemblages are associated with ambient soundscapes. Mar. Ecol. Prog. Ser. 533, 93–107.
281.3 Hz - Recorded at 144 kHz; FFT=512	16 s - averaged over hour-long intervals	Not indicated	To compare three ecoacoustic indices to three traditional species assemblage diversity measures from field surveys of reef fish.	Harris S. A., Shears N. T., and Radford C. A. 2016. Ecoacoustic indices as proxies for biodiversity on temperate reefs. <i>Methods in Ecology and Evolution</i> . 7: 713–724.
22.2 Hz - Resampled at 181.760 Hz; FFT=8192	0.04 s	Noise filter = 5000 $\mu$ V <sup>2</sup> /Hz	To explore the shallow water soundscape of an MPA and to test the ACI as acoustic metric - the index was applied to low frequency band (0.125–0.5 kHz) and to high frequency band (4.0–64.0 kHz)	Buscaino G., Ceraulo M., Pieretti N., Corrias V., Farina A., et al., 2016. Temporal patterns in the soundscape of the shallow waters of a Mediterranean marine protected area. <i>Scientific Reports</i> . 6: 1–13.
39 Hz FFT=512,	0.5 s	Not indicated	To examine variations in SPLs and ACI in relation to fish and benthic communities – applied to low frequency band (ACI Low; 20 Hz to 2 kHz) and high frequency band (ACI High, 2 kHz to 10 kHz)	Bertucci F, Parmentier E, Lecellier G, Hawkins AD, Lecchini D. 2016. Acoustic indices provide information on the status of coral reefs: an example from Moorea Island in the South Pacific. <i>Sci Rep</i> 6: 33326
50 Hz Recorded at 20 kHz FFT = 400	10s	Not indicated	To determine which acoustic measurements best reflect patterns in species diversity – applied to the ‘low band’ (25–1000 Hz) and the ‘high band’ (3000–10 000 Hz).	Staaterman, E.; Ogburn, M.B.; Altieri, A.H.; Brandl, S.J.; Whippo, R.; Seemann, J.; Goodison, M.; Duffy, J.E., 2017. Bioacoustic measurements complement visual biodiversity surveys:

				Preliminary evidence from four shallow marine habitats. <i>Mar. Ecol. Prog. Ser.</i> 575, 207–215.
39.1 Hz (FFT 2048, Low Bands, LB); and 312.5 Hz (FFT 256; High Bands, HB)	0.026 s (LB) 0.0032 (HB)	Noise filter of 5000 $\mu\text{V}^2/\text{Hz}$	To assess the biological sounds of a rocky bottom coastal area - applied to the 'low band' (0–0.62 kHz) and the 'high band' (0.62–40.0 kHz)	Pieretti N., Martire Lo M., Farina A., and Danovaro R., 2017. Marine soundscape as an additional biodiversity monitoring tool: A case study from the Adriatic Sea (Mediterranean Sea). <i>Ecological Indicators.</i> 83: 13–20.
3.9 Hz FFT= 512	60 s. The values were divided by the number of minutes in the recording to reduce the effects of long-duration recordings and were averaged for each hour of the day over the recording period	Not indicated	To explore diel trends in the marine acoustic environment	Rice A. N., Soldevilla M. S., and Quinlan J. A. 2017. Nocturnal patterns in fish chorusing off the coasts of Georgia and eastern Florida. <i>Bulletin of Marine Science.</i> 93: 455–474
15.6 Hz Resampled at 32 kHz; FFT=2048	0.064 s	Noise filter of 2000 $\mu\text{V}^2/\text{Hz}$	To distinguish two different Mediterranean habitats, basing on their soundscapes - applied to the low frequency (0.1-0.5 kHz), Medium frequency (0.5-2 kHz), high frequency (2-20 kHz).	Ceraulo, M. et al. 2018. Acoustic comparison of a patchy Mediterranean shallow water seascape: <i>Posidonia oceanica</i> meadow and sandy bottom habitats. <i>Ecol. Indic.</i> 85, 1030–1043

variable	variable		To assess the ACI effectiveness and potential shortcomings	Bohnenstiehl D.R., Lyon R.P., Caretti O.N., Ricci S.W., Eggleston D.B. 2018. Investigating the utility of ecoacoustic metrics in marine soundscapes. Journal of Ecoacoustics. 2: .
8 kHz, FFT=512	0.5 s	Not indicated	To characterize the acoustic environment of shallow and deeper seamounts., - applied to the low frequency (<2kHz), medium frequency (2-4 kHz)	Carrico R. et al 2020. The Use of Soundscapes to Monitor Fish Communities: Meaningful Graphical Representations Differ with Acoustic Environment, Acoustics 2020, 2, 382–398

Out of these papers (table 1), only Ceraulo et al. (2018) mentioned the use of ACI in the frequency range characterized by delphinidae species; they refer to a class of frequency, called High Frequency (HF), ranging from 2 kHz to 20 kHz; in this case data were resampling at 32 kHz, and a FFT of 2048 points (frequency resolution of 15.6 Hz) was used.

## 2. Settings explorative analysis - The Effect of Operator Choice on the ACI

### 2.1 Pilot study based on hourly data

In order to evaluate the effects of different setting on the ACI index performance, 5 1-hour acoustic files recorded in the ML6 station in Losinj (Croatia) were considered:

two of them containing dolphin whistles and echolocating clicks (File “A” – reference number 13164732, Figure 4; File “B” - reference number 13214922)

two of them containing boat noise at the low frequency (File “C” - reference number 13184735, Figure 5; File “D” reference number 13094721)

one file contained fish sounds (File “E” - reference number 13224923; Figure 6).

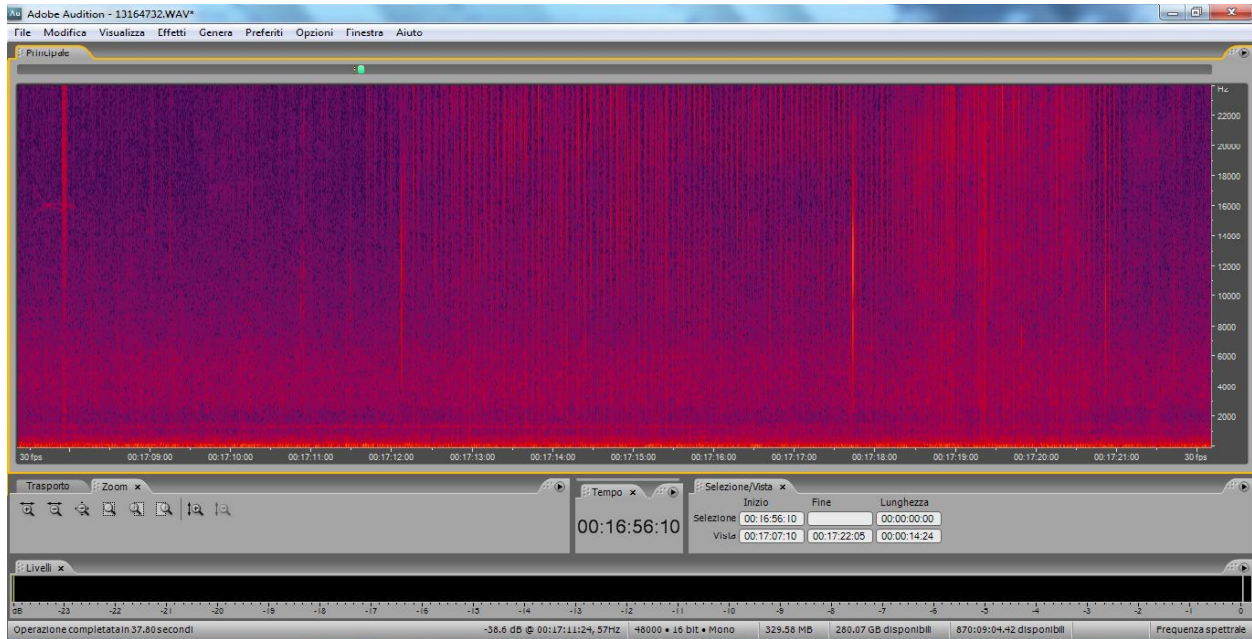


Figure 4. Sonogram of the “File A” (Hanning window, FFT 512, displayed by Adobe Audition)

In the file “A” there are a lot of echolocation clicks (*minutes 4-12, 15-16 and 22-23*); this file was compared with file “C”, where there are a lot of snapping shrimp impulses (*minutes 3-4, 9-10 and 43-44*) at the high frequency whereas at low frequency boat noise is present *after 24 minutes*.

On the contrary to file “A”, in the file “B” there are few echolocation clicks (*minutes 29-30*) and some whistles (*minutes 31-32*) and lots of snapping shrimps; this file was compared with file “D”, where there are a lot of snapping shrimps impulses at the high frequency whereas at low frequency a boat noise passage is clearly evident *between 20 and 22 minutes*.

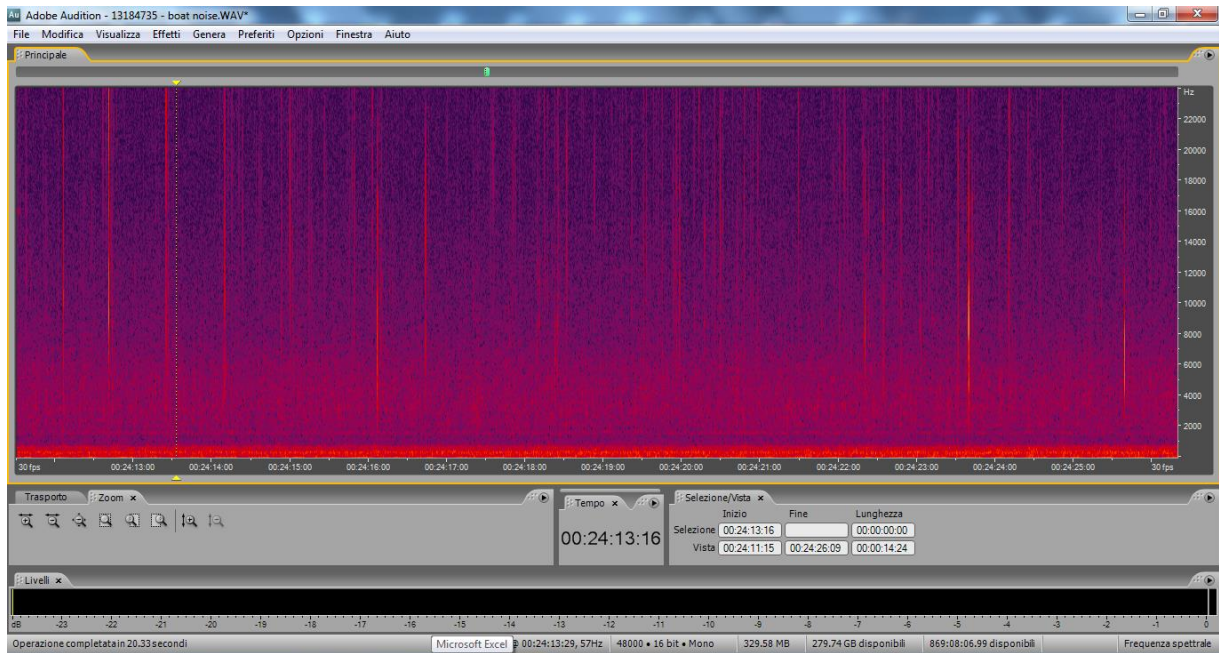


Figure 5. Sonogram of the “File C” (Hanning window, FFT 512, displayed by Adobe Audition)

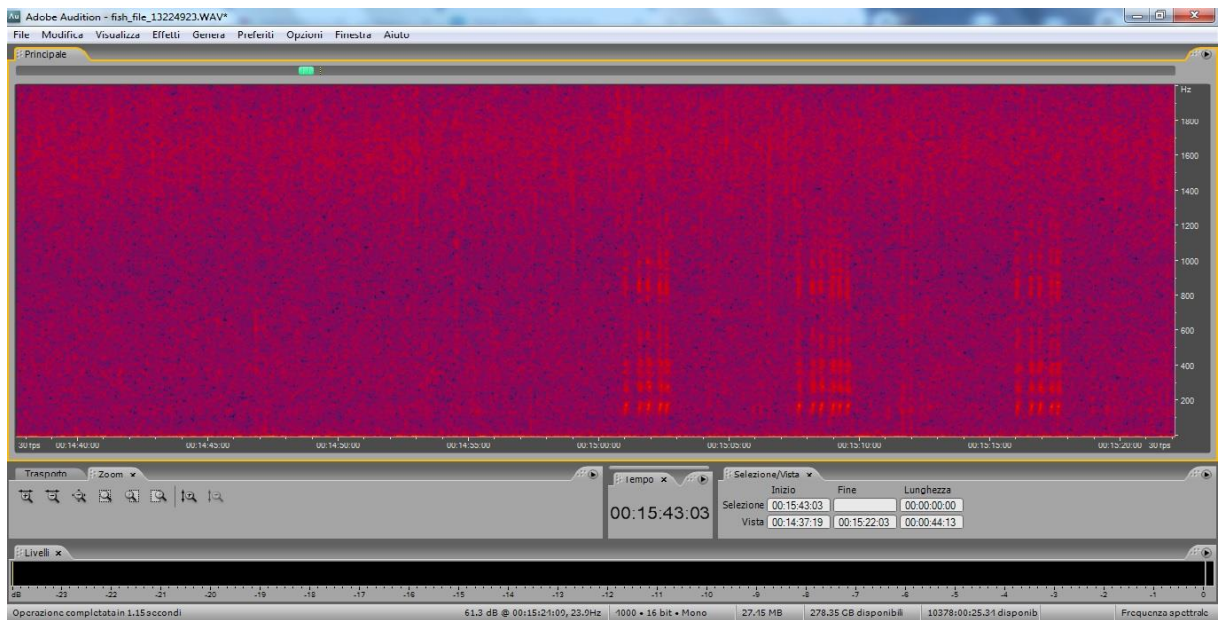


Figure 6. Sonogram of the “File E” (Hanning window, FFT 512, resampled at 4000 Hz; displayed by Adobe Audition)

File “E” includes low frequency sounds (below 1.5 kHz) likely attributable to fish at *minutes 15, 27-28 and 32* (ie. sec 900, 1620 e 1920).

Four FFT settings have been applied on the files in order to evaluate the best configuration for a good correspondence between ACI and the acoustic sources:

FFT 256,  $\Delta f = 86$  Hz,  $\Delta t = 0,01$ ;

FFT 512,  $\Delta f = 43$  Hz,  $\Delta t = 0,02$ ;

FFT 1024,  $\Delta f = 21$  Hz,  $\Delta t = 0,04$ ;

FFT 2048,  $\Delta f = 10,7$  Hz,  $\Delta t = 0,09$ )

Clipping was always considered equal to 60 sec.

*Per* each file, two bands have been considered by applying the ACI :

50-2000 Hz, the so-called “Low Frequency band”, representative of fish sounds and low frequency narrow-band calls produced by dolphins (and easily affected by boat noises)

2000-20000 Hz, the so-called “High Frequency Band”, representative of dolphin sounds as clicks, burst pulse sounds and whistles (and easily affected by snapping shrimps sounds)

The ACI values have been calculated by applying a Matlab code kindly provided by dr. Matt Pine (Ocean Acoustics Ltd) and used in Dimoff et al. (2021).

The ACI was found to be strongly influenced by all settings that must be chosen prior to its calculation. In figure 7 and 8, Aci values (y-axis) are depicted along the time (here indicated as “segment number” since each segment corresponds to 60 sec, as setted in the clipping of the software). ACI values for the files “A” and “B” are displayed by the red curve whereas ACI values for the files “C” and “D” are displayed by the blue curve. Note that the range of values displayed in the y- axis changes according to the considered FFT.



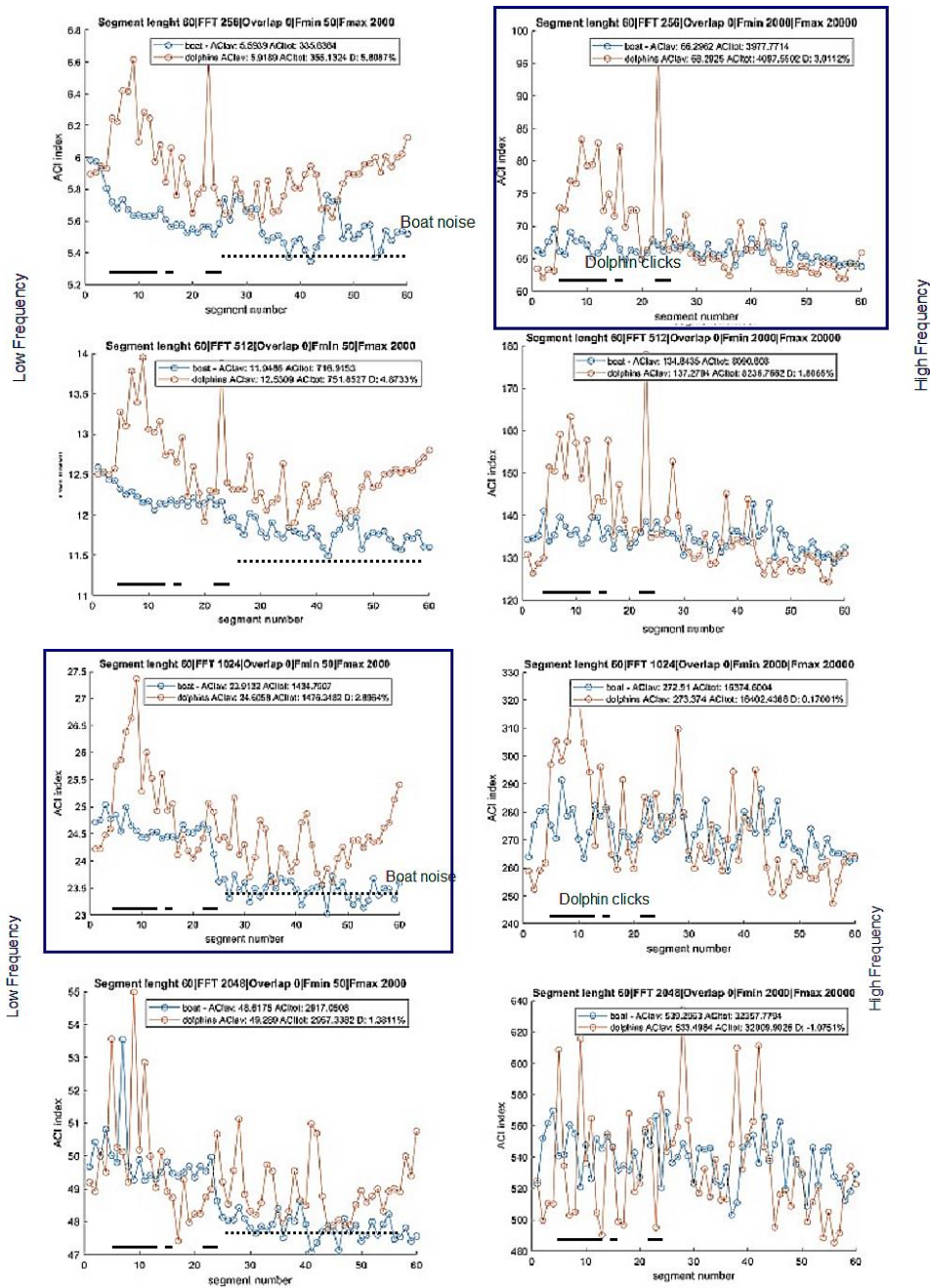


Figure 7. ACI values for the “A” (containing biophony at high frequency) and “file C” (containing boat noise at low frequency) are displayed by the red and blue curves, respectively.

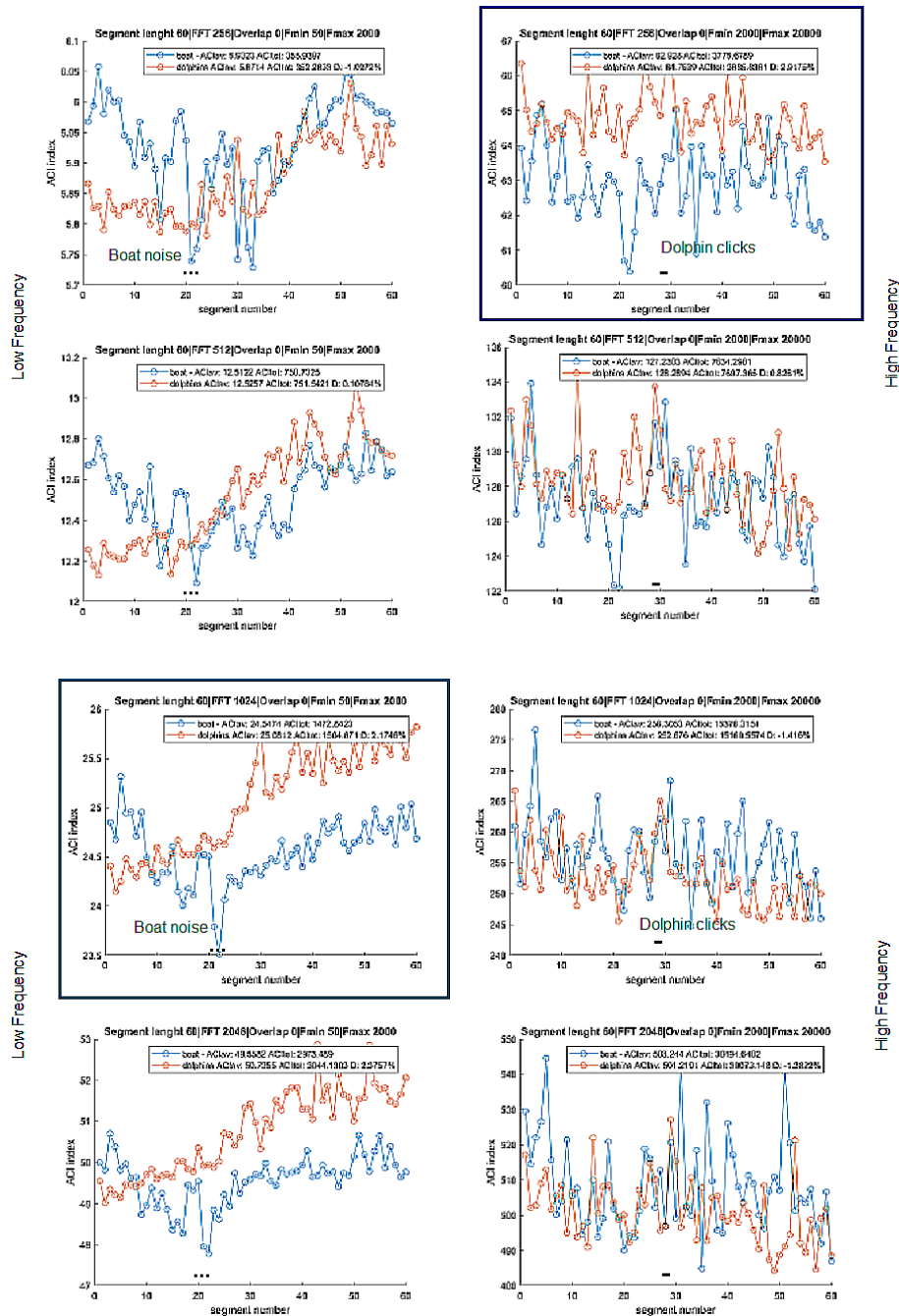


Figure 8. ACI values for the file “B” (containing biophony at high frequency) and file “D” (containing boat noise at low frequency) are displayed by the red and blue curves, respectively.

Once considering the comparisons “A vs. C” files, an increase in the ACI values is evident at the high frequency (“HF”; 2000-20000 Hz) in correspondence of an increase in biophony (*minutes 4-12, 15-16 and 22-23*) when considering the  $\Delta f=43$  e 86 (FFT 512 and 256) but not when the  $\Delta f=21$  e 10,7 (FFTs of 1024 and 2048) are applied. This is confirmed once considering the comparisons “B vs. D” files, where an increase in the ACI values is present at (*min. 29--32*) with  $\Delta f=43$  e 86 but not  $\Delta f=21$  e 10,7.

At the low frequency (“LF”; 50-2000 Hz), a decrease in the ACI values corresponds to the boat noise passages in both the comparisons (“A vs. C” and “B vs. D” files) only when applying the  $\Delta f=21$  e 10,7 (1024-2048 FFT settings ); furthermore  $\Delta f=10,7$  (FFT 2048) is the best option to highlight an increase in the ACI values corresponding to the fish sounds in the acoustic file (*min. 15, 27-28 and 32; ie. sec 900, 1620 e 1920*). The ACIs obtained by applying other FFTs are more influenced by other soundscape components.

In table 2 the ACI total values for HF (2000-20000 Hz) calculated per each 1h-file according to the highlight settings are indicated. File “A”, a large amount of whose file contains biophony due to bottlenose dolphin vocalizations, has the highest ACI values for  $\Delta f=43$  e 86 (FFT 256-512) and, slightly for  $\Delta f=21$  (1024 FFT) settings but not for  $\Delta f=10,7$  (2048 FFT) setting. Despite this, the file “B”, where only a few minutes out of an hour of recordings are characterized by bottlenose dolphin vocalizations, shows lower ACI values with all the FFT settings than file “C” where no biophony is present. This indicated that not only the settings but also the duration of the dolphin vocalizations in the 1h-file affect the total ACI value output, increasing the uncertainty about a positive correspondence between the ACI output and the presence of biophony in the considered file.

Table 2 - ACI total values for HF (2000-20000 Hz) calculated per each 1h-file according to the highlight settings

ACI <sub>tot</sub> values	File A (HF biophony)	File B (HF biophony)	File C (LF anthropophony)	File D (LF anthropophony)
HF ( $\Delta f=86$ )	4097,5	3885,8	3977,7	3775,6
HF ( $\Delta f=43$ )	8236,7	7697,3	8090,6	7634,2
HF ( $\Delta f=21$ )	16402,4	15160,5	16374,6	15378,3
HF ( $\Delta f=10,7$ )	32009,9	30073,1	32357,7	30494,6

Figure 9 shows the ACI plots obtained by processing the file “E” containing the fish pulsed sounds by using different FFTs (clipping 60 sec). Observing the trend of LF (left side of the figure), ACI values calculated with  $\Delta f=10,7$  (2048 FFT) better depicts the fish sounds presente at at *minutes 15, 27-28 and 32* (ie. sec 900, 1620 e 1920).

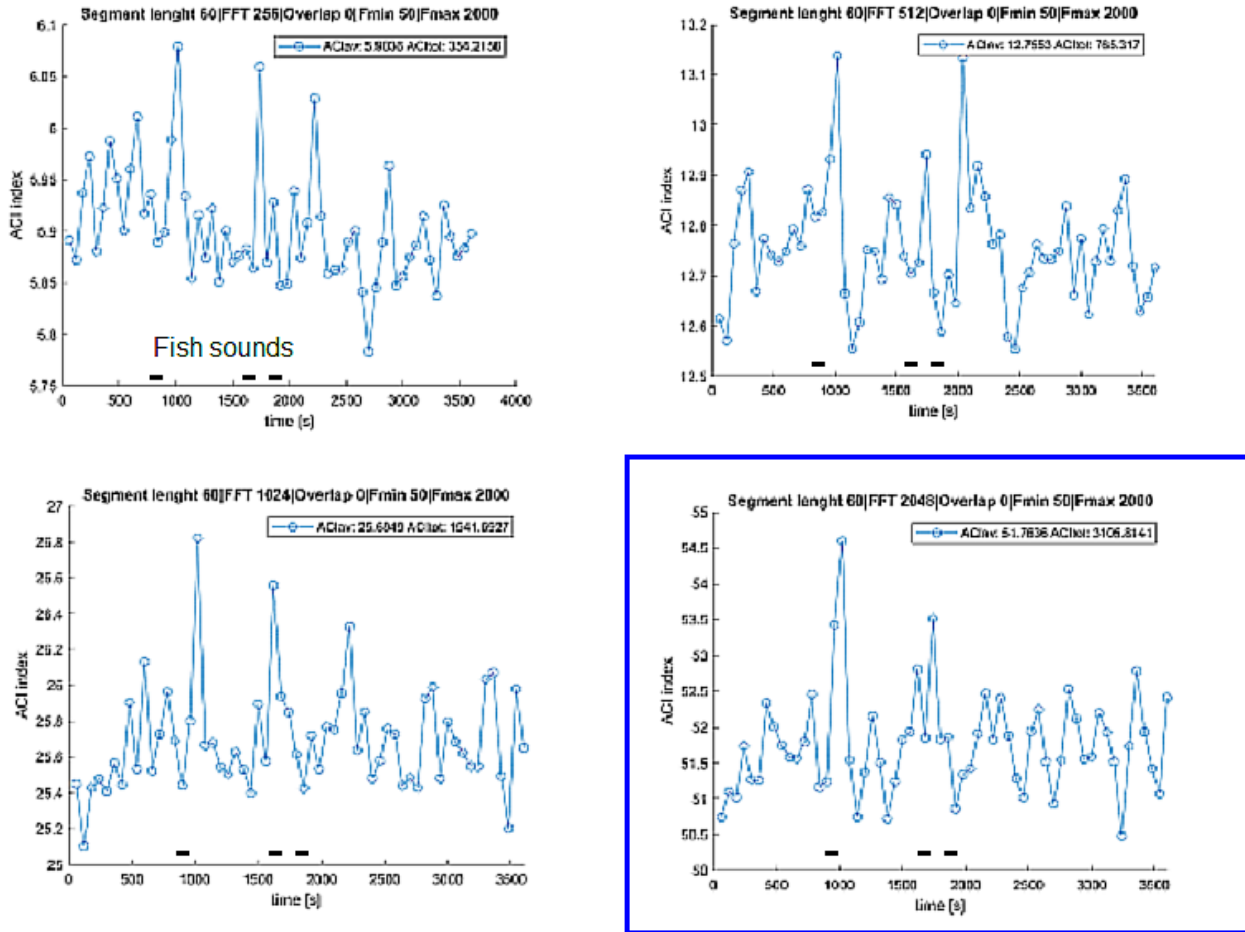


Figure 9. ACI values for the file “E” (containing biophony at low frequency)

In table 3 the ACI total values for LF (50-2000 Hz) calculated per each 1h-file according to the highlight settings are indicated. File “E” containing fish pulsed sounds has the highest ACI values for  $\Delta f$  above 43 Hz (with best resolution when applying the  $\Delta f=10,7/2048$  FFT setting).

It has also to be noticed that boat noise presence affects the ACI total values in file “C” but not in file “D”, where only the passage of one boat was scored. This again indicated that not only the settings but also the duration of boat noise input affects the total ACI value output in 1-h file.

Table 3 - ACI total values for LF (50-2000 Hz) calculated per each 1h-file according to the highlight settings

ACI <sub>tot</sub> values	File E (LF biophony)	File A (HF but not LF biophony)	File B (HF but not LF biophony)	File C (LF anthropophony)	File D (LF anthropophony)
LF ( $\Delta f=86$ )	354	355	352	335	355
LF ( $\Delta f=43$ )	765	751	751	716	750
LF ( $\Delta f=21$ )	1541	1476	1504	1434	1472
LF ( $\Delta f=10,7$ )	3106	2957	3044	2917	2973

## 2.2 Pilot study based on daily data

A 24h cycle of recordings was further analysed by computing ACI 1-h value by applying both  $\Delta f=86$  (256 FFT) and  $\Delta f=10,7$  (2048 FFT) settings and by manually scrolling the files in order to highlight the presence of dolphins/fish vocalizations and/or boat noise presence. The targets were the recordings done by the MS6 located around the Oruda Island (Losinj, Croatia) at 11<sup>th</sup> March 2020.

As it is clearly visible in Figure 10 the trend of the ACI 1-h value (calculated by applying  $\Delta f=86$  settings and calculated for HF) (above) is only partially influenced by the percentage of minutes in the 1-h acoustic files containing dolphin vocalizations (below). It is rather likely that the increase of ACI after sunset is due to an increase of the snapping shrimps activity, whose acoustic activity increases during sunset and sunrise (Bohnenstieh et al 2017; Buscaino et al 2016)

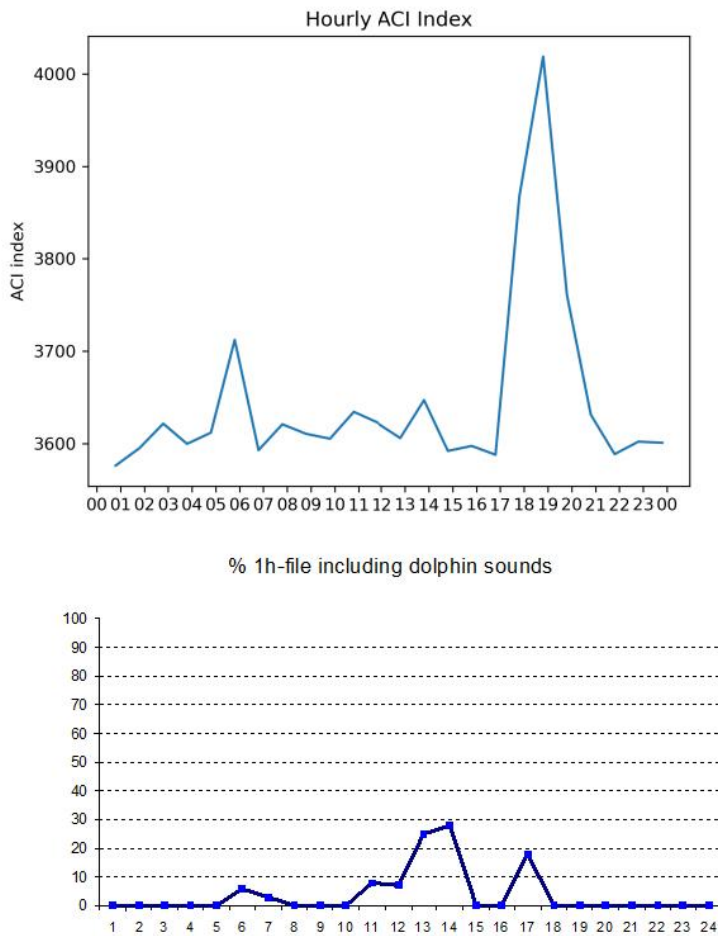


Figure 10. Comparisons between the (above) ACI 1-h values (by applying  $\Delta f=86/256$  FFT settings and calculated for HF) and (below) the percentage of files in the 1-h acoustic files containing dolphin vocalizations.

The ACI calculated for HF using  $\Delta f=86/256$  FFT settings was not correlated with the percentage of minutes containing dolphin sounds (Spearman rank correlations,  $r_s = 0.33$ ,  $p\text{-value} = 0.107$ ).

A better relation between dolphin sound and ACI values is achieved by applying a filter including only the signals whose intensity is above the 30<sup>th</sup> percentile. Although this lowers the ACI peak around sunset, the relation is still limited (Figure 11).

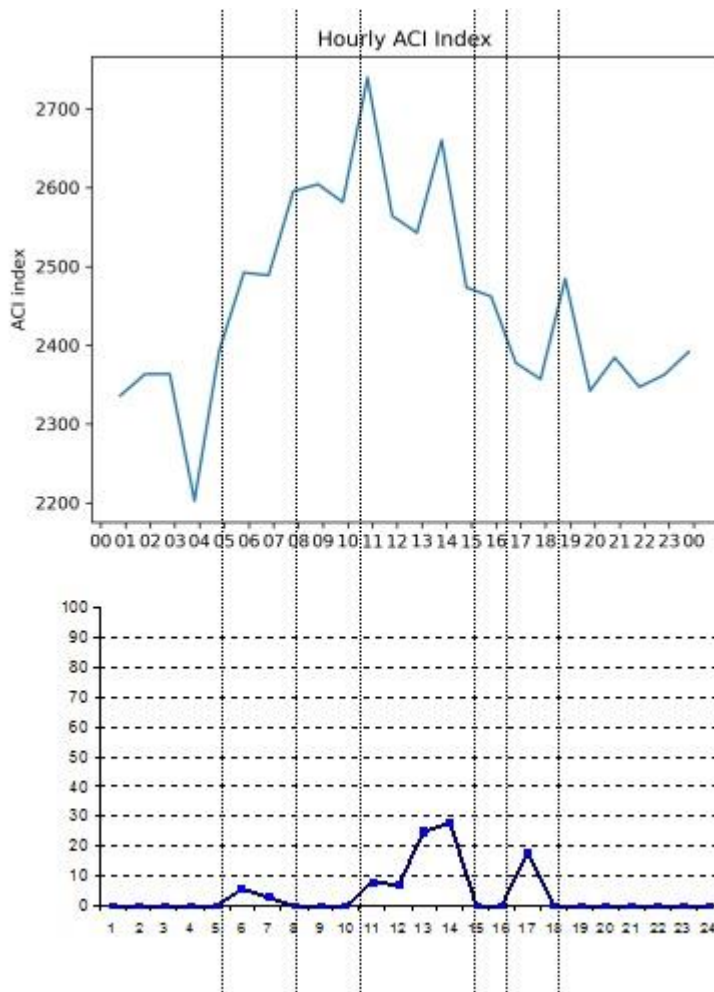


Figure 11. Comparisons between the (above) ACI 1-h values (by applying  $\Delta f=86/256$  FFT settings and calculated for HF but with a reduction of the signal) and (below) the percentage of files in the 1-h acoustic files containing dolphin vocalizations.

Figure 12 highlights the 24-h trend of the ACI 1-h values calculated by applying  $\Delta f=10,7/2048$  FFT settings and calculated for LF (above). In this case ACI seems to respond reasonably well to the percentage of minutes in the 1-h acoustic files containing fish sounds (below), being also influenced by the presence of wideband dolphin clicks. It is less clear how boat noises influence the ACI values.



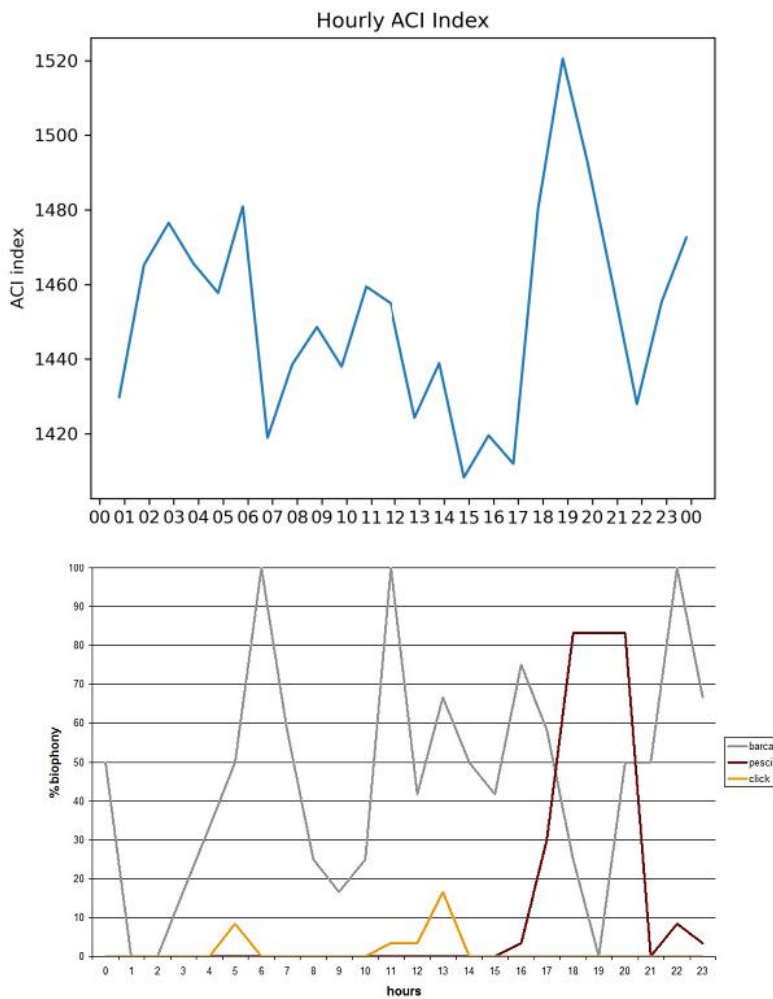


Figure 12. Comparisons between the (above) ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings and calculated for LF) and (below) the percentage of files in the 1-h acoustic files containing fish vocalizations (h 17-20), dolphin vocalizations and boat noises.

The ACI calculated for LF using  $\Delta f=10,7/2048$  FFT settings was positively correlated with the percentage of minutes containing fish sounds (Spearman rank correlations,  $r_s = 0.45$ ,  $p\text{-value} = 0.024$ ,  $N=24$ ) and it was negatively correlated with the percentage of minutes containing boat noises (Spearman rank correlations,  $r_s = - 0.39$ ,  $p\text{-value} = 0.05$ ,  $N=24$ ).

### 3. Application of the ACI on weekly data in order to evaluate the seasonal effect

5-days data has been considered for two different periods, i.e. the winter period (11-15 March) and the summer period (26-30 July), for a total of 240 hours of recordings collected in the Oruda location (Losinj archipelago, Croatia).

These two periods were analysed by computing LF and HF ACI 1-h value by applying both  $\Delta f=86$  (256 FFT) and  $\Delta f=10,7$  (2048 FFT) settings and by manually scrolling the files in order to highlight the presence of dolphins/fish vocalizations and/or boat noise presence. In both cases two fish sound types have been found, one produced by the Roche's snake blenny *Ophidion rochei* (Kever et al. 2016) and one (see Figure 6), whose emitting species is unknown although it has been previously briefly described by Bolgan et al. (2020).

Dolphin sounds have been depicted, including broadband impulsive signals as the echolocation clicks and narrowband frequency-modulated sounds as whistles or low frequency narrow-band calls (LFN, figure 13).

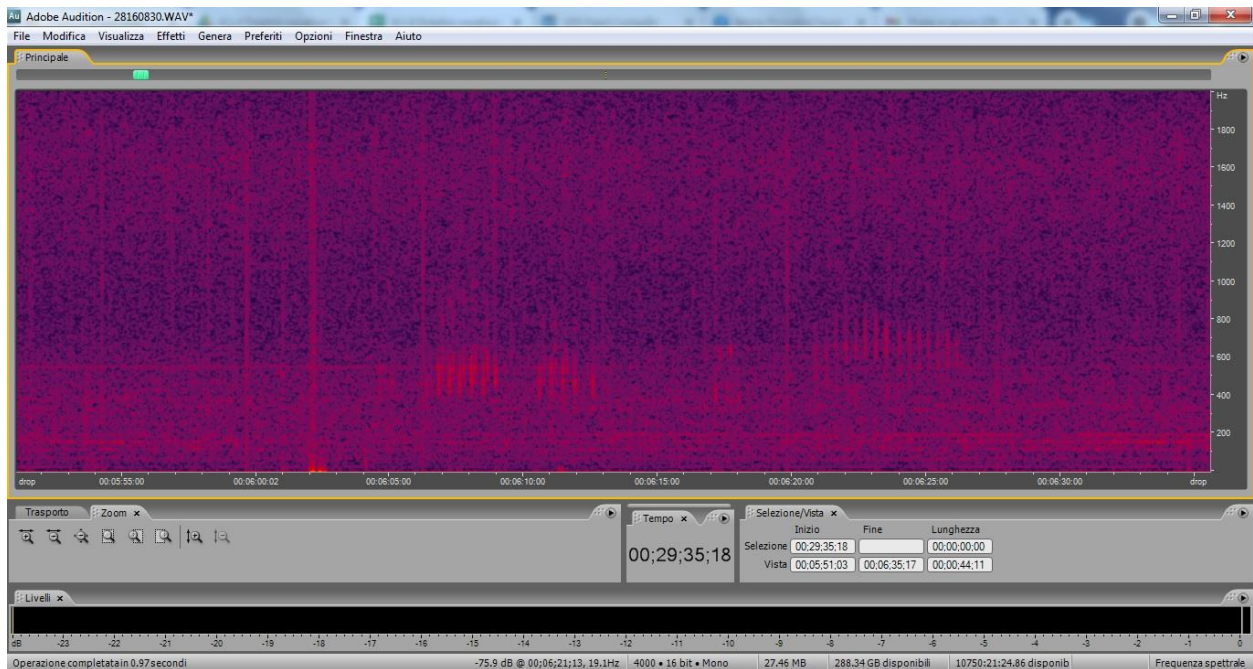


Figure 13. Sonogram related to low frequency narrow-band calls (LFN; Hanning window, FFT 512, resampled at 4000 Hz; displayed by Adobe Audition)

The hourly SPL data has been considered in two different frequency ranges, ie. 50-2000Hz and 2500-20000 Hz, corresponding to the LF (low frequency) and HF (high frequency) ACI ranges.

### 3.1 Winter data

Circadian trends related to the winter week-period are highlighted for the fish and LFN dolphin vocalization components (Figure 14), for the HF dolphin vocalization component (Figure 15) and for the man-made component (Figure 16).

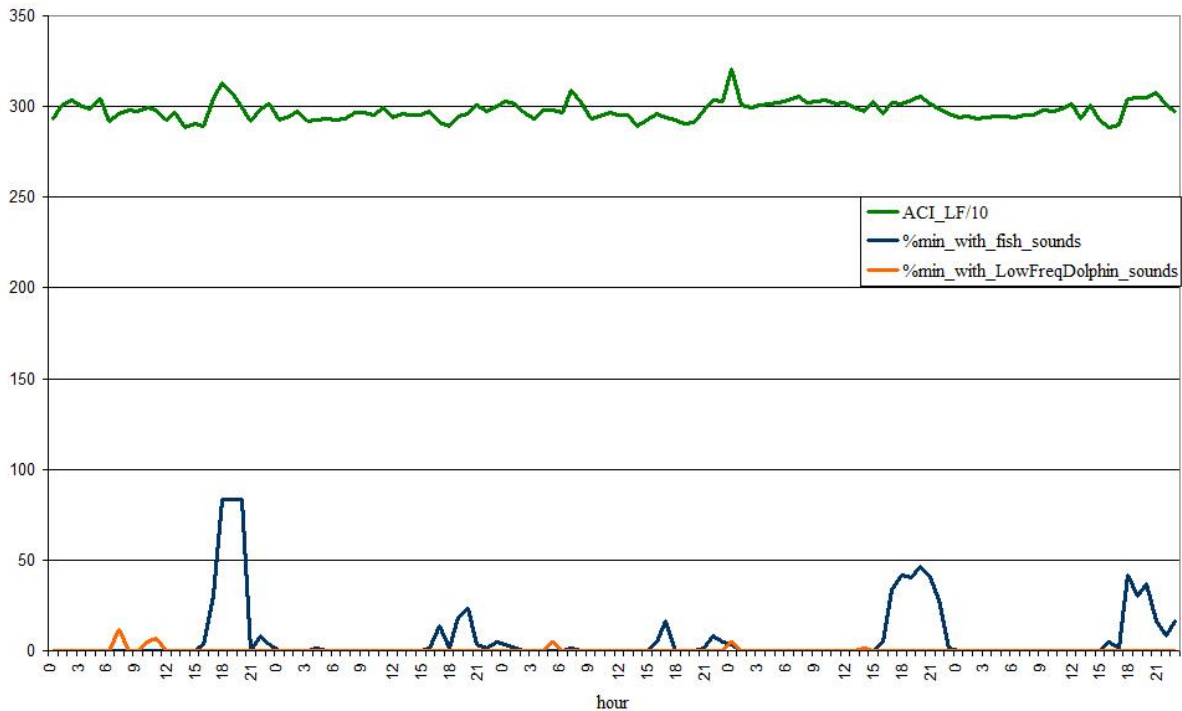


Figure 14. Comparisons between the (above) LF ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings) and (below) the percentage of files in the 1-h acoustic files containing fish vocalizations. ACI original values are divided by 10 for visual comparisons with the other variables.

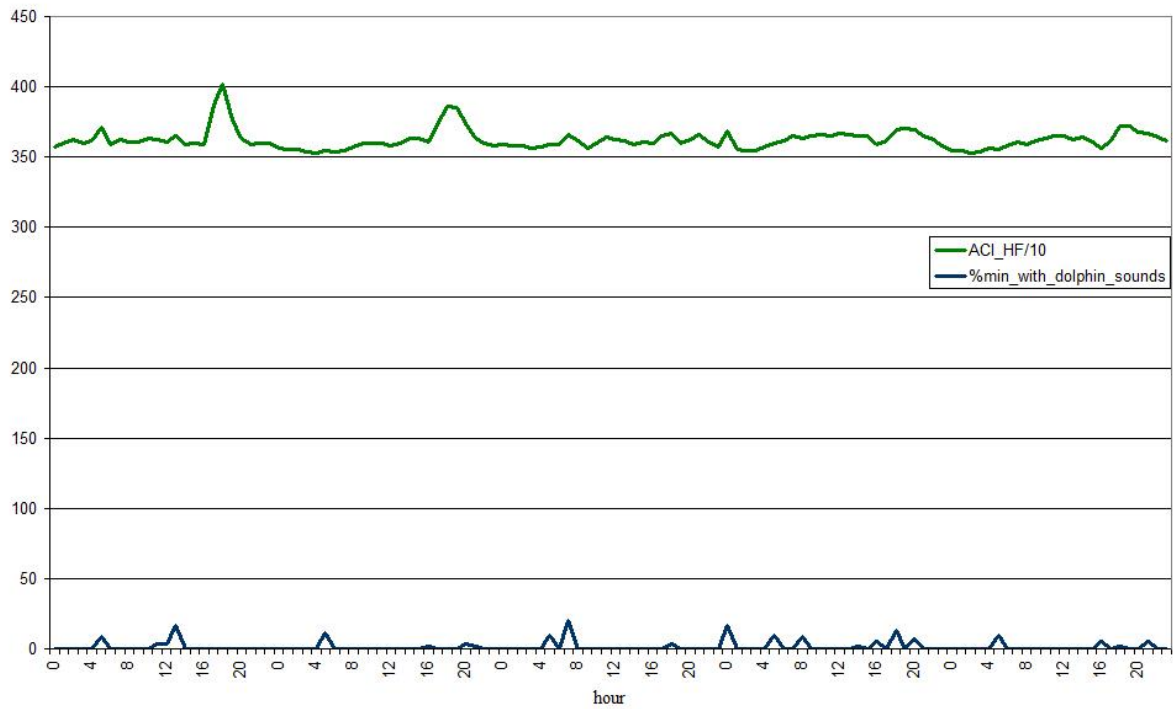


Figure 15. Comparisons between the (above) HF ACI 1-h values (by applying  $\Delta f=86/256$  FFT settings ) and (below) the percentage of files in the 1-h acoustic files containing dolphins vocalizations. ACI original values are divided by 10 for visual comparisons with the other variables.

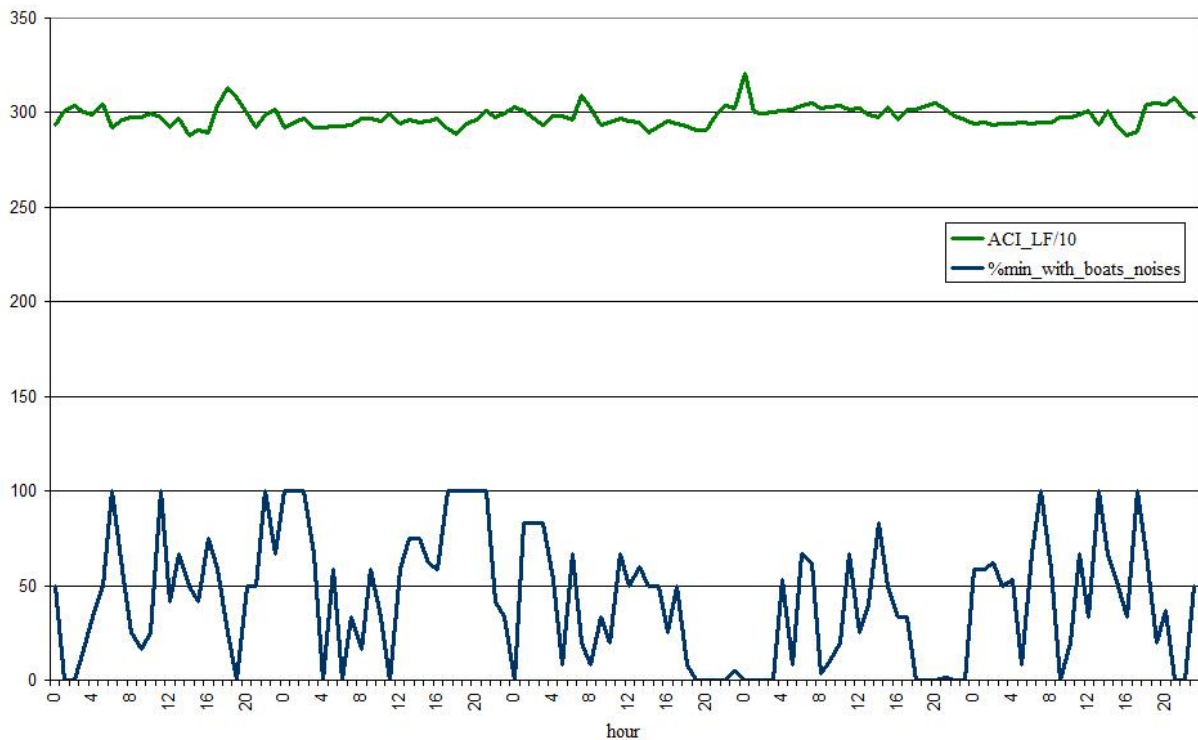


Figure 16. Comparisons between the (above) LF ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings) and (below) the percentage of files in the 1-h acoustic files containing boat noises. ACI original values are divided by 10 for visual comparisons with the other variables.

Fish sounds are produced mainly after the sunset (h 16-23) whereas both LF and HF dolphin vocalizations are more randomly distributed along the day. Boat noise is extremely present in the files reaching up to the 100% of the recording for some hours; interestingly it is present both at daytime and at nighttime.

The ACI calculated for LF using  $\Delta f=10,7/2048$  FFT settings was positively correlated with the percentage of minutes containing fish sounds (Spearman rank correlations,  $r_s = 0.34$ ,  $p\text{-value} = 0.000$ ,  $N=120$ ) and it was negatively correlated with the percentage of minutes containing boat noises (Spearman rank correlations,  $r_s = -0.35$ ,  $p\text{-value} = 0.00$ ,  $N=120$ ). No correlation has been found between LF ACI value and the percentage of minutes containing LFN dolphin sounds (Spearman rank correlations,  $r_s = 0.87$ ,  $p\text{-value} = 0.344$ ,  $N=120$ ).

In March the ACI calculated for HF using  $\Delta f=86/256$  FFT settings was positively correlated with the percentage of minutes containing HF dolphin sounds (Spearman rank correlations,  $r_s = -0.207$ ,  $p$ -value = 0.023,  $N=120$ ).

### 3.2 Summer data

Circadian trends related to the summer week-period are highlighted in figure 17 for the fish and LFN dolphin vocalization components, figure 18 for the HF dolphin vocalization component and figure 19 for the man-made component. Comparing with the winter case, it appears clear that circadian patterns are more evident during the summer for the fish, which are vocally active from about 17-18 to 4 in the morning (UTC), but not for the dolphin vocal activity; that is rare and randomly distributed along the day. Boat noise is mostly present during the daytime.

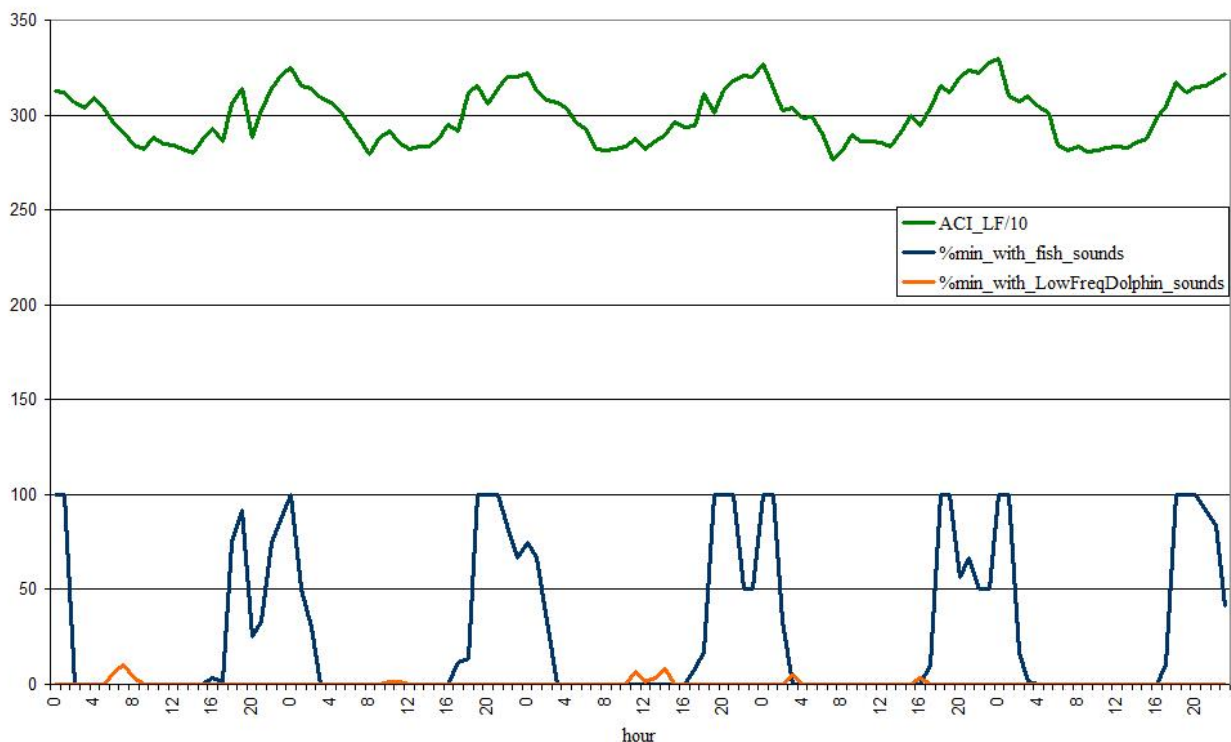


Figure 17. Comparisons between the (above) LF ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings) and (below) the percentage of files in the 1-h acoustic files containing fish vocalizations. ACI original values are divided by 10 for visual comparisons with the other variables.

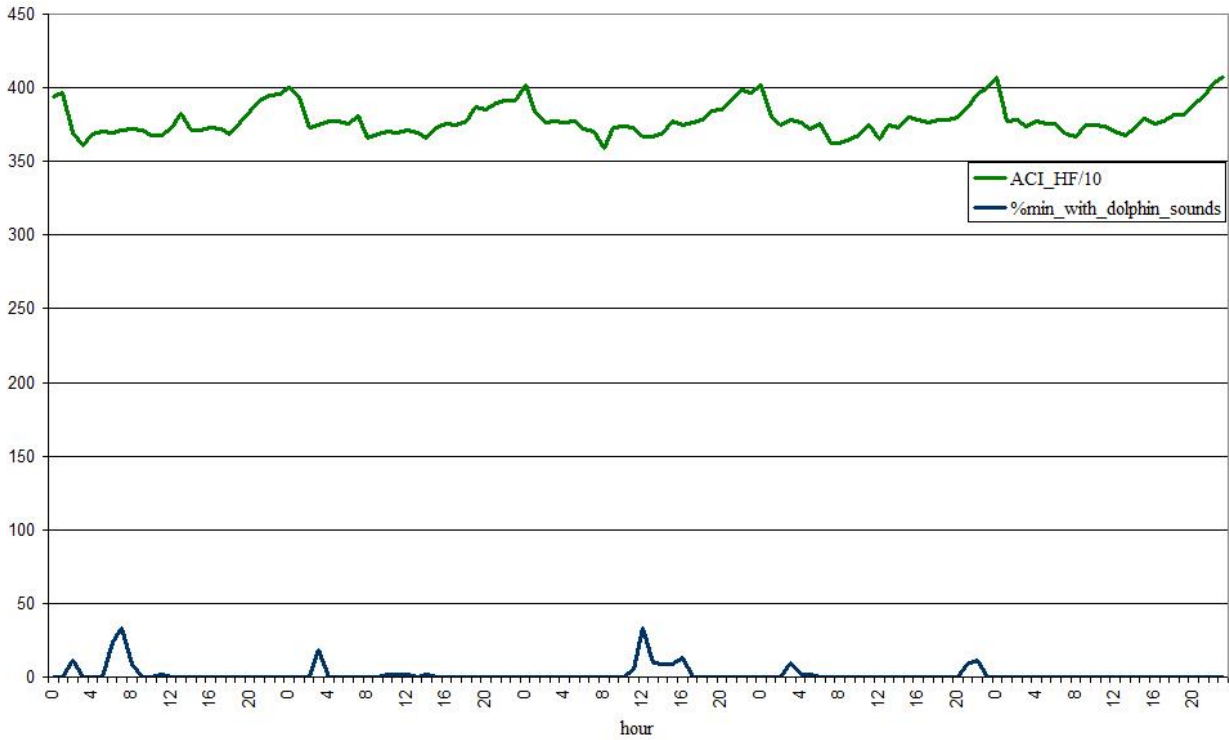


Figure 18. Comparisons between the (above) HF ACI 1-h values (by applying  $\Delta f=86/256$  FFT settings ) and (below) the percentage of files in the 1-h acoustic files containing dolphins vocalizations. ACI original values are divided by 10 for visual comparisons with the other variables.

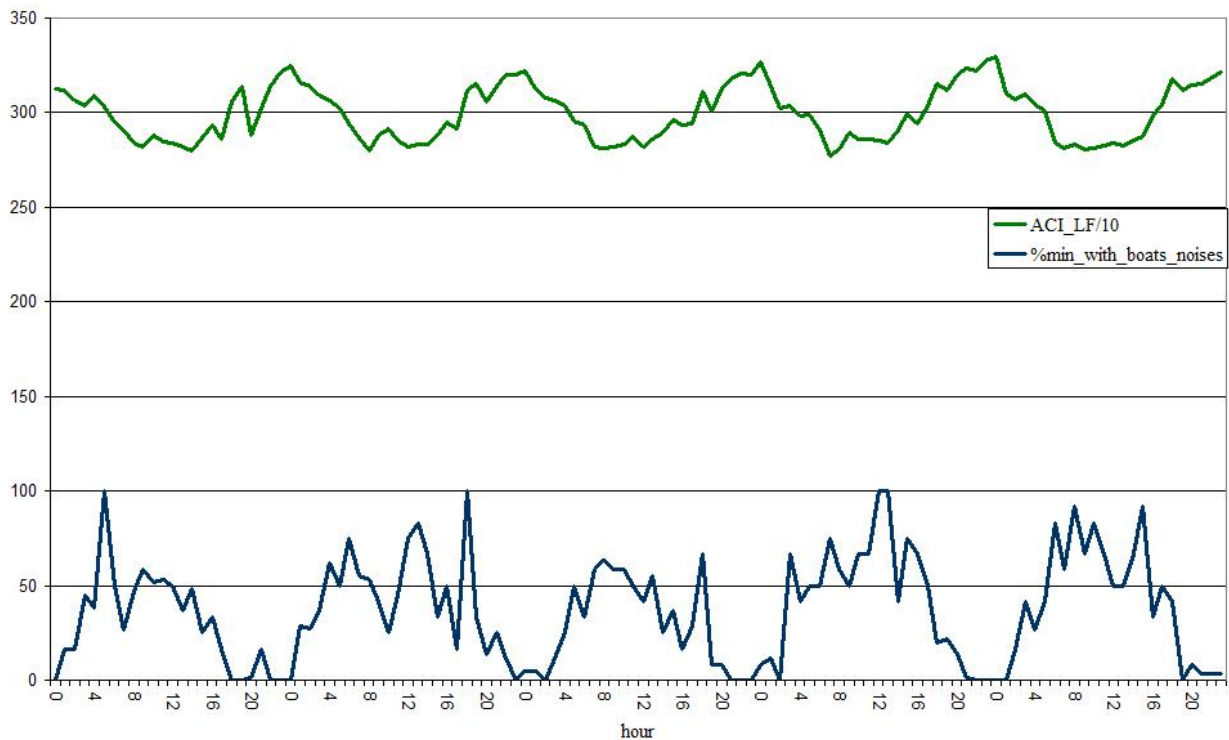


Figure 19. Comparisons between the (above) LF ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings) and (below) the percentage of files in the 1-h acoustic files containing boat noises. ACI original values are divided by 10 for visual comparisons with the other variables.

In July, the ACI calculated for LF using  $\Delta f=10,7/2048$  FFT settings was positively correlated with the percentage of minutes containing fish sounds (Spearman rank correlations,  $r_s = 0.76$ ,  $p\text{-value} = 0.000$ ,  $N=120$ ) and it was negatively correlated with the percentage of minutes containing boat noises (Spearman rank correlations,  $r_s = -0.71$ ,  $p\text{-value} = 0.00$ ,  $N=120$ ). A negative correlation has been found also between LF ACI and the percentage of minutes containing LFN dolphin sounds (Spearman rank correlations,  $r_s = -1.83$ ,  $p\text{-value} = 0.046$ ,  $N=120$ ); this is likely due to the higher abundance of LFN sounds during the hours of light, in contrast to the fish case.

In July, the ACI calculated for HF using  $\Delta f=86/256$  FFT settings was negatively correlated with the percentage of minutes containing HF dolphin sounds (Spearman rank correlations,  $r_s = -0.251$ ,  $p\text{-value} = 0.006$ ,  $N=120$ ) and not correlated the SPL 2500-20000 Hz values (Spearman rank correlations,  $r_s = -0.71$ ,  $p\text{-value} = 0.441$ ,  $N=120$ ).



### 3.3 Comparisons

When considering ACI values obtained on files collected in the same location for two different periods of the year, temporal variations related to seasonality and circadian rhythms are evident (Figures 20 and 21).

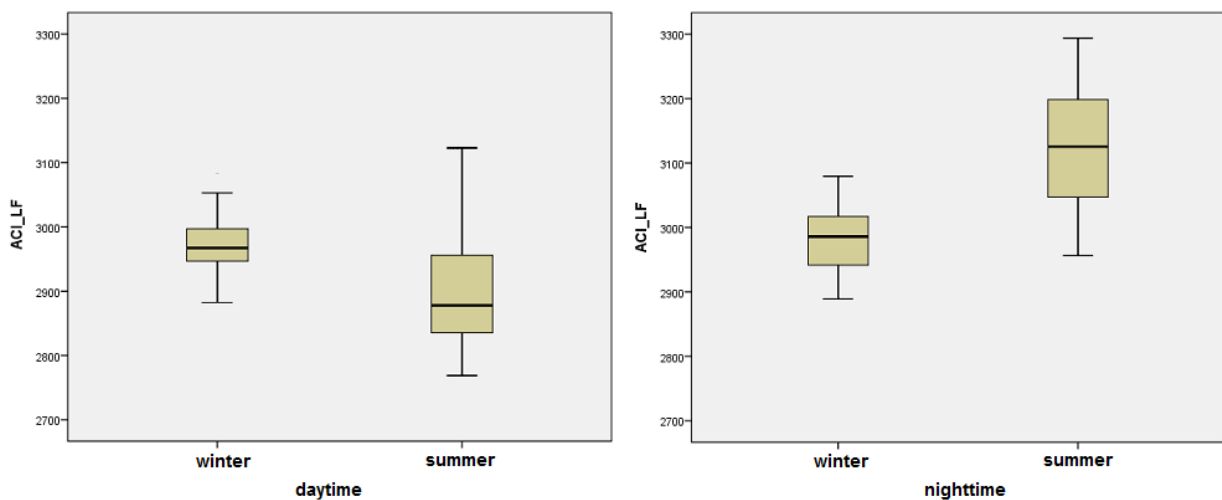


Figure 20. Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and maximum) of ACI LF measured on daytime and nighttime during the two considered seasons.

A significant variation in the ACI LF values between the considered seasons is found during both day and night (U-Mann Test,  $P=0.000$  for both cases). Interestingly, however, in summer the ACI LF values are lower than in the winter data during day ( $N=50$ ) but higher during night ( $N=75$ ).

In fact in winter data ACI LF does not change between day and night (U-Mann Test,  $P=0.178$ ), nor does the percentage of boat noise or LFN sounds in the recorded samples (average  $47 (\pm 4)$  vs.  $38 \% (\pm 4)$  of minutes with boat noises; U-Mann Test,  $P=0.075$ ; average  $0.5 (\pm 0.2)$  vs.  $0.15 \% (\pm 0.1)$  of minutes with LFN sounds; U-Mann Test,  $P=0.236$ ), although the percentage of fish sounds is significantly higher at night ( $2.3 (\pm 1)$  vs  $10.5 (\pm 3)$  % of minutes with fish sounds per recorded hour; U-Mann Test,  $P=0.004$ ).

On the opposite in summer a significant variation is evident in ACI LF values according to the circadian rhythm (U-Mann Test,  $P=0.000$ ), in accordance to the case of fish sounds that are present for an average of 16 % ( $\pm 4$ ) of the minutes per recorded hour during the day vs. an average of 45 % ( $\pm 6$ ) minutes per recorded hour during the night. The fish vocal component explains the above observed difference since LFN sounds are more abundant during the day than the night (0.6 ( $\pm 0.2$ ) vs 0.1 ( $\pm 0.1$ ) % of minutes with fish sounds per recorded hour; U-Mann Test,  $P=0.043$ ) and no variation in % minutes per recorded hour of boat noise presence can be highlight between day and night for the summer (U-Mann Test,  $P=0.691$ ).

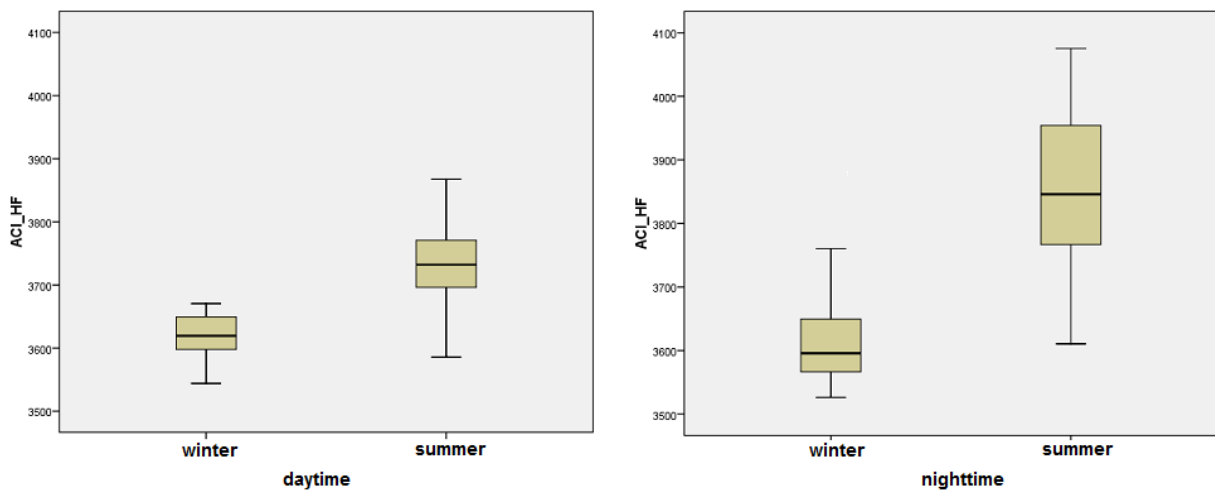


Figure 21. Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and maximum) of ACI HF measured on daytime and nighttime during the two considered seasons.

Also, in the case of ACI HF values a significant variation between the considered seasons is observed during both day and night (U-Mann Test,  $P=0.041$  for both cases). It can be excluded that this difference is related to the dolphin sounds percentage distribution since during winter no variation in this parameter is found between day and night (1.3 ( $\pm 0.5$ ) % vs 1.6 ( $\pm 0.4$ ) %; U-Mann Test,  $P=0.730$ ) and in summer the percentage of minutes with dolphin sound *per* recorded hour is higher during day vs. night (47 ( $\pm 3$ ) % vs 19 ( $\pm 3$ ) %; U-Mann Test,  $P=0.000$ ), showing therefore an opposite trend than ACI HF. It is rather likely that ACI HF responds to the snapping shrimp acoustic activity that typically peaks during sunset, night and sunrise (Bohnenstiehl et al 2017; Buscaino et al. 2016).

Table 4 show the variation of the considered parameters between the two periods irrespective of the recording daily time.

Table 4 Average  $\pm$  SD of the here considered parameters between the two seasons, irrespective from the recording daily time; statistical significant variation is further indicated

	ACI_LF	%min_fish	%min_boats	ACI_HF	%min_dolphins
March	2978 $\pm$ 5	6.7 $\pm$ 1.4	42.5 $\pm$ 2	3622 $\pm$ 6.5	1.4 $\pm$ 0.3
July	2995 $\pm$ 13	27.3 $\pm$ 3.5	1.7 $\pm$ 0.5	3780 $\pm$ 9.5	37 $\pm$ 1.4
Mann-Whitney U	7065,000	5623,500	1775,500	980,000	1391,000
P	,802	,001	,000	,000	,000

#### 4. Application of the ACI on weekly data in order to evaluate the spatial effect

5-days data has been considered for two different recording locations, one located inside the Losinj archipelago (Oruda island) and the other one at a station facing the Adriatic Sea (Susak island), along the summer period (26-30 July), for a total of 240 hours of recordings.

These two periods were analysed by computing LF and HF ACI 1-h value by applying both  $\Delta f=86$  (256 FFT) and  $\Delta f=10,7$  (2048 FFT) settings and by manually scrolling the files in order to highlight the presence of dolphins/fish vocalizations and/or boat noise presence. Further the hourly SPL data has been considered in two different frequency ranges, ie. 50-2000Hz and 2500-20000 Hz, corresponding to the LF (low frequency) and HF (high frequency) ACI ranges.

On the contrary to the case of Oruda recordings, in Susak fish sound types, which have been already described, are rarely found whereas few new sound types have been randomly spotted. Interestingly, very low frequency signals (Figure 22) are often present in the recordings. Dolphin HF and LF sounds have been depicted.

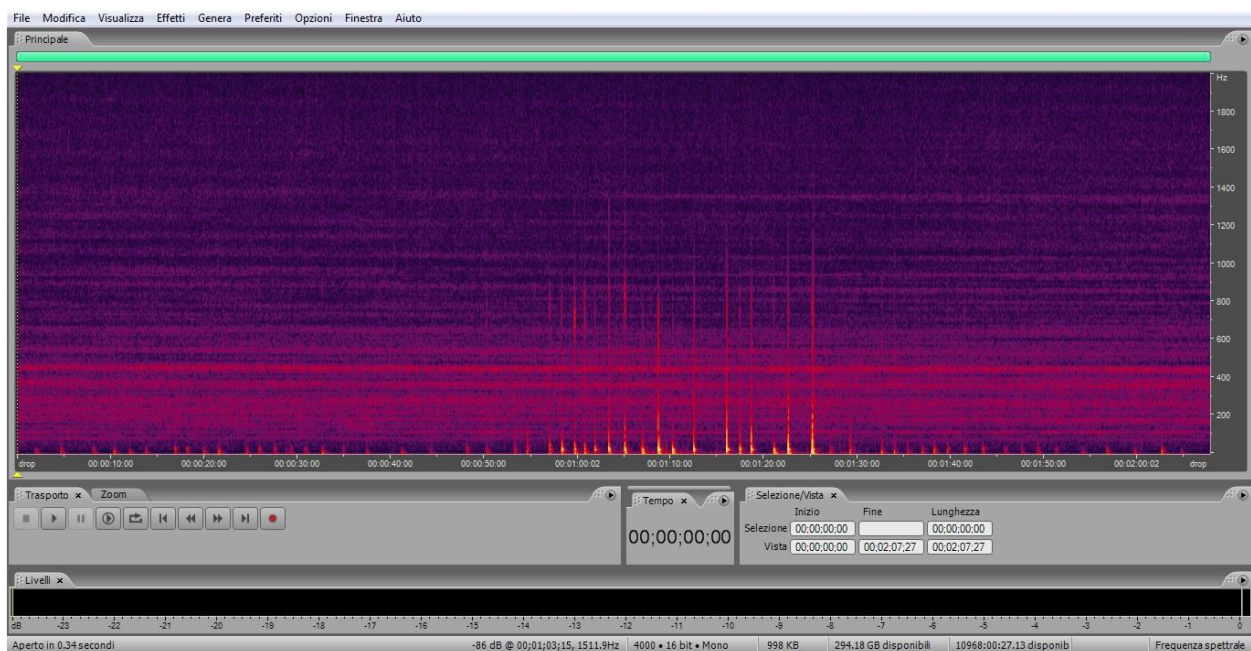


Figure 22. Sonogram related to low frequency signals (Hanning window, FFT 512, resampled at 4000 Hz; displayed by Adobe Audition)

Data related to Oruda location are described in 3.2 paragraph.

#### 4.1 Susak data

Circadian trends related to the summer week-period are highlighted in figure 23 for the fish and LFN dolphin vocalization as well as the low frequency signal components, in figure 24 for the HF dolphin vocalization component and in figure 25 for the man-made component. Comparing this case with the Oruda case, it appears clear that there is a lack of circadian patterns in the low frequency patterns (including fish sounds) as well as in the LF and HF dolphin vocalization component, that is rare and randomly distributed along the day. Boat noise is dominating the local soundscape both during the daytime and nighttime.

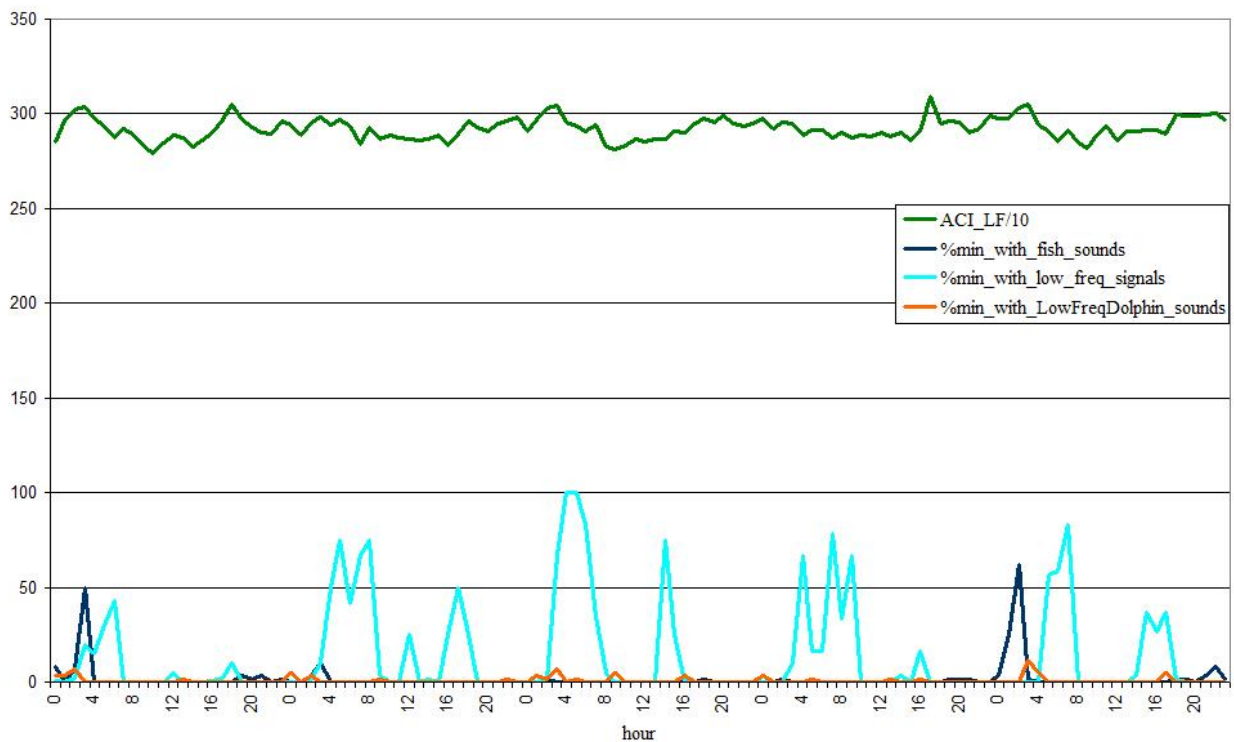


Figure 23. Comparisons between the (above) LF ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings) and (below) the percentage of files in the 1-h acoustic files containing fish vocalizations and low frequency signals. ACI original values are divided by 10 for visual comparisons with the other variables.

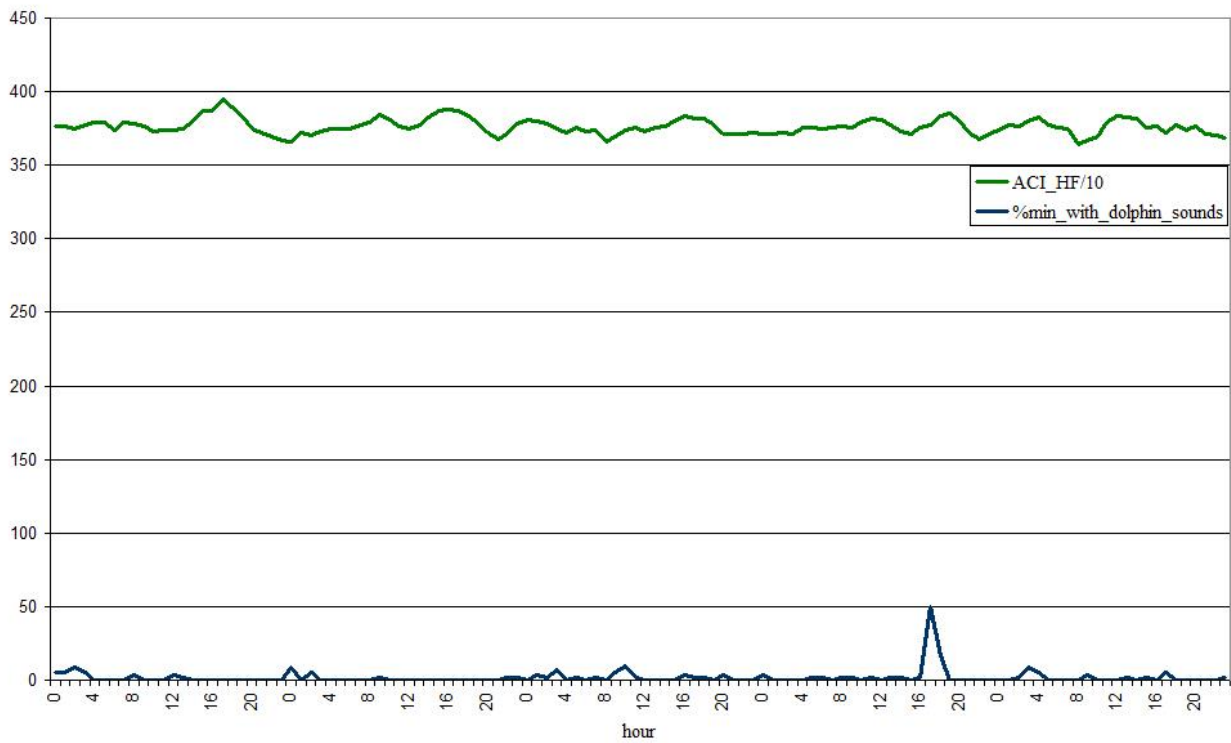


Figure 24. Comparisons between the (above) HF ACI 1-h values (by applying  $\Delta f=86/256$  FFT settings ) and (below) the percentage of files in the 1-h acoustic files containing dolphins vocalizations. ACI original values are divided by 10 for visual comparisons with the other variables.

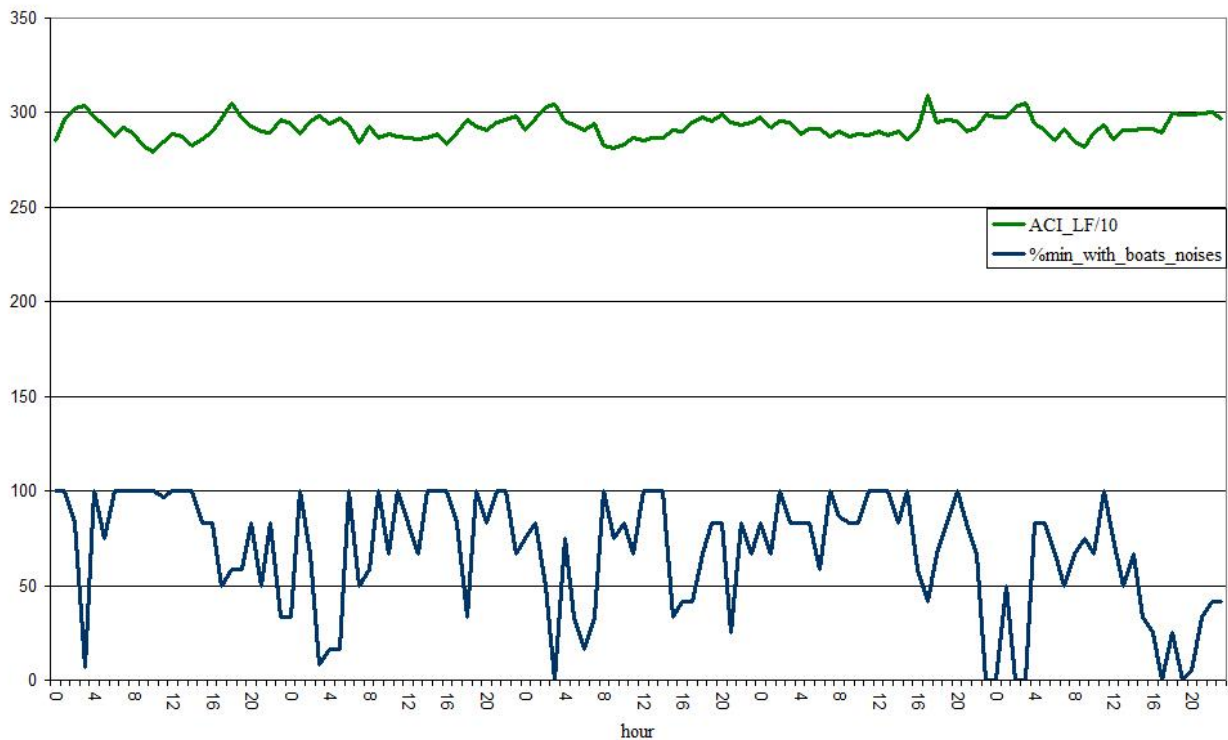


Figure 25. Comparisons between the (above) LF ACI 1-h values (by applying  $\Delta f=10,7/2048$  FFT settings) and (below) the percentage of files in the 1-h acoustic files containing boat noises. ACI original values are divided by 10 for visual comparisons with the other variables.

In July in Susak recordings, the ACI calculated for LF using  $\Delta f=10,7/2048$  FFT settings was positively correlated with the percentage of minutes containing fish sounds (Spearman rank correlations,  $r_s = 0.45$ ,  $p\text{-value} = 0.000$ ,  $N=120$ ) but not with the percentage of minutes containing low frequency signals (Spearman rank correlations,  $r_s = -0.38$ ,  $p\text{-value} = 0.682$ ,  $N=120$ ); it was also negatively correlated with the percentage of minutes containing boat noises (Spearman rank correlations,  $r_s = -0.52$ ,  $p\text{-value} = 0.00$ ,  $N=120$ ). No correlation has been found between LF ACI and the percentage of minutes containing LFN dolphin sounds (Spearman rank correlations,  $r_s = 0.126$ ,  $p\text{-value} = 0.169$ ,  $N=120$ ).

In Susak, the ACI calculated for HF using  $\Delta f=86/256$  FFT settings was not correlated with the percentage of minutes containing HF dolphin sounds (Spearman rank correlations,  $r_s = -0.14$ ,  $p\text{-value} = 0.877$ ,  $N=120$ ).

## 4.2 Comparisons

When considering ACI values obtained on files collected in the different locations for the same period of the year, spatial variations are evident (Figures 26 and 27).

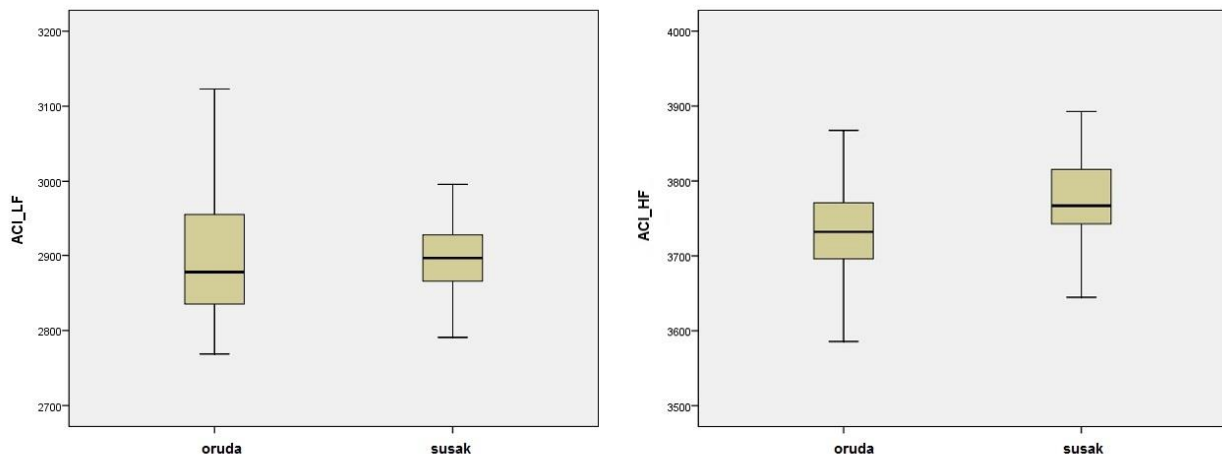


Figure 26. Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and maximum) of ACI LF and HF measured on daytime for the two considered locations.

Considering the daytime data, a significant variation in the the percentage of minutes containing fish sounds, HF dolphin sounds and boat noise has been found (U-Mann Test,  $P=0.000$  in all the cases,  $N=240$ ), with Oruda presenting more fish (16.62 ( $\pm 4$ ) % vs 0.2 ( $\pm 0.07$ )) and HF dolphin sounds (47.6 ( $\pm 3$ ) % vs 1.7 ( $\pm 0.7$ )) but less boat noise (2.02 ( $\pm 0.7$ ) % vs 73 ( $\pm 3.2$ )) than Susak. Abundance of LFN dolphin sounds does not differ between the two recording stations (U-Mann Test,  $P=0.393$ ).

Surprisingly, a significant variation in the ACI HF but not in the ACI LF values between the considered recording locations (U-Mann Test,  $P= 0.440$  and  $P=0.000$ , respectively,  $N=90$ ) has been observed. In its turn, the significantly lower average ACI HF value in the recording in Oruda -despite a significantly higher percentage of HF dolphin sounds - confirms that ACI HF cannot be used as a proxy of dolphin vocalizations. Less clear is the role of ACI LF, which positively correlated with fish sounds and negatively with boat noises in both locations, but it appears similar in its average value when comparing them.



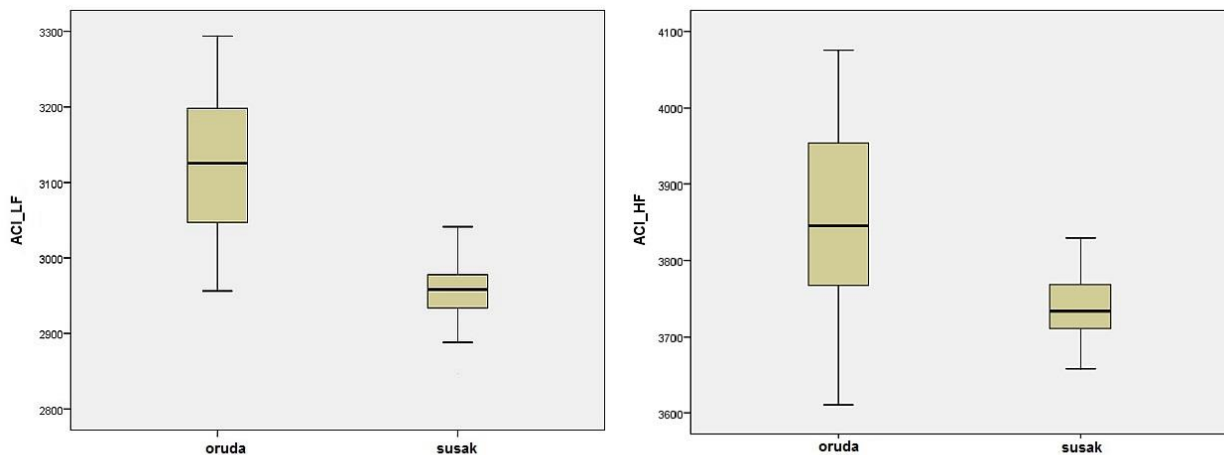


Figure 27. Box plot (central line: median; box limits: first and third quartile; whiskers: minimum and maximum) of ACI LF and HF measured at nighttime for the two considered locations.

In accord to the case of daytime, also during the nighttime a significant variation in the the percentage of minutes containing fish sounds, dolphin sounds and boat noise has been found (U-Mann Test,  $P=0.000$  in all the cases,  $N=240$ ), with Oruda presenting more fish ( $45.2 (\pm 6) \%$  vs  $4.3 (\pm 1.8)$ ) and dolphin sounds ( $19.7 (\pm 3.4) \%$  vs  $1.6 (\pm 0.3)$ ) but less boat noise ( $1.4 (\pm 0.5) \%$  vs  $58 (\pm 5)$ ) than Susak. Abundance of LFN dolphin sounds differ statistically between the two recording stations during summer (U-Mann Test,  $P=0.000$ ), with Oruda presenting less LFN dolphin sounds ( $0.1 (\pm 0.1) \%$  vs  $1.3 (\pm 1.3)$ ) than Susak.

A significant higher values for both the ACI HF and LF values has correspondingly been observed for Oruda than Susak locations (U-Mann Test,  $P=0.000$  in both cases,  $N=90$ ). Here the ACI variations correspond to the biological variation between the target areas. Still it cannot be excluded that the increase observed in the high frequency component is not influenced by the activity of the snapping shrimps, given that in both locations the HF ACI values were found to be not correlated with the percentage of minutes containing dolphin sounds.

Table 5 show the variation of the considered parameters between the two locations irrespective from the recording daily time.

Table 5 Average  $\pm$  SD of the here considered parameters between the two locations irrespective from the recording daily time; statistical significant variation is further indicated.

	ACI_LF	%min_fish	%min_boats	ACI_HF	%min_dolphins
Oruda	2995 $\pm$ 13	27 $\pm$ 3	1.7 $\pm$ 0.5	3780 $\pm$ 9.5	37 $\pm$ 2
Susak	2921 $\pm$ 5	1.7 $\pm$ 0.6	67 $\pm$ 2	3762 $\pm$ 5	1.7 $\pm$ 0.4
Mann-Whitney U	5525	4788	557	7169	1600
	0.002	0.000	0.000	0.954	0.000

## 5. Summary and general conclusions

The Acoustic Complexity Index (ACI) as set out in Pieretti et al. (2011) quantifies the relative change in sound intensity across all frequencies of a soundscape, while being minimally affected by constant anthropogenic noise. The ACI was developed on the assumption that with increased diversity of species, there would be an increase in the complexity of biological sound produced. On the other hand, ACI has been shown to have a number of drawbacks due to biophony, geophony or anthropophony. For example, the ACI has shown to be increased heavily by snapping shrimp, which produce a high intensity broadband ‘snap’, meaning an increased ACI when diversity has only marginally increased (McWilliam and Hawkins, 2013); in contrast, chorusing behaviour can heavily decrease ACI (Kaplan et al., 2015). ACI is also affected by geophony such as wind and rain (McWilliam and Hawkins, 2013) or by any sounds which are not repetitive or consistent in intensity sounds, such as boat engines (Pieretti et al., 2011).

The main goal of this study was to analyze the differences in the ACI response by taking into account different biological sound producers (fish and dolphins) in relation to temporal and spatial variabilities. The different sounds of these marine animals occupy differently the acoustic spectrum, reducing the overlap of signals along time and/or frequency dimensions. Given that most of the fish sounds are characterized by frequencies below 2 kHz (Amorim 2006) whereas the bottlenose dolphin emits sounds characterized by higher frequency range (Au, 2004), two different frequency bands have been considered, ie. low frequency band below 2 kHz (LF) and high frequency band above 2 kHz (HF). The choice to split the analysis based on the frequency bands of the principal biologic components recorded, helped the results interpretation.

Further two pilot studies have been considered in order to highlight the effects of the settings (i.e. spectral and temporal resolution of the ACI algorithm, amplitude filter) on the ACI output, being the ACI strongly influenced by all settings chosen prior to its calculation (Bolgan et al. 2018).

The variable results obtained by applying different settings on the same recordings processed at hourly and daily levels confirms that the application of acoustic indices alone, without a knowledge of the type of signals present in a specific site, might result in interpretations that do not accurately reflect the biophony of the area. The application of a validation procedure, such as the one presented in this study with a subsample of the data on which manual scrolling has been carried out, is needed in order to assure the quality of the information extrapolated by the acoustic index.

Here the best available *ad hoc* setting configuration chosen for the ACI to be representative of variation in fish and dolphin sound abundance results by applying the  $\Delta f=10,7$  (sampling rate 44.1 kHz, FFT 2048 Hanning) and  $\Delta f=86$  (sampling rate 44.1 kHz, FFT 256 Hanning) settings for the LF and HF ACI, respectively.

The interpretation of the information provided by the ACI was therefore carried out by using these specific settings for each of the considered situations.

The ACI analysis was applied on 2 set of 5-days data related to two different conditions:

a recording site (Oruda, Losinj archipelago, Croatia) evaluated both during summer and during winter time and two recording sites (Oruda and Susak, Losinj archipelago, Croatia) monitored during the summer period, when the biophony is usually higher in temperate water.

In order to compare the ACI outputs with the real biophony, the latter has been evaluated by manually scrolling 360 hours of recordings. The abundance of both the biophony and the anthropophony (here generated by boat noises) has been calculated in terms of the percentage of minutes containing the tested component per hour of recording. Further the SPLs (dB re 1 uPa) for both LF and HF have been calculated.

Analysis of the acoustic data revealed that the low-frequency (0.1–2 kHz) soundscape in Oruda was dominated by two single call type: the Roche's snake blenny *Ophidion rochei* (Kever et al. 2016) and one sound type (see Figure 6), whose emitting species is unknown although it has been previously briefly described by Bolgan et al. (2020). These vocalizations were present in both seasons but they were a massive acoustic presence in summer, showing a clear circadian pattern. On the contrary fish vocalizations were rare and the chorus was absent in Susak, here evaluated for the summer period. Snapping shrimps were typically present everywhere.

Different types of sound produced by bottlenose dolphins were found: broadband impulsive signals (clicks/burst), ranging from a few kHz up to 120 kHz, used to explore the surrounding environment (Au, 2004), and modulated narrowband whistles used for individual recognition, contact maintenance and group coordination (Janik and Sayigh, 2013); both LF and HF dolphin sounds were depicted at both sites but no evident circadian rhythm could be highlight.

In all the considered tested situations the ACI calculated for LF using  $\Delta f=10,7/2048$  FFT settings was found to be positively correlated with the percentage of minutes containing fish sounds and negatively correlated with the percentage of minutes containing boat noises. On the other hand, no correlation or negative correlation has been found between LF ACI and the percentage of minutes containing LFN dolphin sounds.

More in detail, temporal variations related to seasonality and circadian rhythms were evident when considering ACI values obtained on files collected in the same location for two different periods of the year. In particular the circadian rhythm of the fish vocal component drives the ACI variation during summer, so that ACI LF values represent a good proxy for the fish biophony in this period of the year. This is confirmed when comparing the ACI LF values between locations, since higher values have been found

in Oruda, characterized in summer by higher fish vocal and lower boat noise components compared to Susak. As a final remark, it has to be reminded that, when the ACI is applied to fish vocal communities, it does not discriminate between sound abundance and sound diversity, as was the case of the two sound types in the present study.

On the other hand, the ACI calculated for HF using  $\Delta f=86/256$  FFT settings was found to respond differently to the abundance of HF dolphin vocalizations according to recordings that differ in space and time, since this correlation in Oruda was positive in March but negative in July whereas no correlation was found between the two variables in July in Susak. The ACI HF values are known to be affected by the acoustic activity of the snapping shrimps that present circadian and seasonal trends, increasing during the summer and from dawn to dusk (Buscaino et al 2016). This could be a variable that affects the observed results but it has not been quantitatively evaluated in the present study.

Overall these considerations support the finding that neither ACI LF nor ACI HF *per se* does provide a sufficient and valuable tool to evaluate dolphin sound abundance in the study area.

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