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IN

BIOLOGIA MARINA

Studio del paesaggio sonoro sottomarino nell'area antistante il Parco del Conero (sito Natura 2000, Mar Adriatico)

Investigation of the underwater soundscape in front of the Conero Park (Natura 2000 site, Adriatic Sea)

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ABSTRACT

Underwater sound plays an important role for marine organisms, as it allows efficient intra- and interspecific short- and long-range communications, overcoming the transmission of signals of other kinds, such as light or chemical ones that are easily dispersed. The study of marine acoustics is therefore essential for a correct understanding of the communication dynamics that are established between organisms and between them and the surrounding environment. Passive Acoustic Monitoring (PAM) could be a good non-invasive method for short- and long-term monitoring of any ecosystem, including the most inaccessible systems. This study focuses on the application of an acoustic index, called ACI (Index of acoustic complexity), which is based on the difference between the intensity of the sounds between one temporal step and the next. The underlying theory emphasizes that sounds of biological origin (e.g., vocalizations of fish) have an intrinsic variability of intensity between a sound emission and the subsequent, instead of sounds of abiotic origin (anthropogenic or linked to weather conditions) that have no variability and are constant over time. Consequently, from the ACI calculation we should get higher values for files with

biological sounds than the files without them. The ACI index was applied to audio recordings made with a hydrophone placed in front of the Natural Park of Monte Conero by the CNR-IRBIM of Ancona, in the context of a European Union project, SOUNDSCAPE, focused on monitoring noise pollution. Thanks to these data, ACI was analysed from several records to investigate its seasonal and daily variability.

The results show that the index provides higher values on audio containing biotic sounds (vocalizations of fish) than audio with very limited presence of organisms. Moreover, it can be noticed how the audio of the summer months have higher ACI values than in the winter months, confirming the presence of greater vocalizations during the summer due to the reproductive period. Similarly, summer night-time audio has higher ACI values than day-time audio, as vocalizations of many species are more concentrated at night. The use of this index could represent a useful complementary tool to investigate marine biodiversity, by improving our knowledge on biological sounds and their spatial and temporal changes, potentially also due to human and climate-induced impacts. .

RIASSUNTO IN ITALIANO

Il suono in mare ricopre un ruolo di notevole importanza per gli organismi marini, poiché permette efficienti comunicazioni intra- e interspecifiche a corto e a lungo raggio, con una efficienza di trasmissione maggiore di segnali di altro genere, come quelli luminosi o chimici che si disperdono più facilmente. Lo studio dell'acustica marina pertanto risulta essenziale per una migliore comprensione delle dinamiche comunicative che si instaurano fra gli organismi e fra questi e l'ambiente circostante. Il monitoraggio acustico passivo (PAM) può rappresentare un utile metodo non invasivo per il monitoraggio a breve e lungo termine di qualsiasi ecosistema, compresi quelli maggiormente inaccessibili. Questo studio è incentrato sull'applicazione di un indice acustico, denominato ACI (Indice di complessità acustica) che si basa sulla differenza fra le intensità dei suoni fra un intervallo temporale e il successivo. La teoria alla base sottolinea come i suoni di origine biologica (es. vocalizzi di pesci) abbiano una intrinseca variabilità di intensità fra un'emissione sonora e la successiva, a differenza invece dei suoni di origine abiotica (antropogenica o legata alle condizioni meteo-marine), che non hanno variabilità e risultano costanti nel tempo. Di conseguenza dal calcolo ACI si dovrebbero ottenere valori più alti per file con suoni biologici rispetto a file senza di essi. L'indice ACI è stato applicato su registrazioni audio effettuate dal CNR-IRBIM di Ancona, con un idrofono posizionato di fronte al Parco Naturale del Monte Conero nell'ambito di un progetto europeo, SOUNDSCAPE, incentrato sul monitoraggio dell'inquinamento acustico. Grazie a tali dati, l'ACI è stato analizzato in diverse registrazioni per valutarne la sua variabilità stagionale e giornaliera. I risultati mostrano come l'indice fornisca valori più alti su audio contenenti suoni biotici (vocalizzi di pesci) rispetto ad audio in cui la componente biologica è molto limitata. Inoltre, si può notare come gli audio dei mesi estivi abbiano valori ACI più alti rispetto ai mesi invernali, a conferma della presenza di maggiori vocalizzi durante l'estate, plausibilmente legati al periodo riproduttivo. Allo stesso modo, gli audio delle ore notturne estive presentano valori ACI maggiori degli audio delle ore diurne, in quanto i vocalizzi di molte specie si concentrano maggiormente di notte. In conclusione, l'uso di questo indice potrebbe rappresentare un utile complemento per lo studio della biodiversità, consentendo di approfondire le conoscenze sui suoni biologici e lo loro variazioni spazio-temporali, potenzialmente connesse anche alle attività antropiche e ai cambiamenti climatici in atto.

1. INTRODUCTION

1.1 Characteristics of the sound

A propagating sound wave consists of alternating compressions and rarefactions of molecules within an elastic medium (liquid or gas or solid), which are detected by a receiver as changes in pressure. Acoustic waves are characterized by different properties including the amplitude, the frequency, the wavelength, the speed, and the intensity (Fig. 1.). The amplitude (a) of a sound wave is proportional to the maximum distance a vibrating particle is displaced from rest, i.e., the peak pressure reached in one cycle. The frequency (f) of a sound wave is the rate of oscillation or vibration of the wave particles, i.e., the rate that pressure cycles from high to low and again to high. Frequency is measured in cycles/s or Hertz (Hz). The wavelength (λ) of a wave is the distance between two successive compressions or the distance the wave travels in one cycle of vibration. The speed (c) of a wave is the rate at which vibrations propagate through an elastic medium and is characteristic of that medium. Consequently, the speed of sound in water is approximately 1500 m s^{-1}, while the speed of sound in air is approximately 340 m s^{-1}.

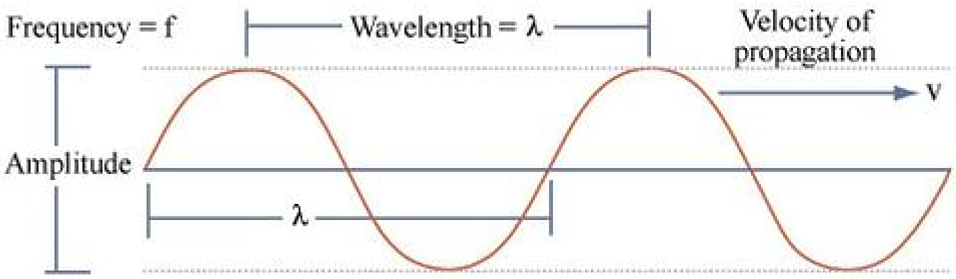


Figure 1.A graphic representation of a sound wave (https://www.tamingthesru.com/physics/)

The speed of sound in water will depend on the density of the water column, which is a function of temperature (t), depth (pressure) and salinity. As temperature decreases with increasing depth, speed decreases. A change in temperature of 1°C, results in a 3 m s^{-1} in speed. A change in salinity of 1 result in a 1.3 m s^{-1} change in speed. As pressure increases with depth, speed increases. Below the Sound Speed Minimum (Vmin) pressure has the greatest influence on speed, as temperature and salinity remain constant at depth. A change in pressure of 1 Pa results in a 1.7 m s^{-1}change in speed.

The intensity of a sound is defined as the acoustical power per unit area in the direction of propagation, i.e., intensity is a measure of the mechanical (kinetic) energy and potential energy carried by a propagating wave per unit area. Intensity is proportional to the square of the acoustic pressure.

$$I = \propto p^2$$

Where acoustic pressure (p) is defined as the sound force per unit area, it is usually measured in micropascal (µPa) (Simmonds et al., 2004).

1.2 Importance of underwater sounds

The soundscape of the ocean is characterized by three different types of sound sources called geophony, biophony, and anthrophony. The underwater sound is an important component of the ocean life. Marine animals have sensory systems used for orientation, feeding, predator detection, navigation, and social interactions (Williams et al., 2015). Sound propagates faster and further in water than in air, allowing efficient vocal communication between animals, that covers spatial scales larger than other sensory signals such as light or chemicals. The acoustic channel is the primary mode available for social interactions. Consequently, marine organisms have evolved several receptors to detect sounds (Duarte et al., 2021). The great majority of sounds emitted by fish are produced in a social context and involve interaction between individuals. Sound production commonly occurs in fish when an individual is disturbed by a predator or subjected to a noxious stimulus, as shown by the gurnard (*Trigla lucerna)* often accompanied by a strong visual display (Hawkins, 1986). In gobies, vocalizations are very important for the courtship of the partner. Some results suggest that painted goby females select males on the basis on their vocal capacity (Amorim et al., 2013) .

1.3 Geophony

The geophony includes all sounds produced by natural phenomena. Several hydrodynamic processes continuously occur in the ocean, even at zero sea state (for example in case of reduced turbulence of the wave motion), producing different types of sound. The ambient noise is composed by turbulent-pressure fluctuations effective in the band 1 cps to 100 cps (cycles per second, 1 cps = 1 Hz, 1 Hz to 100 Hz) and wind-dependent noise from bubbles and spray resulting, primarily, from surface agitation, 50 cps to 20 kcps (50 Hz to 20 kHz). The wind stress can be also correlated to the ice-cover, as in the Southern Ocean. The increase of the sea-ice concentration, area, and thickness, determines a gradual sound level decrease. Contrary, highest spectral levels occur in ice-free conditions (Menze et al., 2017). Precipitation is also a basic noise deriving from a spray of water droplets (rain) and rigid bodies (hail). The effects of precipitation are most noticeable at frequencies above 500 cps (Hz) but may extend to as low as 100 cps (Hz) if heavy precipitation occurs when wind speeds are low (Wenz, 1962). In addition, the tectonic activity in the earth's crust (volcanoes and earthquakes) generates natural noise (Simmonds et al., 2004) (Harland & Harland, 2017).

1.4 Biophony

The biophony concerns biotic sounds that have been recorded on a large scale of phylogenetically different organisms, from small invertebrates such as the snapping shrimp, *Alpheus heterochaelis* (Say, 1818) (Au & Banks, 1998b), to big cetaceans (Adam, 2009a; Amorim et al., 2015; di Iorio et al., 2012). Biogenic sounds have two different forms, a passive form to listen and orient in the environment and an active one as they produce sound to communicate or to search for prey or for objects (e.g., Eco-localization). The passive communication is firstly used to hear conspecifics for communication purposes, but it also allows an efficient perception of the surrounding environment to perceive the noise or the sounds of a possible predator approaching or the presence of a prey. Consequently, the range of frequencies audible by a species is often wider the range of frequencies emitted (Pavan, 2015) and biotic emission frequencies ranging from ultrasound to infrasound. The active communication could be between partners during the breeding seasons or between individuals belonging to the same population as a guide for the migration. Social functions proposed for baleen whales' sounds include long-range contact, assembly calls, sexual advertisement, greeting, spacing, threat, and individual identification (Dudzinski et al., 2009).

In some cases, the use of sound is a by-product of other animals' activity such as feeding or nest building (Boyd et al., 2011).

1.4.1. Use of sound in Vertebrates

1.4.1.1. Role of sound in marine mammals

Cetaceans communicate within and between species in a variety of ways, along long distances, and most of this communication is in the form of acoustic signals. Marine mammal vocalizations cover a very wide range of frequencies, from < 10 Hz to > 200 kHz (Simmonds et al., 2004a). Cetacean communication has a variety of functions such as: intrasexual selection; intersexual selection; mother/calf cohesion; group cohesion; individual recognition; danger avoidance. In Odontocetes, acoustic signals can be divided into three categories: "impulsive sounds" (i.e., broadband clicks), "squeaks" or "whistles," which are narrowband and "complex sounds," being some combination of these two categories (Lammers et al., 2003). Vocalizations of baleen whales are significantly lower in frequency than are those of Odontocetes; frequencies are rarely above 10 kHz. Their sounds can be categorized as low-frequency moans; simple calls (impulsive, narrowband) complex calls (broadband pulsatile AM or FM signals); and complex "songs," in some cases with regional and interannual variations in phrasing and spectra.

Except for the vocalizations of baleen whales, which can be detected for hundreds of kilometres, the contributions of marine mammals to the ocean sound ambient are localized in space. There is diurnal and seasonal variability in the occurrence of vocalizations, although in some locations marine mammal sounds are consistent features of the ambient (Reidenberg, 2017; Payne & Webb, 1971; van Opzeeland & Hillebrand, 2020).

Not only cetaceans but also sirenians communicate by sound emission. Manatees (*Trichechus spp.*) and dugongs (*Dugong dugon*) possess a rich repertoire of sounds. In pinnipeds most of the signals of aquatic mating species occur in the water, whereas terrestrial breeding pinnipeds produce a wide variety of vocalizations while hauled out. These signals are often loud, directional, broadband, and highly repetitive (Frankel, 2009).

1.4.1.2. Role of sound in fish

Sound production commonly occurs in fishes for many purposes. Firstly, during reproductive activity where the calls may directly influence the behaviour of prospective mates. Sometimes the sounds accompany complex visual displays as a part of a more complex signalling system. Often the calls are produced by the male fish, which in many cases shows territorial behaviour. However, there are numerous examples of fish producing sounds while competing for example for food or space. Where fish swim in coordinated groups or schools, the motions set up in the water by the swimming fish may well be important in maintaining the cohesion of the school under poor visual conditions. One disadvantage in using sound to promote cohesion of the school once assembled is that predators may intercept the sounds, perhaps eliminating any anti-predator advantages provided by the shoaling habit. Various studies have shown that predatory fish, and especially sharks, may horne in on the incidental sounds produced by struggling or injured prey (Larsson, 2014).

Many of these sounds involve striking two bony structures against one another. The swim bladder, an organ located in the abdominal cavity of most fish that contains air and regulates buoyancy, amplifies the fundamental frequency, and matches the impedance of the sound to water (Fig. 2). As a result, sounds produced by fish are pulsed signals with the energy mostly below 1 kHz. The major contributions are from those species that participate in chorusing behaviour. Biological choruses occur when many animals are calling simultaneously. Fish choruses are known to increase the ambient noise levels in certain locations, at certain times of the day, for example, the "sunset chorus" that lasts for a few hours after sundown and at certain times of the year (often the spring and early summer months) by 20 dB or more in the 50 Hz to 5 kHz band over sustained periods of time (National Research Council, 2003).

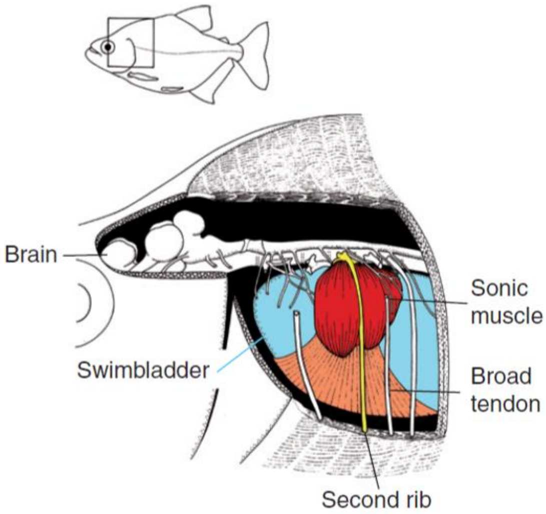


Figure 2. Line drawing of lateral view showing swim bladder region of a black piranha (Serrasalmus rhombeus) (Ladich, 2011))

1.4.2. Use of sound in Invertebrates

Small invertebrates are less charismatic than large vertebrates and for this reason there are few studies on them and their ecology. Nevertheless, invertebrates such as small crustaceans play an important role in the soundscape, especially in the benthic ecosystem, where there is low-light condition. The acoustic soundscape of the benthic zone is a cacophony of snaps, squeaks, hums, grunts, and rasps produced by animals such as snapping shrimp (Au & Banks, 1998), clawed lobsters (Henninger & Watson, 2005), hermit crabs (Jansson, 1972).The acoustic modality can play important roles: attracting mates, repelling rivals, deterring predators, or maintaining territories. From the literature it is highlighted a diurnal variability of sound production, as in *Hemisquilla californiensis* (Stephenson, 1967), which is most active during crepuscular periods (Staaterman et al., 2011).

1.5 Anthrophony

Anthrophony describes all noises produced by anthropogenic activities. Anthropogenic noises are generated by a variety of sources, and they are classified in impulsive and non-impulsive sounds. Impulsive noise produces sounds that are typically transient, brief (less than 1 second), broadband, and consist of high peak sound pressure with rapid rise time and rapid decay (Fisheries, 2018). There are two types of impulsive sound:

- Single pulse: single acoustic event with a > 3 dB difference between received level using impulse vs equivalent continuous time constant. Example: single explosion, sonic boom, single airgun, water gun, pile strike, or sparker pulse, single ping of certain sonars, depth sounders, and pingers.

- Multiple pulses: multiple discrete acoustic events within 24h with > 3 dB difference between received level using impulse vs equivalent continuous time constant. Example: serial explosions, sequential airgun, water gun, pile strikes, or sparker pulses, certain active sonar (IMAPS), some depth sounder signal.

Contrary, non-impulsive noise produce sounds that can be broadband, narrowband, or tonal, brief, or prolonged, continuous, or intermittent and typically do not have a high peak sound pressure with rapid rise/decay time that impulsive sounds do (Fisheries, 2018).

- Non-pulses sounds are defined as a single or multiple discrete acoustic events within 24h < 3 dB difference between received level using impulse vs equivalent continuous time constant. Example: vessel/aircraft passes; drilling, infrastructure constructions or other industrial operations, certain sonar system (LFA, tactical mid-frequency), acoustic harassment/deterrent devices, acoustic tomography sources (ATOC), some depth sounder signal (Southall et al., 2008).

  Analysis of noise from ships revealed that their propulsion systems are a dominant source of radiated underwater noise at frequencies < 200 Hz (Ross D, 1976). Cavitation at the propeller blade tips was found to be a significant noise mechanism across all frequencies, though the higher frequencies do not propagate far. Cavitation occurs when the local static pressure drops below a critical value; it can be characterized as boiling of the seawater, brought about by static pressure decrease. Cavitation noise includes both broadband noise due to bubble collapse, and tonal components that are related to blade passage frequency and higher harmonics. Additional sources of ship noise include rotational machinery that produces tones, and reciprocating machines that produce sharp pulses at a constant repetition rate, resulting in multiple harmonics of the repetition frequency. On average, noise levels were found to be higher for the larger vessels, and increased vessel speeds resulted in higher noise levels (Hildebrand, 2009).

1.6 Descriptor 11 of the MSFD

The Marine Strategy Framework Directive (MSFD) (2008/56/EC) (MSFD) requires European Member States to develop strategies for their marine waters that should lead to programmes of measures that achieve or maintain Good Environmental Status (GES) in European Seas.

Good Environmental Status means ‘the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy, and productive within their intrinsic conditions, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations’.

To assess the status of their marine waters, European Member States must follow 11 high-level criteria described as ‘qualitative descriptors’ in the Directive:

1. Biological diversity is maintained. The quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic, and climatic conditions.
2. Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems.
3. Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock.
4. All elements of the marine food webs, to the extent that they are known, occur at normal abundance and diversity and levels capable of ensuring the long-term abundance of the species and the retention of their full reproductive capacity.

5. Human-induced eutrophication is minimised, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms, and oxygen deficiency in bottom waters.
6. Sea-floor integrity is at a level that ensures that the structure and functions of the ecosystems are safeguarded and benthic ecosystems, in particular, are not adversely affected.
7. Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems.
8. Concentrations of contaminants are at levels not giving rise to pollution effects.
9. Contaminants in fish and other seafood for human consumption do not exceed levels established by Community legislation or other relevant standards.
10. Properties and quantities of marine litter do not cause harm to the coastal and marine environment.
11. Introduction of energy, including underwater noise, is at levels that do not adversely affect the marine environment.

As regards the Descriptor 11 (Noise/Energy), the EU Directive highlights the importance of underwater noise measurements, focusing on evaluation and monitoring, which is the subject of further development, also in relation to mapping. Both long duration and short duration sounds are included due to their different impacts on organisms. Commercial activities are considered a source of high-level noise affecting relatively broad areas for which introduce regulated conditions subject to a license.

Two indicators were published for Descriptor 11 in the EC Decision 2010/477/EU on criteria and methodological standards on GES of marine waters (2010/477/EU, 2010):

11.1. Distribution in time and space of loud, low, and mid frequency impulsive sounds

- Proportion of days and their distribution within a calendar year over areas of a determined surface, as well as their spatial distribution, in which anthropogenic sound sources exceed levels that are likely to entail significant impact on marine animals measured as Sound Exposure Level (in dB re 1 $\mu Pa^2.s$) or as peak sound pressure level (in dB re 1 μPa_{Peak}) at one metre, measured over the frequency band 10 Hz to 10 kHz (11.1.1).

11.2. Continuous low frequency sound

- Trends in the ambient noise level within the 1/3 octave bands 63 and 125 Hz (centre frequency) (re 1μPa RMS; average noise level in these octave bands over a year) measured by observation stations and/or with the use of models if appropriate (11.2.1) (Tasker et al., 2010).

The monitoring of indicator 11.1 recommended by the technical sub-group (TSG) of Noise provides the set-up of a register of the occurrence of impulsive sounds that are sounds for which the effective time duration of individual sound pulses is less than ten seconds and whose repetition time exceeds four times this effective time duration. In this interpretation, it is proposed that all sounds of duration less than 10 s that are not repeated are also impulsive. The most important sounds of this type are represented by airguns, pile-driving, explosives, and sonar working at relevant frequencies and some acoustic deterrent devices. Also, boomers, sparklers, and scientific echo sounders are included. This register represents the first step to establish the current level and trend in these impulsive sounds. In particular, the register is useful to estimate the spatial and temporal impact on the environment (the total period and total habitat loss by impulsive noise sources) and for determining the baseline level. In this way it is possible to describe the pressure of the environment of impulsive noise sources. Information is collected through the year and throughout regional seas, to permit an assessment of possible cumulative impacts of displacement on marine species at the population level. The choice of the upper limit of the frequency band (10 Hz to 10 kHz) in the Commission Decision 2010 is because sounds at higher frequencies do not travel as far as sounds within this frequency band. Although higher frequency sounds may affect the marine environment, they do so over shorter distances. This choice of bandwidth, therefore, also excludes most depth-finding and fishery sonars. The indicator is focused on those impulsive sound sources that are most likely to have adverse effects, and the sources that generate sound in this frequency band. The source levels should include all classes of high intensity sounds that are known to affect the marine environment adversely; the activities that generate such sounds are routinely licensed or are assessed (van der Graaf AJ et al., 2012).

To complete Descriptor 11, TSG Noise suggests the following interpretation of indicator 11.2: Trends in the annual average of the squared sound pressure associated with ambient noise in each of 2/3 octave bands, one centred at 63 Hz and the other at 125 Hz, expressed as a level in decibels, in units of dB re 1 μPa, either measured directly at observation stations, or inferred from a model used to interpolate between or extrapolate from measurements at observation stations. Continuous sound is defined as an imprecise term meaning a sound for which the mean square sound pressure is approximately independent of averaging time. No precise threshold is specified for this type of sound as for impulsive one (van der Graaf AJ et al., 2012). It is suggested a combined use of measurements and models (and possibly sound maps) to ascertain levels and trends of ambient noise in the relevant frequency bands. In this way, the trend estimation is more reliable and cost-effective. TSG Noise proposes the development of international standards for the measurement, modelling, and data storage of ambient noise. A monitoring system between Member States within a sub region is advised (Dekeling et al., 2014).

1.7 Effects of anthropogenic noise

Anthropogenic noise can affect marine organisms in three different ways, anatomical, physiological, and behavioural level. Major effects are represented by hearing damage, including permanent threshold shifts, and other non-auditory tissue damage from exposure to very loud sounds; temporary threshold shifts from acoustic overexposure; masking of sounds hindering the perception of acoustic information and changing hormone levels, leading to stress responses and lack of sleep (Dooling et al., 2015). The effects of anthropogenic noise can range from small, short-term behavioural adjustments to large behavioural or physiological changes resulting in death (Kunc et al., 2016).

Anthropogenic noise can be also classified as an endocrine stressor inducing changes in cortisol level. For example, the exposure to boat noise can induce a relatively acute response in different species regardless of their hearing sensitivities (Wysocki et al., 2006). An increase in ship noise results in increased cortisol levels and a decrease in ship noise determines a decreased cortisol level, suggesting a positive and direct correlation between noise and the HPA axis (hypothalamic–pituitary–adrenal axis) (Rolland et al., 2012).

Buscaino et al. (2010) in a controlled experiment carried out on the sea bream and sea bass motility showed an increased swimming activity due to an acoustic stimulus. They concluded that anthropogenic noise at low frequencies can influence the swimming activity of fish. Consequently, increased swimming activity and the associated metabolic costs could compromise other biological activities, such feeding, regulation due to environmental perturbation, migration, and reproduction. Other studies (Banner & Hyatt 1973; Lagardère 1982) observed a strong reduction of egg survival and reproductive performance and growth rates in farmed fish species exposed to high sound levels. This suggests that noise pollution may have a negative impact on many aspects of the ecology of a fish. The effects of noise pollution on invertebrates are poorly studied, but probably the continuous noises, such as the frequent passage of boats, mask or stop the emission of sound made by some invertebrates, such as the California mantis shrimp (*Hemisquilla californiensis,* Stephenson, 1967) (Staaterman et al., 2012).

1.8 Acoustic indexes to assess the biodiversity

The passive acoustic monitoring (PAM) is a non-invasive method for studying marine organisms in their natural environment, without the introduction of any interference or stressing factor. This is a cost-effective tool enabling the achievement of accurate assessment of soniferous species, even at large spatial scales (Pieretti & Danovaro, 2020). PAM is often faster than traditional methods because data can be collected and analysed in real-time. This monitoring allows two types of study: ecoacoustics and bioacoustics.

Ecoacoustics is defined as a theoretical and applied discipline interested in studying sound along a broad range of spatial and temporal scales to tackle biodiversity and other ecological questions. The use of sound as a means by which to deduce ecological information allows ecoacoustics to investigate the ecology of populations, communities, and landscapes (Sueur & Farina, 2015). Ecoacoustics is strictly related to bioacoustics, but differs markedly because ecoacoustics considers sound to be a component and an indicator of ecological processes, whereas bioacoustics is an animal behaviour discipline that considers mainly sound as a signal of information between individuals (Rossing, Thomas D., 2007).

Recently, ecologists have developed ecoacoustic indices as proxies of species assemblage diversity in both terrestrial and marine environments. These new tools allow access to habitats otherwise difficult to study with traditional methods. Acoustic diversity has been a good proxy for overall vocal animal richness (Depraetere et al., 2012a). In fact, recent research has demonstrated that when more species are present within a community, there is an increase in diversity of signals across the spectrum of frequencies.

The most used indexes as reported by Pieretti and Danovaro (2020) are the so-called intensity indexes which give simple quantitative or statistical summaries of the acoustic energy of the soundscape depending on the frequency or the time domain. One of the most used indices in biodiversity assessment, the Shannon and evenness indices, was applied on sound emitted by animal communities by computing two acoustic sub-indices H_f (spectral entropy), and H_t (temporal entropy). These two indices were multiplied to obtain an acoustic entropy named H, ranging between 0 and 1, with low values indicating pure tones and high values sound with numerous and even frequency bands (Sueur et al., 2014). The normalized difference soundscape index (NDSI) has been designed instead to estimate the level of anthropogenic disturbance on the soundscape by computing the ratio of human-generated (anthrophony) to biological (biophony) acoustic components found in field collected sound samples (Kasten et al., 2012). Another important index is the Acoustic richness (AR) index which is based on the temporal entropy and amplitude of the signal. Depraetere et al. (2012b) revealed an expected gradient of diversity with higher diversity values, in the young forest where they sampled, that potentially provides a higher number of microhabitats.

Harris et al. (2016) proposed four criteria to evaluate the robustness of these indices in temperate marine environments, as follow:

1. Be positively correlated with traditional species assemblage measurements in relevant frequency ranges.
2. Be robust to changes in spectral resolution.
3. Be robust to the inclusion of natural noise interference (i.e., Wind) in the acoustic data set.
4. Be robust to the inclusion of anthropogenic noise interference in the acoustic data set.

In their study, they evaluated three ecoacoustic indexes (H, AR and ACI) on marine recordings concluding that the ACI index meets all the criteria and consequently can be considered a successful index. First, the ACI is positively correlated with species assemblage diversity.

1.8.1 Acoustic Complexity Index (ACI)

The Acoustic Complexity Index (ACI) is an algorithm introduced by Farina and Morri (2008) to produce a direct and quick quantification of the birds' vocalizations by processing the intensities registered in audio-files. The ACI formula is based on the observation that many biotic sounds, such as bird songs, are characterized by an intrinsic variability of intensities, while some types of human generated noise present very constant intensity values. This index can represent a useful tool to determine changes in behaviour and composition of a vocalizing community and, consequently, to better monitor animal dynamics in a quick and non-invasive way.

The ACI can be calculated according to a formula with few steps. Based on 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 (Δf_l):

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

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

$$D = \sum_{k=1}^{n} d_k \quad \text{for :} \quad j = \sum_{k=1}^{n} \Delta t_k; \quad n = \text{number of } \Delta t_k \text{ in } j \qquad (2)$$

where D is the sum of all the d_k contained in j. To obtain the relative intensity and to reduce the effect of the distance of the birds from the recording microphone, this result is then divided by the total sum of the intensity values registered in j:

$$\mathrm{ACI} = \frac{D}{\sum_{k=1}^{n} I_k} \qquad (3)$$

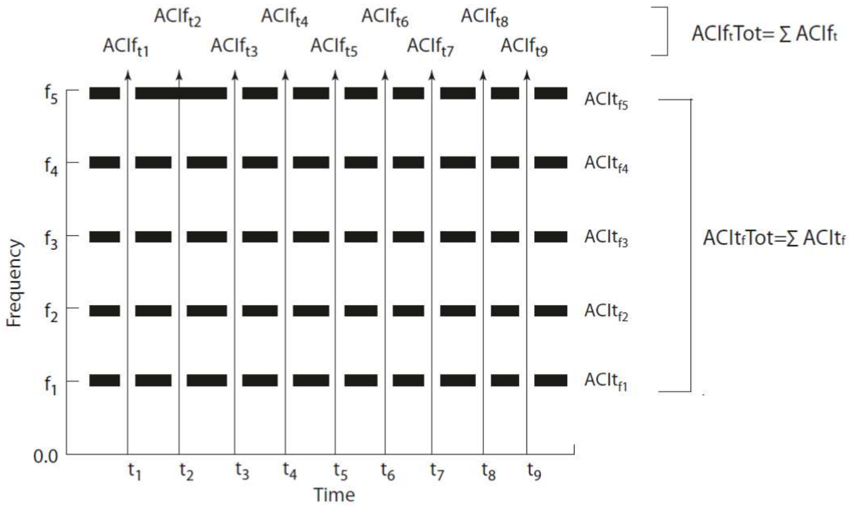
where the ACI is calculated in a single temporal step (j) and in a single frequency bin (Δf_l) (Pieretti et al., 2011).

In addition, the ACI algorithm computes the relative intensity [the sum of all the d_k values (differences in intensities) is divided for the total sum of intensities], reducing the variability introduced by the singing organisms being different distances from the recording microphones. Despite this standardization, the index remains moderately sensible at the proximity/remoteness of the sound source and a close sound will result in a higher ACI value then an identical sound emitted in a more distant location. (Pieretti et al., 2011).

The ACI metric is composed of two different indices:

- **ACI$_{tf}$** calculates the amount of information present along every spectral line at selected intervals of time.

- **ACIft** calculates the amount of information across all the spectral lines at each temporal step (for convention f = frequency, t = time).



When in an audio there are biophonic signals (that usually have different patterns according to time or frequency orientation) the two indices extract different aspects of acoustic information. In this way, ACItf and ACIft are used with their specific explicit attributes: ACItf can describe the distribution of frequencies to build the acoustic signature and ACIft can define the distribution of acoustic information along time (Farina et al., 2016).

1.9 The physical and acoustic-biological characteristics of the Adriatic Sea

The Adriatic Sea (Fig. 3) is an important shallow basin with an elongated shape in the Mediterranean Sea. The basin can be divided into three parts considering the increase in depth. The first part, the Northern Adriatic Sea, is shallow with a gradual slope of depth up to 100 m, an average bottom depth of about 35 m and includes the Gulf of Venice. The Central Adriatic Sea, starting at the south of Ancona, is 140 m deep on average but drops quickly at 200 m at the Jabuka Pit (about 280 m deep), an important nursery area for fishes and crustaceans. The third part, the Southern Adriatic, also has a deep at its centre, the South Adriatic Pit (ca. 1200 m deep). The bottom rises again in the Otranto Strait where the Adriatic opens on the Ionian Sea.

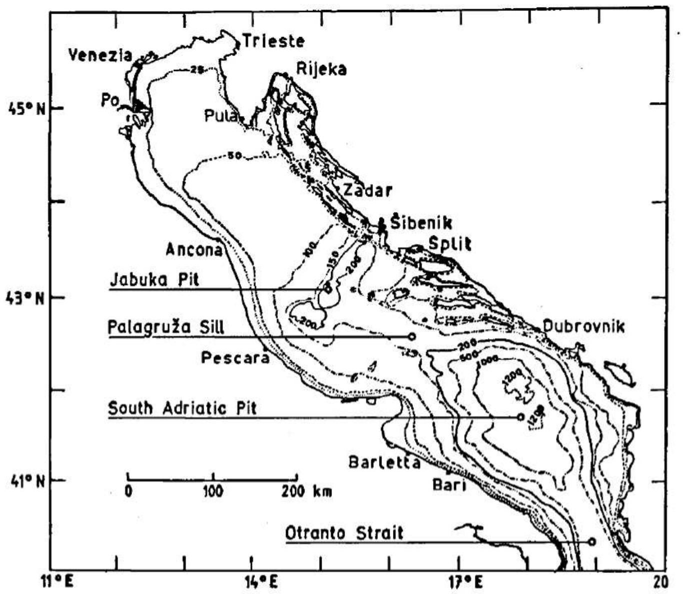


Figure 3. Position and topography of the Adriatic Sea.

The major fresh water source is the Po River. The general surface circulation of the Adriatic Sea may be described as a large-scale cyclonic meander, with a northerly flow along the eastern coast and a southerly return flow along the western coast (Orlié et al., 1992). The eastern coast is generally high and rocky whereas the western coast is low and mostly sandy (Artegiani et al., 1997). An intense trawling, touristic and shipping activity contribute to its low water transparency (Pieretti et al., 2017).

The Northern Adriatic Sea is often characterized by high turbidity due to the Po River outflow and the shallow depth of the basin. Therefore, there are the presence of different species that emit sound signals to communicate in that dark context and in opaque water. Typical are nocturnal fish species that emit characteristic sounds to attract the partner during the reproductive period as for the *Chromis chromis* (Linnaeus, 1758), a small fish, living near rocky reefs which reproduces from June to September. This species also uses sounds to approach a conspecific (Picciulin et al., 2002). *Sciaena umbra* (Linnaeus, 1758) (Fig. 4) is also a typical vocalizer fish, living on hard substrate, especially along the coasts of the gulf of Trieste (Bonacito et al., 2002). Gobies family (Perciformes; Gobiidaeis) is equally studied for the various species exploiting specific vocalizations for the intraspecific communication (Malavasi et al., 2008). The cusk-eel *Ophidion rochei* (Müller, 1845) can be defined as a cryptic endemic Mediterranean fish living at depths ranging from a few metres to 150 m (Dulčić et al., 2002). This sand-dwelling fish is a nocturnal predator that hides during daylight hours. This fishes spawn from June through the end of September (Codina et al., 2012). This specie emits specific vocalizations at low frequencies, which can be easily detected and recognizable (Fig. 5). For that reason, a passive acoustic monitoring can reveal the presence of this species despite its crypticity (Picciulin et al., 2019). Therefore, the Adriatic Sea hosts many different fish producing-sound species that were adapted to the conditions of the barely visible waters to survive. Also, local marine mammals, such as bottlenose dolphins, produce specific vocalizations (e.g., whistles), easily recognizable in spectrograms (Fig. 6) (Corrias et al., 2021; Picciulin et al., 2022). Various crustaceans produce high-frequency mechanical sounds, as for the snapping shrimp (Synalpheus sp., *Alpheus* sp.) (Budelmann, 1992). In spectrogram snapping shrimps are often characterized by high-frequency repeated clicks as shown in Fig. 7.

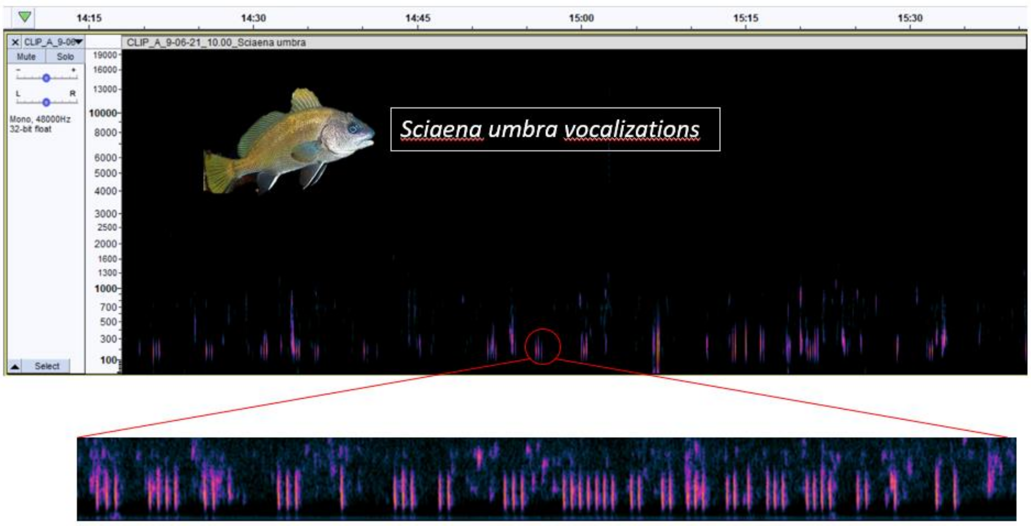


Figure 4. Spectrogram representing *Sciaena umbra* vocalizations.

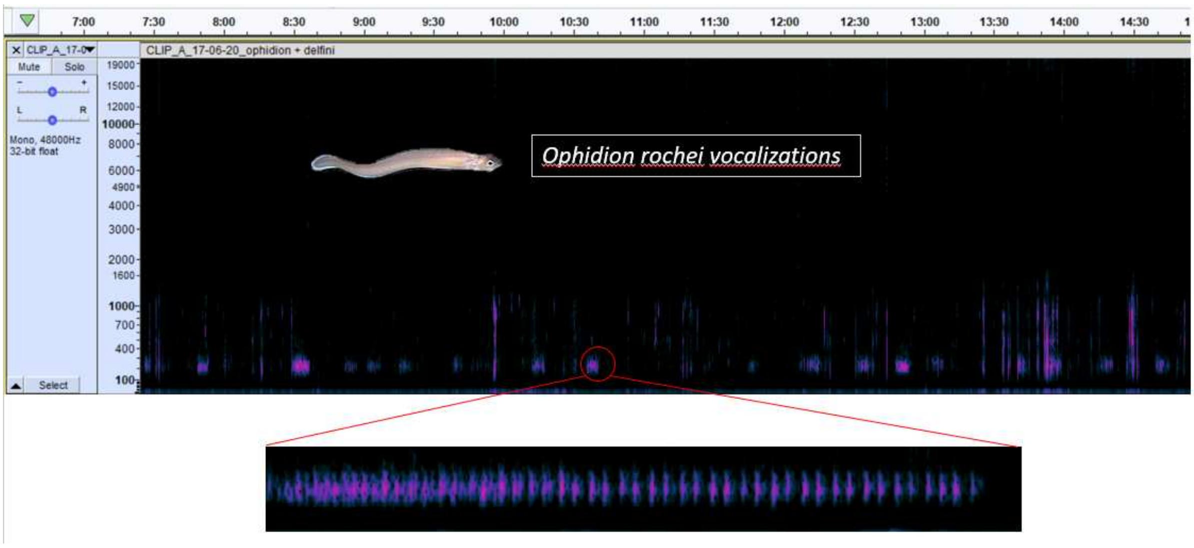


Figure 5. Spectrogram representing *Ophidion rochei* vocalizations

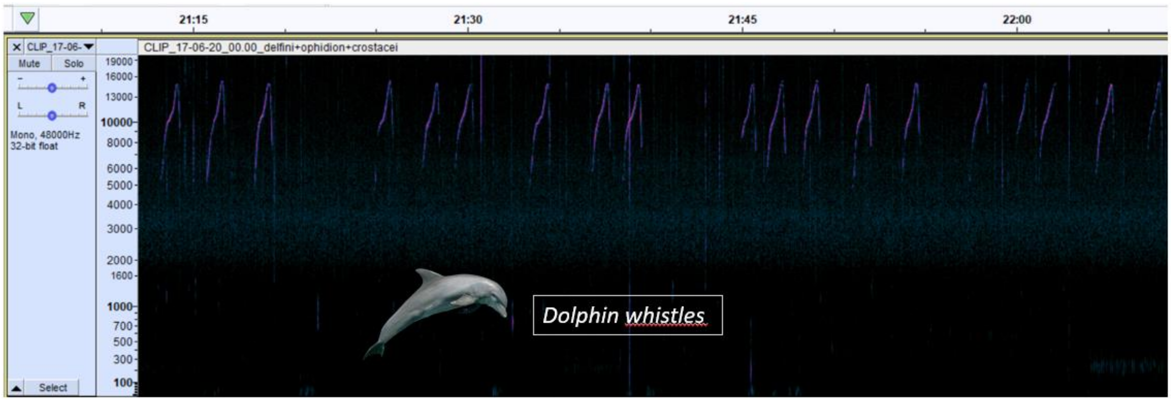


Figure 6. Spectrogram of dolphin whistles.

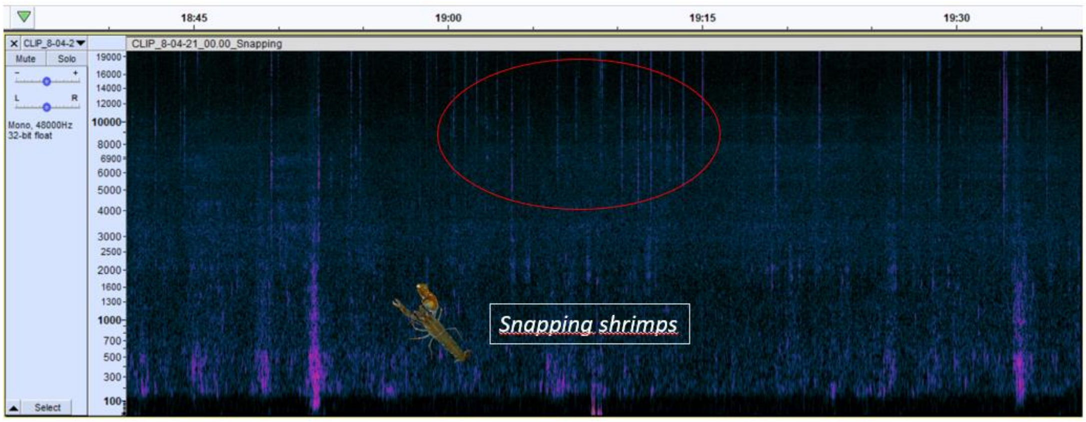


Figure 7. Spectrogram of snapping shrimps clicks.

1.9.1 The coast of Mount Conero as a potential marine protected area

In 2000 the project for the establishment of the "Marine Protected Area" of Mount Conero was presented. The MPA would include the tract of sea in front of the Conero Regional Park, between the Port of Ancona in the North and the mouth of the river Musone in the South, up to the bathymetry of 12 m, for a total extension of about 60 km (Fig. 8). It would comprise a coastal stretch along 25-30 km, with cliffs on the sea consisting of limestone and marly-sandy formations, typical of the Conero Promontory. Formations and continuous sea erosion gives rise to caves, ravines, and particular cliffs.

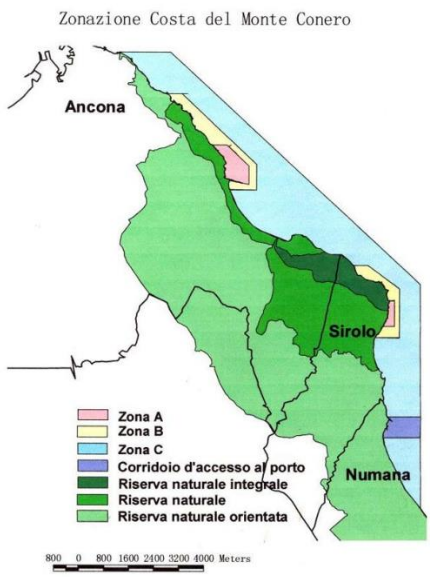


Figure 8. Zonation of the potential MPA of the Conero Coast.

The coast of Mount Conero differs from north to south. From Ancona to the “Scoglio del Trave” there are rocks overlooking the sea, small and pebble beach, steep slopes with little vegetation. South of the “Scoglio del Trave” a pebble-sandy beach is present. Instead, the area of Portonovo is characterized by sandy-gravel beach. Still different is the area to the south of Numana with a low and vegetation-covered coast and sandy-pebble beach.

Usually, the seabed is characterized by gravel and sands that gradually decrease their grain size towards the open sea where, after the clayey liming belt, there are again sands. These latter are called by geologists "wreck sands" and by fishermen "sabbioni", important fishery area (Fabi et al., 2003) .

On the 10th of July 2020, the “Promoter Committee for the construction of the Marine Protected Area of Conero” was established. The Committee wants to disseminate scientific information on Marine Protected Areas and their need to protect marine biodiversity, and the natural environment in general. They will organise initiatives to promote the MPA proposal and promote knowledge of the protection of the sea, and the Conero Coast in particular. The main points in favour of the presence of an MPA is protecting biodiversity and increasing ecosystem productivity; greater resilience and maintenance of ecosystem services; reduction of the costs faced from the public administrations for depuration, defence of the coasts from the erosion; increased fishing in the surrounding areas (spill-over); protection of geological features or processes; protection of cultural values; more recreational and tourist opportunities; Increase in value for goods and services produced within or around the area; Creation of new possibilities for education, teaching and scientific research, including research and development of new products and new molecules of food, medical and pharmaceutical interest. They support the importance of the rocky bottom that serves as a reserve of biodiversity and allows the dispersion of marine species. The hard substrates are represented by natural rocks, such as the “Scogli delle due sorelle” or the “Scoglio del Trave, and artificial substrates such as breakwater barriers or the wreck of the motor ship Nicole, sunk in 2003 about two miles off Numana. The breakwater barriers wind along the entire Adriatic coast giving rise to a system of artificial hard substrates (the Great Adriatic Barrier) that hosts a wide variety of organisms. The waters of this area of the Adriatic Sea, particularly rich in nutrients and phytoplankton, host peculiar organisms that sometimes reach exceptional size and/or density. In a few meters of water, it is possible to admire dozens of species of crustaceans and colourful nudibranchs, giant sponges, and numerous varieties of anemones (Comitato promotore per la realizzazione dell'Area Marina Protetta del Conero, 2020).

The plan of the marine protected area of the Conero has not been uniformly accepted by all the institutions of Ancona, let alone from all the citizens. A "Committee against the Conero MPA" has been established, which have joined representatives of fishermen, both sports and professionals, users of boating and nautical activities, underwater sports, and tour operators. Also, local administrations of Ancona, Numana and Sirolo opposed. The "Committee against the Conero MPA" argues that Marine Protected Areas already established in Italy are financially bankrupt. The funds to manage them are insufficient. The Ministry finances only the costs of establishment but not the subsequent management. In their opinion, already from the depth of ten meters an "inert" sandy or muddy seabed extends. Moreover, they complain that in no existing MPA are allowed activities that are part of the cultural heritage of Ancona such as: use pots for sport fishing, collect "moscioli" or practice spearfishing (Comitato contro l'AMP del Conero, 2020).

1.10 *The SOUNDSCAPE project: sound mapping and monitoring network in the Northern Adriatic Sea*

The SOUNDSCAPE project (Soundscapes in the north Adriatic Sea and their impact on marine biological resources), is an Interreg Italy-Croatia Project (Vukadin P. & Dadić V., 2021), co-funded by the European Union. SOUNDSCAPE Project represents the first extensive study on underwater noise in the Mediterranean Sea. The main objective of the project is to create a cross-border technical, scientific, and institutional cooperation to face together the challenge of assessing the impact of underwater environmental noise on the marine fauna and in general on the North Adriatic Sea ecosystem. This cooperation aims to ensure an efficient protection of marine biodiversity and to develop a sustainable use of marine and coastal ecosystems and resources. The objectives of the project are to be pursued in three ways: Implementing a shared monitoring network for a coordinated regional and transnational assessment of the underwater noise, evaluating the noise impact on marine biological resources, developing, and implementing a planning tool for straightforward management.

The Soundscape project set up an international cross-border network of nine monitoring stations (Fig. 9) with the same type of instruments and shared protocols for data acquisition. Broadband acoustic data were collected continuously for 15 months, covering all seasonal variations in underwater noise sources. The processing of the SOUNDSCAPE data helped to split the noise levels between a wide band of frequencies (50 Hz - 22100 Hz), including the MSFD request frequencies. The SOUNDSCAPE Project provided a shared assessment on annual and seasonal noise level distribution in the North Adriatic Sea. The Project data confirm the relevance of underwater noise pollution in the North Adriatic Sea and the need of evaluating its impact on biodiversity within the maritime management. In fact, there are no extensive data on underwater noise in the area and the knowledge on noise pollution and its impact on biodiversity is very limited. The monitoring results will be used to fill the knowledge gap about underwater noise levels in the Northern Adriatic Sea but also to support setting up and validating the soundscape model. The monitoring station MS3 Ancona, managed by CNR-IRBIM of Ancona, is located south of the Ancona harbour. The maritime traffic is dense with all categories of vessels (cargo, passenger and recreational), and anthropogenic underwater noise pressure is expected to be moderated/high. The point is in shallow water in a Natura

2000 site coastal area, so it is an interesting area for monitoring the impact of underwater noise to marine fauna.

Figure 9. Maps representing the 9 monitoring stations within the SOUNDSCAPE Project. Focus on MS3, Station of Ancona

1.11 Aim of the thesis

Given the importance of sound in the marine ecosystem, in this study we investigated the use of an acoustic index to study the complexity of underwater soundscape.

Typically, acoustic indexes are developed for terrestrial animal sounds studies, especially in the field of ornithology. Contrary, in the marine environment these indexes are still little investigated and applied, due to the high sensitivity of these to background noise, that can easily compromise the analysis.

Therefore, in this work we applied an acoustic index, called ACI (complexity acoustic index), analysing underwater sounds recorded with a hydrophone. First, we verified if there was a correspondence between the ACI application and the presence of fish vocalizations. In fact, when an audio presents fish sounds, provides ACI values greater than one that does not have them. Thanks to this confirmation, it was possible to apply the ACI index in this study.

The data for this current work have been acquired by CNR-IRBIM institute of Ancona, which was involved in the EU Interreg Italy-Croatia Project SOUNDSCAPE: Soundscapes in the North Adriatic Sea and their Impact on Marine Biological Resource. The SOUNDSCAPE Project set up a monitoring network with nine stations in the North Adriatic Sea to assess the underwater noise, in within the Marine Strategy Framework Directive.

For this study, the data has been recorded for a period of one and a half years (February 2020-July 2021) using a hydrophone located in front of

Mount Conero Park, representing the Station MS3 Ancona of the monitoring network.

In this way, it will be also possible to verify if the ACI index could be applied in the future to marine acoustic studies.

2. MATERIALS AND METHODS

2.1 Study area

The monitoring station MS3 Ancona, managed by CNR-IRBIM of Ancona, is located in shallow water not far from the coast, in front of Habitat Natura 2000 protected area of Mount Conero Regional Park. The equipment was positioned near a mussel farm to provide protection from fishing trawling and gillnets, situated at 15 m depth.

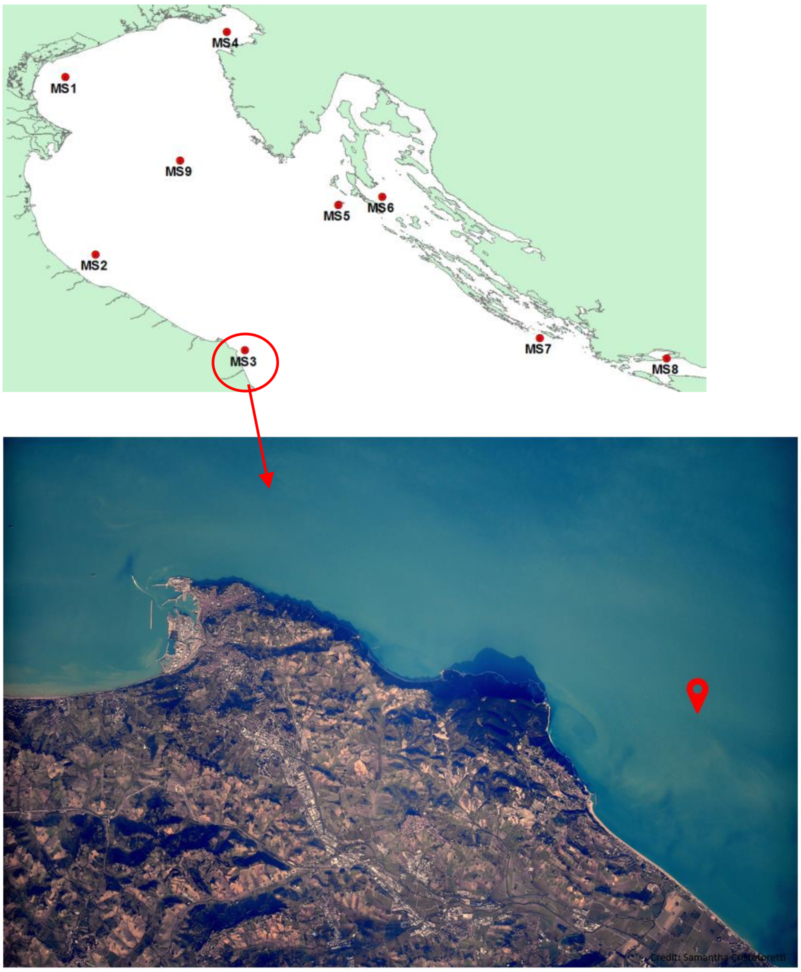


Figure 10._Above) Spatial distribution of the nine monitoring stations within SOUNDSCAPE Project. Below) MS3 station Ancona with satellite map picture provided by Samantha Cristoforetti

2.2. Data collection

Audio wav.files have been collected from the underwater recordings made by the SOUNDSCAPE Project using an autonomous passive underwater acoustic recorder Sono.Vault (by Develogic Subsea Systems GmbH, Hamburg, Germany) with omnidirectional broadband Neptune Sonar D60 Hydrophone characterized by a sensitivity around −192.7 dB re 1V/µPa (Vukadin et al., 2019). The instrument includes a programmable recorder, a battery set and a 1TB-SD memory card. The recorder was set to record continuously at a sampling rate of 48 kHz, providing a recording bandwidth of approximately 22 kHz with 16-bit resolution.

Hydrophone equipment consists of 4 principal components:

1. Sono.Vault passive recorder
2. Heavy anchoring block
3. Floating buoys
4. Acoustic releaser*

*Acoustic releaser added later between the anchoring block and the passive recorder. It is not represented in the figures 11 and 12.

Figure 11. Hydrophone equipment, on board Tecnopesca II vessel, managed by CNR-IRBIM within SOUNDSCAPE project.

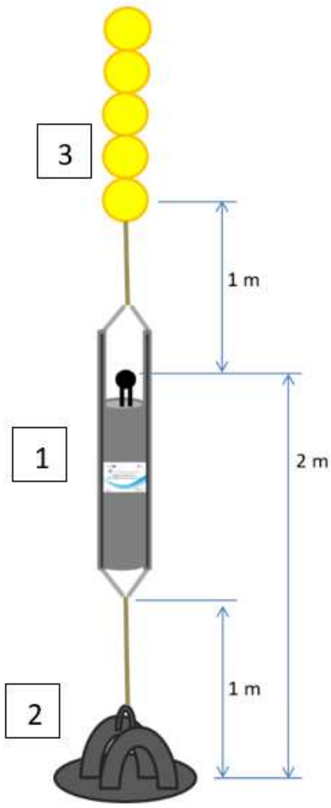


Figure 12. Graphic representation of the hydrophone equipment and its components.

The entire equipment was located at 15 m depth, above a sandy bottom. The Sono.Vault passive recorder, with a weight of 55 kilograms, was located at 2 m from the bottom to avoid the reflections of the echo of the seabed, thanks to a floating buoys system that verticalized the instrument. The buoys in column have a diameter of 25 cm and were 1 meter away from the recorder to avoid the encumbrance in the water and a partial acoustic reception of the underwater sounds around.

The monitoring was carried out for a period of one and a half years. The first deployment of the hydrophone equipment was made on 2020-02-21 and the period of recording was until 2021-07-11. The recovery of the instrumentation was approximately every three months. In this way, data have been downloaded, battery replaced, and instrument cleaned and calibrated. Data collection was in continuous.

2.3 Data analysis

2.3.1. Raw data cleaning

The ACI index calculates the difference between two consecutive intensities, but in the calculation, it counts all the sounds recorded in the file, including the background noise (e.g., the sound of waves, the noise of hydrophone ropes, indistinct underwater noise). These different noises, if not excluded, disturb the ACI index, causing an incorrect increase in values. In fact, ACI values must be higher for biotic sounds and not for background noise.

Consequently, a preliminary cleaning of the background noise was made to the .wav files with a software named *Audacity,* before the ACI analysis.

Audacity is an Open-Source tool for editing and recording audio. It is free and easy to use (De Maestri, 2009). (Fig.13-16).

Processing the audio-file with Audacity software

1) Importing the .wav file

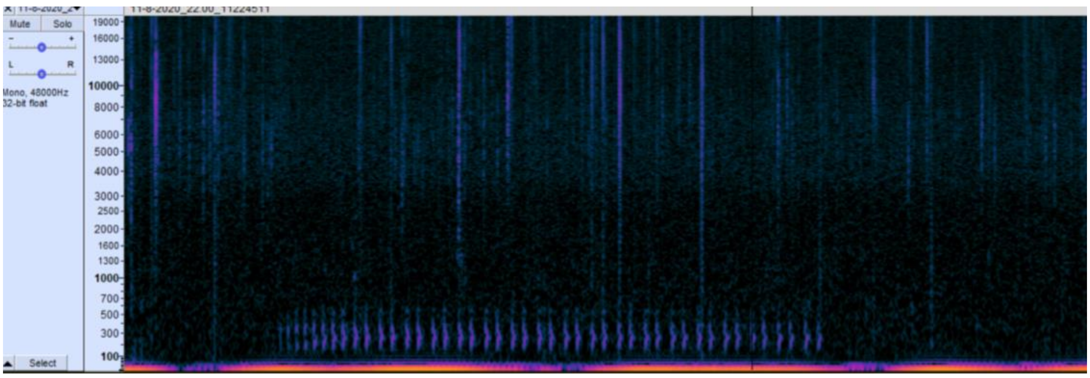


Figure 13_Spectrogram of an audio without any filter.

2) Application of “Filter Curve EQ” option from 60 Hz to 1000 Hz to select the frequency range target for this study

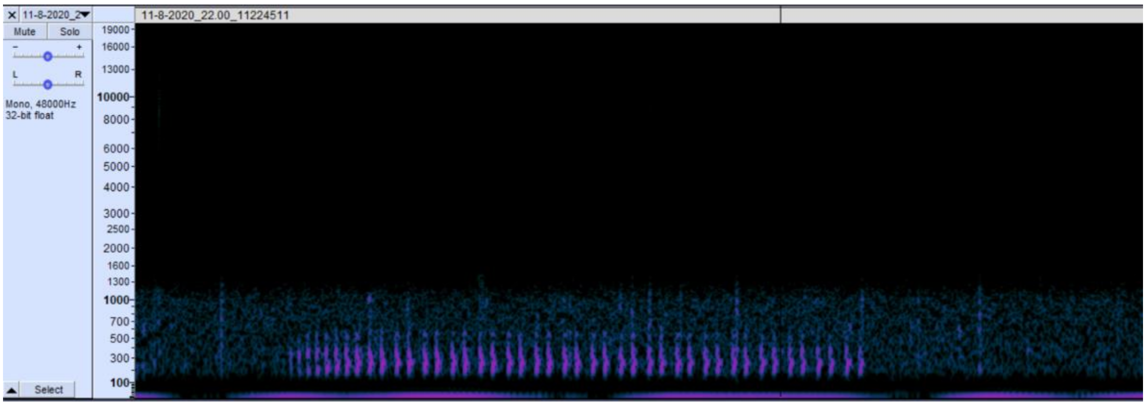


Figure 14_Spectogram of an audio after the application of the "filter curve EQ" 60-1000 Hz.

3) Manual reduction of the background noise with the “Noise reduction” option

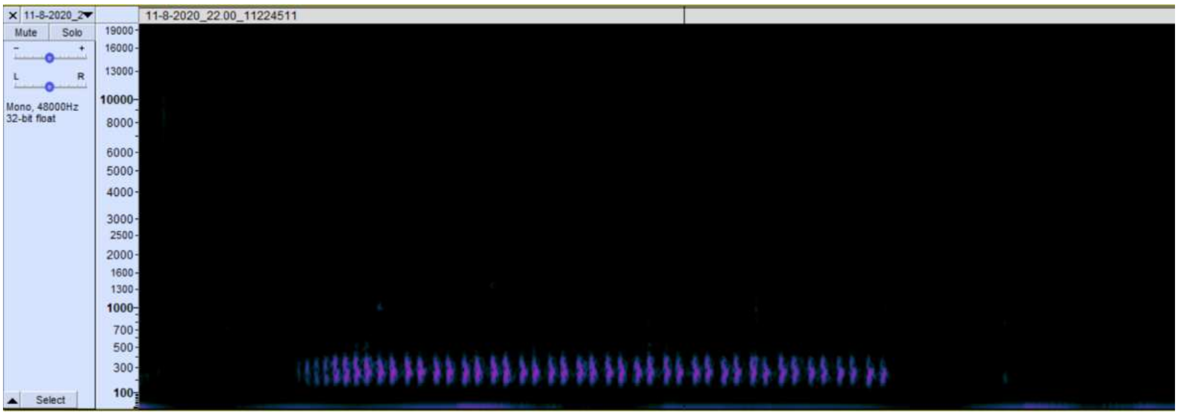


Figure 15_Spectogram of an audio after "Noise reduction" filter application.

4) Sound amplification of the entire audio track, there are the same dB values for all analysed files.

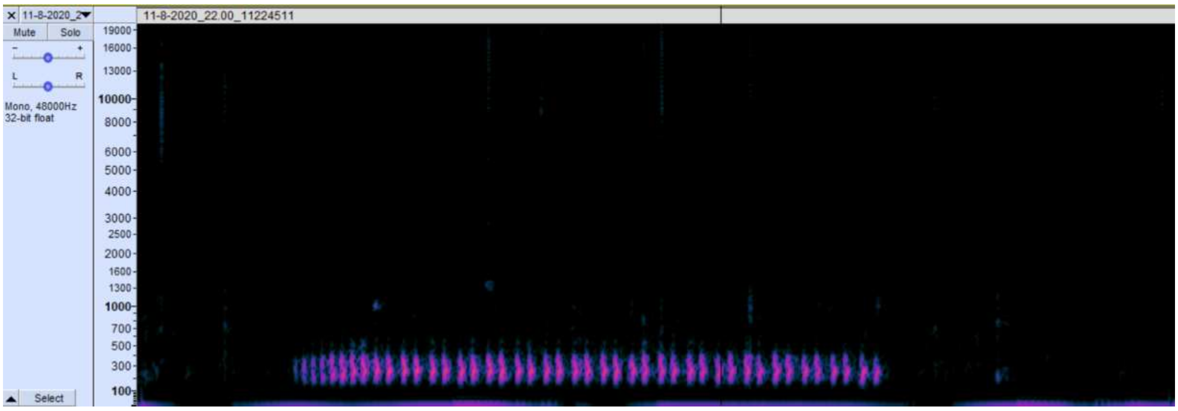


Figure 16_Spectrogram of an audio after amplification (10 dB)

2.3.2. Processing data

After a preliminary transformation of the raw data with Audacity, the processed wav. files are loaded in the SonoScape programme for the calculation of the ACI index.

SonoScape is a new software to process Waveform Audio Files format (wav) using Acoustic Complexity metrics (ACIft, ACItf), developed under the MATLAB® platform (Farina & Li, 2021a). The programme has options that allows to manage single and large collection of sonic files.

Parameter setting

- *Importing Files*: To import a .wav file, users can first select the folder that contains the file from the "Folder List", and then select the specific file from the "File List". To make the program run better, there must be only one file per folder.
- *Setting the Time Scale:* every file can be subdivided according to the time scale measured in seconds. For example, for a file of 60 seconds, a time scale of 1 means to obtain 60 elaborations of 1 s each.

  In this experiment, a time scale of 60 s has been set to correctly highlight audio content and to have a better temporal resolution in 30 min files.

- *Setting the Energy Filter:* this parameter is divided into two options,
    1) the "High" [Near field], empirically fixed, excludes from the ACI calculation every element of the sonic matrix that is > the fixed value. This is useful to exclude from computation high intensity sounds like the passage of an airplane.
    2) "Low" [Far field], empirically fixed, excludes from the ACI calculation every element of the sonic matrix < the fixed value. This subroutine excludes faint sounds.

For this current experimental project, only the "Far field" was used, setting a range of **0.4 – inf.** The 0.4 value represents the highest ACI average value calculated for silence audio (with only low background noise and no biotic sound). This way we can exclude most of background noise from ACI calculations for audio with fish vocalizations.

- *The Frequency Filter* is divided into two subsections:
    1) "Low frequency filter" (LFF): This parameter excludes from ACI computation a number of frequencies bands equal to the input.
    2) "High frequency filter" (HFF): This parameter excludes from ACI computation a number of frequencies bands higher than a threshold.

For this study the **values 0** was applied, for both LFF and HFF, because in the preliminary cleaning of raw data, some frequencies were cut and only the range from 60 Hz to 1000 Hz was selected. This choice is because of the major presence of fish vocalizations in that range frequencies (Fay, 2008), and because the ACI analysis made on fish sounds give good results. Contrary, marine mammals' vocalizations are typically at high frequency, above 1000 Hz, but their ACI analysis don't respond in a good way.

- *Clumping*: is a procedure of temporal aggregation of data before the application of the ACI metrics. The size of the clumping depends on the goal of investigation, and it is important for finding the best temporal resolution in which to consider the elements of the sonic matrix. In our case, a clumping value was set at **6.**

Once the parameters have been established, it is possible to perform the analysis of the acoustic complexity and saving the calculations. Results are automatically stored in specific folders named "Results" by the software. The SonoScape manages data files in a hierarchical structure (Farina & Li, 2021b).

After the ACI calculation, results can be visualized in a graph. An ACI Visualizer configuration panel appears for users to select the combination of parameters with which to display the graph. The graphic visualization of ACI is then available (Fig.17).

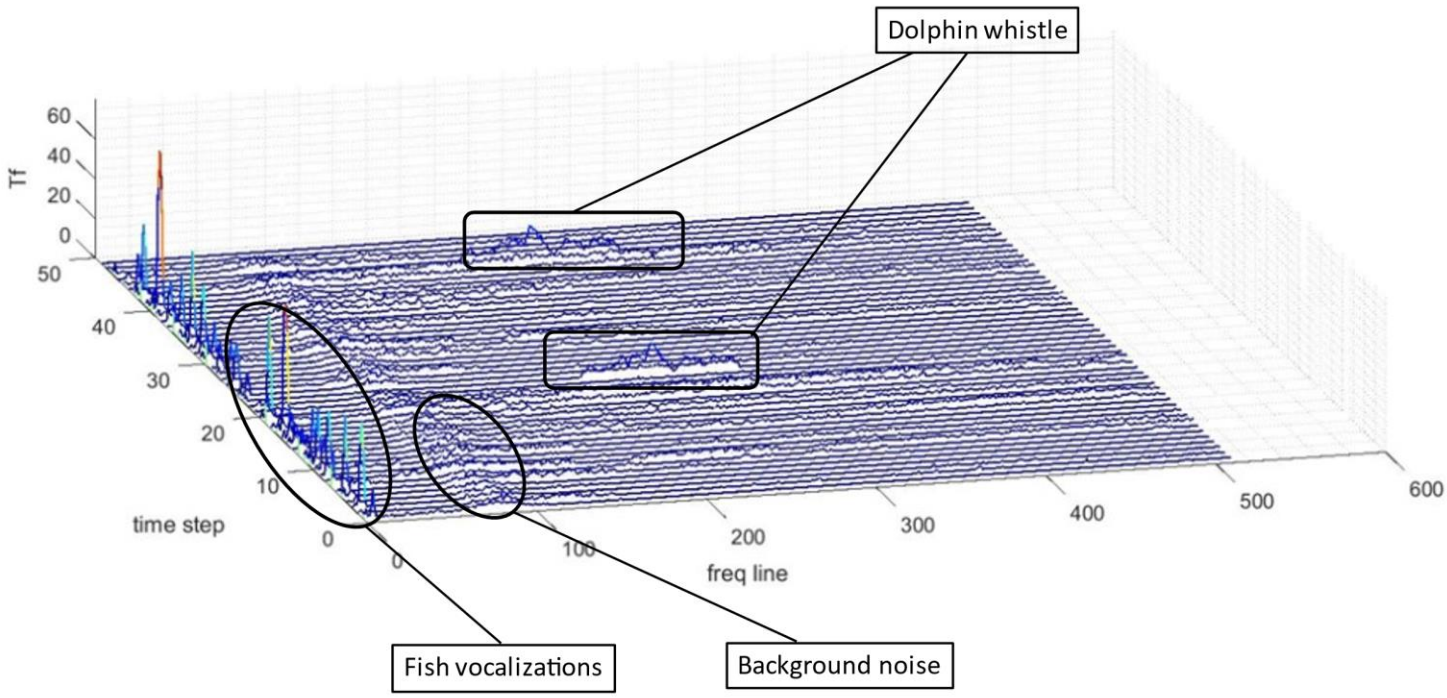


Figure 17_ACI_Tf graph of a 7 min audio with biotic signals. On the X-axis frequency line is represented; on the Y-axis time step of 10 s is presented; on the Z-axis ACItf values are calculated.

2.4. Statistical analysis

We used the Sonoscape program for the calculation of ACI index changing the various parameters, to verify how much they influenced the results and which values applied to them are better for a good resolution of data.

Selection criteria for audio files

a. **Timescale**

   First, the timescale was tested because many studies used short audio-files, composed by few minutes, but for this project half-hour files were used. For each second from which the wav. file is composed, ACI values will be processed, so the choice of the time scale is important to decide the best time resolution of the data according to the objective of the studies (Fig.18).

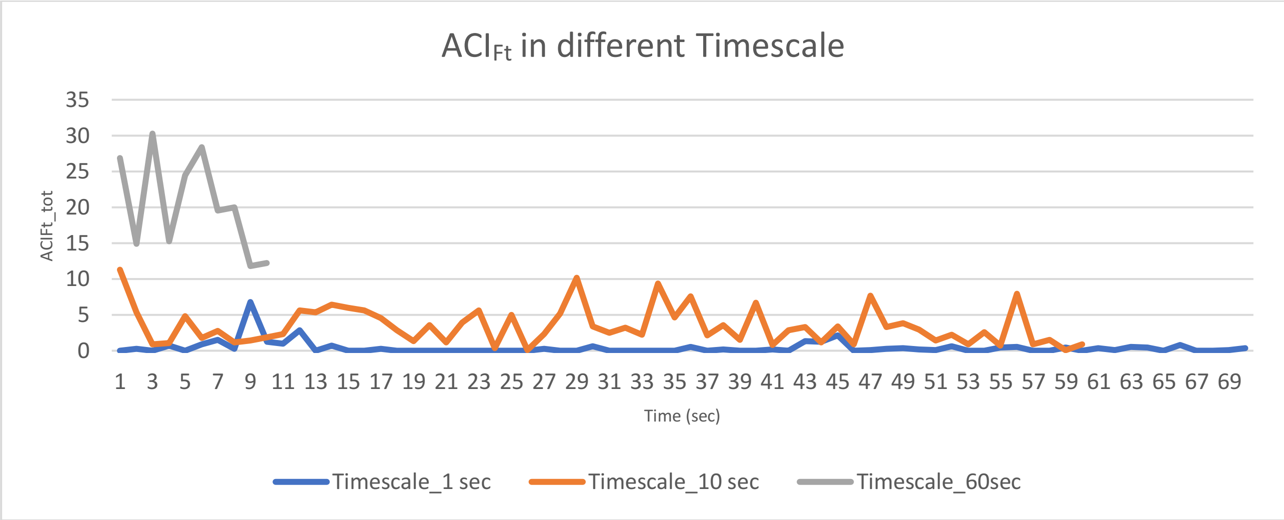


Figure 18_ACI index calculated in different timescale (applied on the same 10 min-audio).

b. Presence/absence of biotic sounds

Audio whose spectrograms show biotic signals i.e., vocalizations of fish, have higher ACI values than silent audio, where no biotic or other sounds are recorded (Fig.19-23).

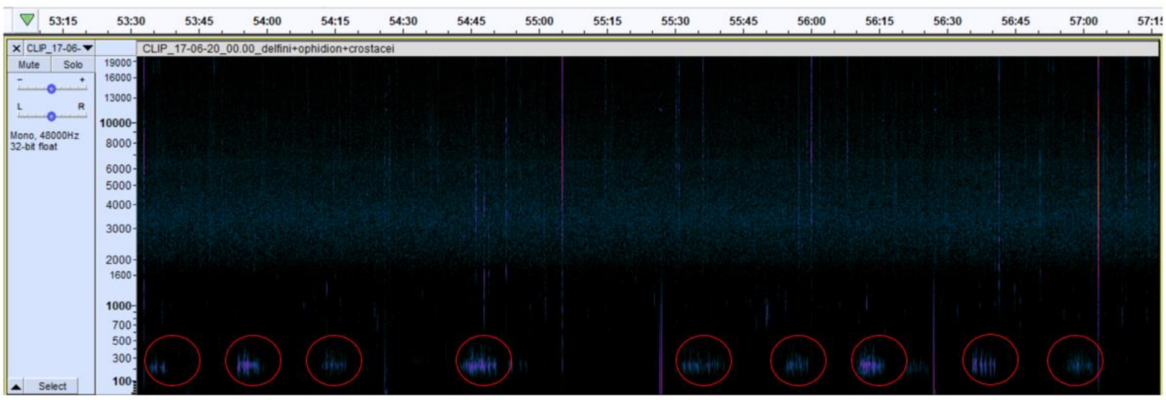


Figure 19_Spectrogram of an audio with presence of fish. Red circles highlight fish vocalizations.

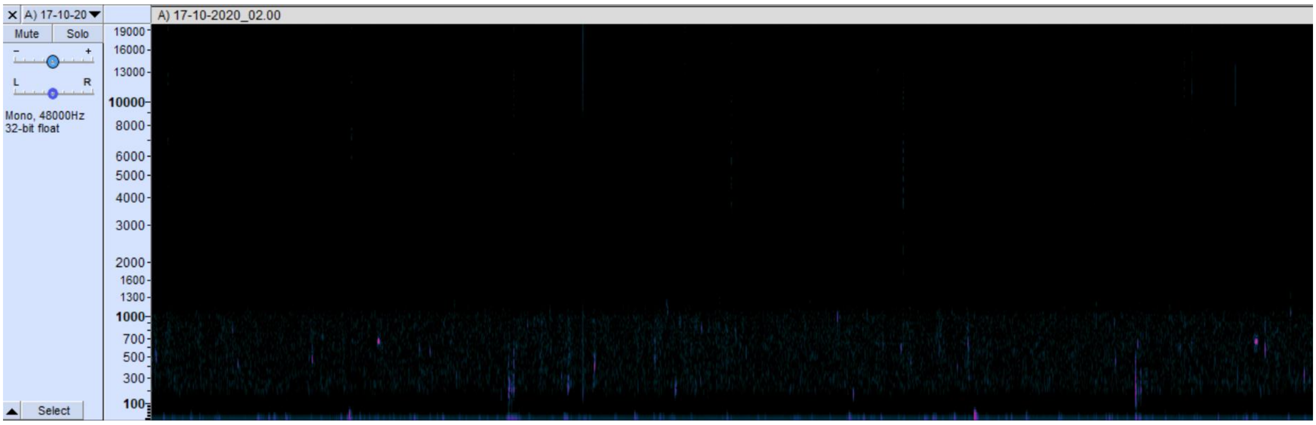


Figure 20_Spectrogram of an audio with absence of fish.

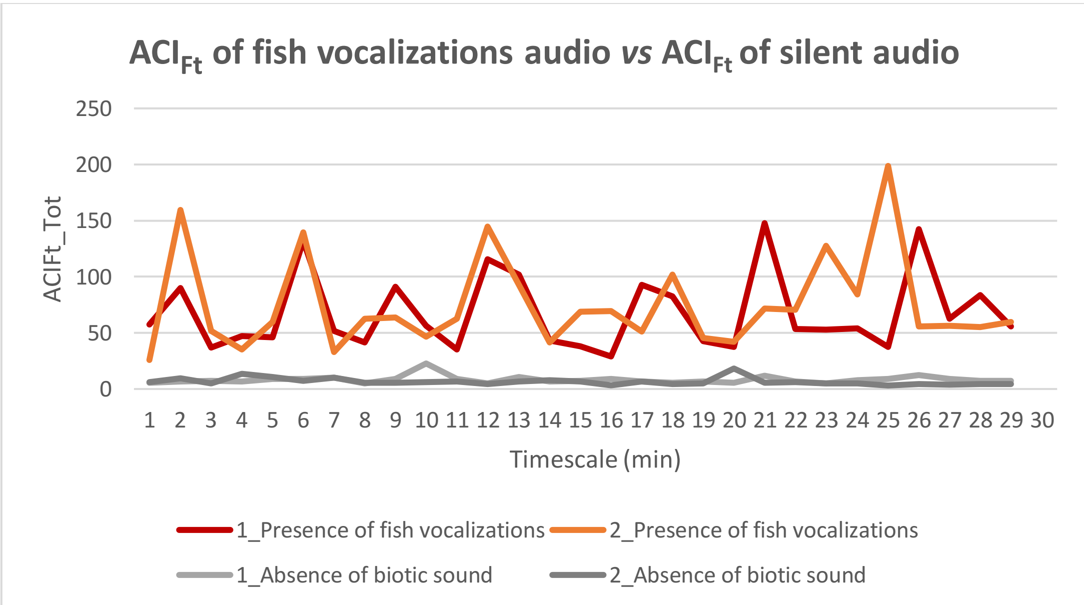


Figure 21_ACI$_{Ft}$ graph representing audio with presence of fish vocalizations (red and orange lines), which show higher ACI values than silence audio, with absence of biotic sound (dark and light grey lines). Parameter setting: Timescale_60 sec/ energy filter_far field: 0.4-inf / frequency filter 0-0 / clumping 6.

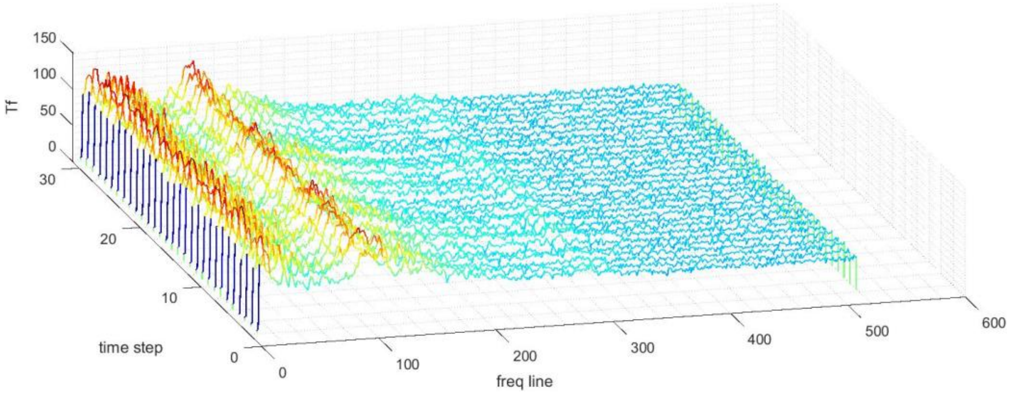


Figure 22_Sonoscape graph representing the presence of fish vocalizations: the red-yellow peaks.

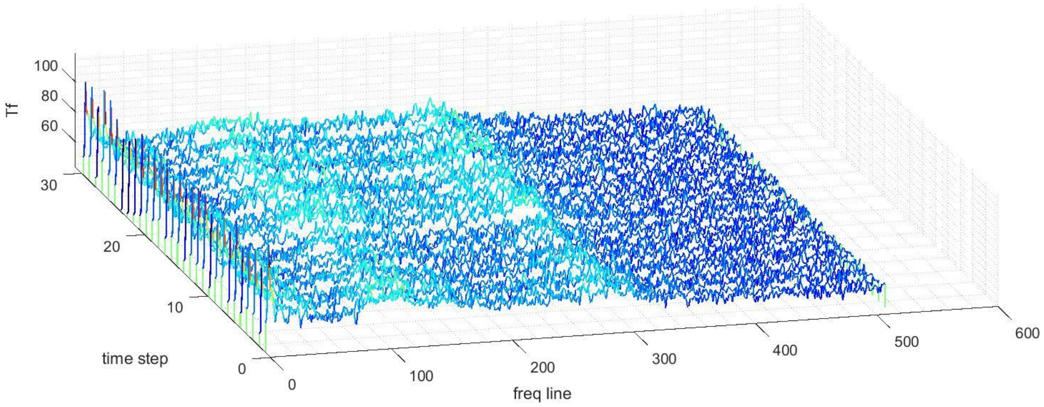


Figure 23_Sonoscape graph representing absence of fish vocalizations: no red-yellow peaks.

c.ACI values for biotic sounds

Theoretically audio with a different number of fish vocalizations have different ACI values. However not always audio with more vocalizations have higher ACI values (Fig. 24-25), because the index calculates much more differences in the amplitude than the number of pulses emitted by fish, many of which are low in intensity. This means that in case of an audio having few fish pulses but high intensities, ACI values could be higher than an audio with more fish vocalizations but lower in intensities.

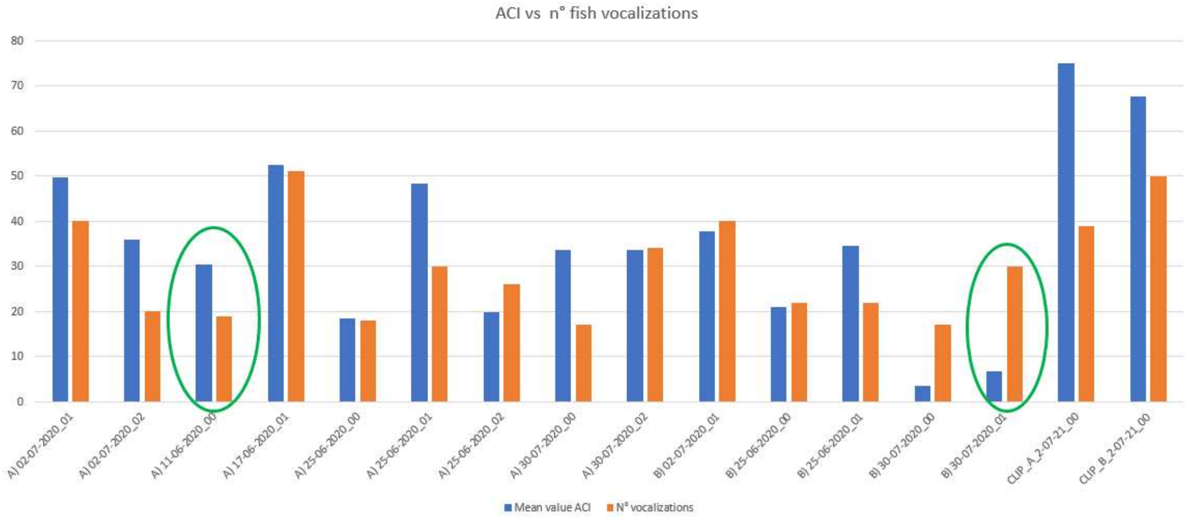


Figure 24_Graph representing the comparison between the Mean value of ACI and the number of fish vocalizations. As shown by audio "B) 30-07-20_01" number of vocalizations (N°= 30) is higher than the number of "A) 11-06-20_00"(N°= 17), but ACI is lower. This is because the audio "B)30-07-20_01" has vocalizations with lower intensities than "A) 11-06-20_00" which has a smaller number of fish pulses but stronger intensities.

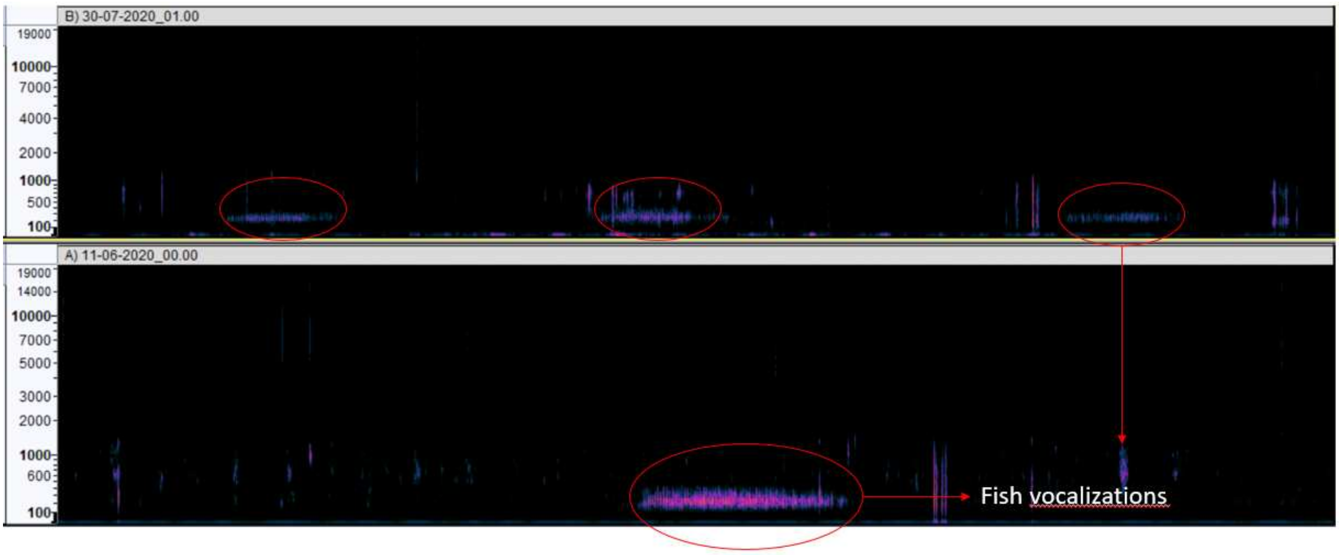


Figure 25_Spectrograms of audio with fish vocalizations (in this case belonging to Ophidion rochei. Above: spectrogram showing a greater number of fish vocalizations but with lower intensities; below: spectrogram showing a single fish vocalization in the same temporal step but stronger in intensities than audio above.

d. Exclusion of files with excessive noise

From the graphs and ACI values it is possible to see how the audio files containing excessive noises, such as the passage of a boat, the heavy rain, and the noise of nearby engines, give very high ACI values, even greater than the values obtained by audio with biotic sounds. This is probably due to the sound peaks that are recorded by these files, which give a high difference in sound amplitude between one temporal step and another. In Fig. 26 it is observed as on average the ACI values of audio containing vocalizations of fish (blue line) are greater than the ACI values of the file with the presence of a passing boat (orange line), except for the high peak corresponding to the approach of the boat to the hydrophone (Fig.27).

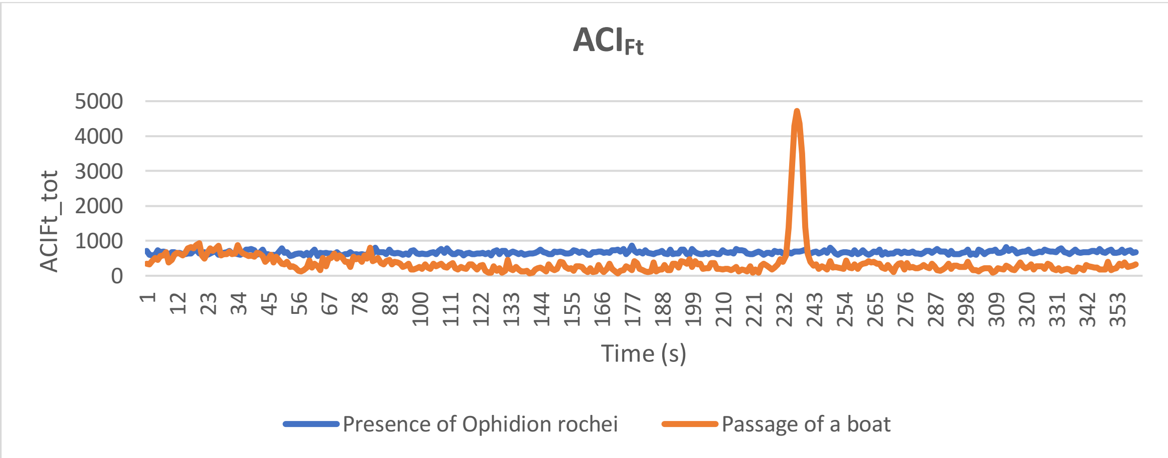


Figure 26_Presence of excessive noise e.g., Passage of a boat between 231-241 s. Parameter setting: Timescale_10 s.

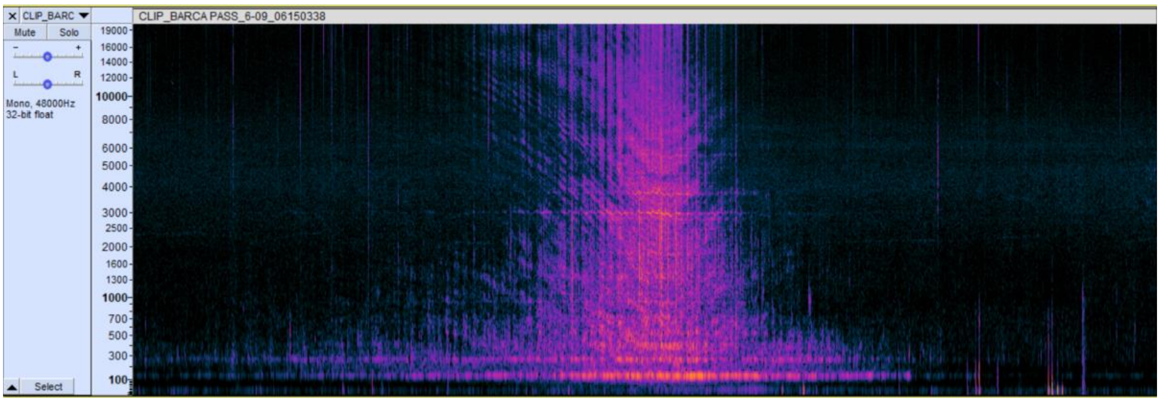


Figure 27_Spectrogram of an audio describing the Passage of a boat.

e. Clumping

Different clumping values provide different time resolutions, aggregating the sonic matrix data differently. There is a linear increasing trend between the increase in clumping value and the increase in ACI values.

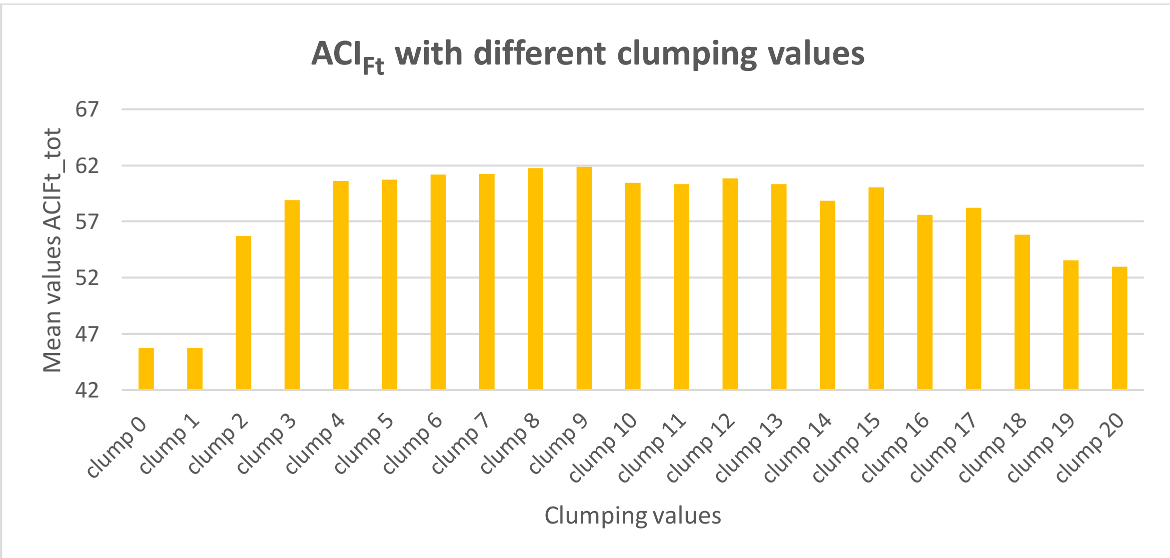


Figure 28_Trend of ACI$_{Ft}$ after the application of the clumping procedure for values from 0 to 20.

From the graph (Fig. 28), we can observe that clumping values from 4 to 9 have similar results, reaching a plateau. For this study, a clumping value of 6 was chosen because it represents one of the highest clumping values and it can correctly aggregate our data, providing a good temporal resolution. The selection of higher clumping values can determine the loss of detail of the recorded vocalizations, coming to group all the data together and no longer getting a significant difference in the ACI values between a group and the other.

f. Frequency filter

The choice of the frequency range determines different values of ACI. The filter only displays the selected frequency bands in a defined range. In our study, during the previous raw data cleaning, only the frequency range, 60 Hz -1000 Hz, was selected, because corresponds to the frequency utilized by fish sounds. Therefore, the frequency filter in the Sonoscape program was not applied.

3. RESULTS

3.1 Descriptive analysis

In this study, we analysed the seasonal pattern of ACI values and how they change in relation to the day-night cycle. We have randomly selected 6 audio files for each month (from February 2020 to July 2021), choosing 3 representative hours of the day and 3-night hours. We collected a total of 108 files. The respective spectrograms were visually and audibly scrutinized with the Audacity program to verify the quality of the recorded sounds and to ensure that they met two selection criteria:

- the presence/ absence of fish vocalizations in the file, to make an ACI comparison between audio with sounds of biological origin and audio with only silent abiotic background.
- the presence/absence of loud noise to be discarded as this would disturb the ACI calculation. We have in fact discarded files containing nearby boat passages or adverse weather conditions, such as heavy rain or very rough sea. Analysing these audios, we found too high ACI values, higher than the ACI values of files containing biotic sounds. These values would clash with the ACI theory that audio with biotic

sounds would have higher values than audio with abiotic sounds.

From the 108 files collected, only 67 met such criteria.

The 67 selected audios have been cleaned using audacity filters:

- A filter curve was applied to select the frequency range 60 Hz-1000 Hz, useful for the representation of fish vocalizations sounds.
- A noise reduction filter was applied to reduce the background noise, which would distort the ACI values during the calculation.
- Finally, an amplification of 10 dB was made to highlight all sounds found in the file, especially to better accentuate fish vocalizations.

Analysed audios were uploaded on SonoScape software to calculate ACI values. We applied the same filters to all files to standardise the data. Each uploaded file was a 30 min audio. A timescale of 60 s was applied, in this way we got about 30 ACI values, one for each 60 s, for a single file. We obtained a total of 1911 ACI values. The ACI average for each month has been calculated.

3.2 Output of the statistical analysis

Our data have a non-normal distribution. We carried out a comparison of ACI values between the 4 seasons to investigate the presence of significant differences, using the Kruskal-Wallis non-parametric test. We found a significant difference through all the comparisons between all seasons ($p < 0.01$), as shown in Fig. 28. Using a non-parametric Wilcoxon test we conducted also a pairwise comparisons, finding a significant difference ($p < 0.01$), especially between summer and winter season and summer and spring, as shown in the table 1.

Table 1_Significant differences between seasons through pairwise comparisons using non-parametric Wilcoxon test

	Autumn	Spring	Summer	Winter
Spring	$< 2e^{-16}$	-	$< 2e^{-16}$	0.00076
Summer	6.9e-14	$< 2e^{-16}$	-	$< 2e^{-16}$
Winter	1.5e-07	0.00076	$< 2e^{-16}$	-
Autumn	-	$< 2e^{-16}$	6.9e-14	1.5e-07

In detail (Table2), the ACI values in summer were higher than those of the other seasons. In particular, summer season displayed the highest mean (30.63 ± 1.54) and median (19.75) values of ACI, (table 2). Also, autumn was characterised by high ACI values, while winter and spring have the lowest values (Fig.29).

Table 2_: Descriptive statistics of ACI values seasonal distribution

	n	Mean	Median	Min	Max
Autumn	317	16.63 ± 0.83	11.72	1.28	107.59
Spring	609	8.93 ± 0.35	8.84	0.34	95.59
Summer	435	30.63 ± 1.54	19.75	0	236.24
Winter	549	12.06 ± 0.48	8.83	0	71.09

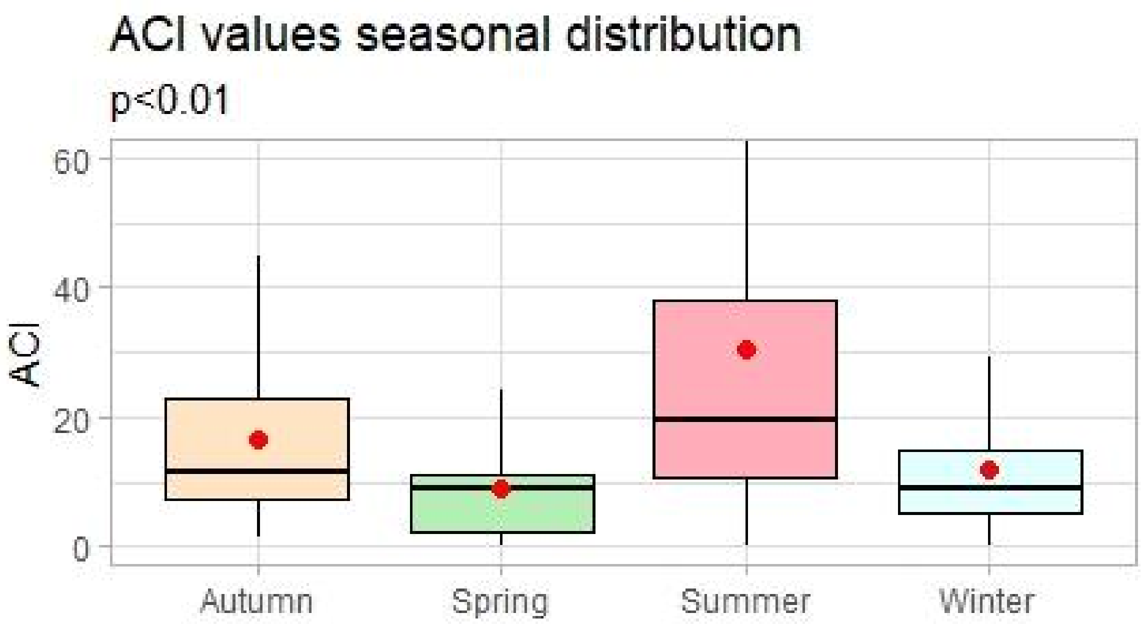


Figure 28_Boxplot graphic representation of ACI values seasonal distribution. On Y-axis ACI values are shown; on the X-axis seasons are represented. The red dots indicate the mean, showing that summer mean is the highest. The black centre lines indicate the median value, within summer has the highest value.

From the dataset, a subset of data has been extracted for only the summer season to compare ACI values between daily and night hours. A non-parametric Wilcoxon test have been performed. Results display a significant difference between summer day and night ACI values ($p < 0.01$). We found that summer nocturnal ACI values are higher that daily ones, as shown in the table 2 and in Fig. 29.

Table_2: Descriptive statistics of ACI values summer day-night distribution.

	n	**Mean**	**Median**	**Min**	**Max**
Day	203	18.68 ± 1.15	15.31	0	134.84
Night	232	41.09 ± 2.52	29.4	3.16	236.24

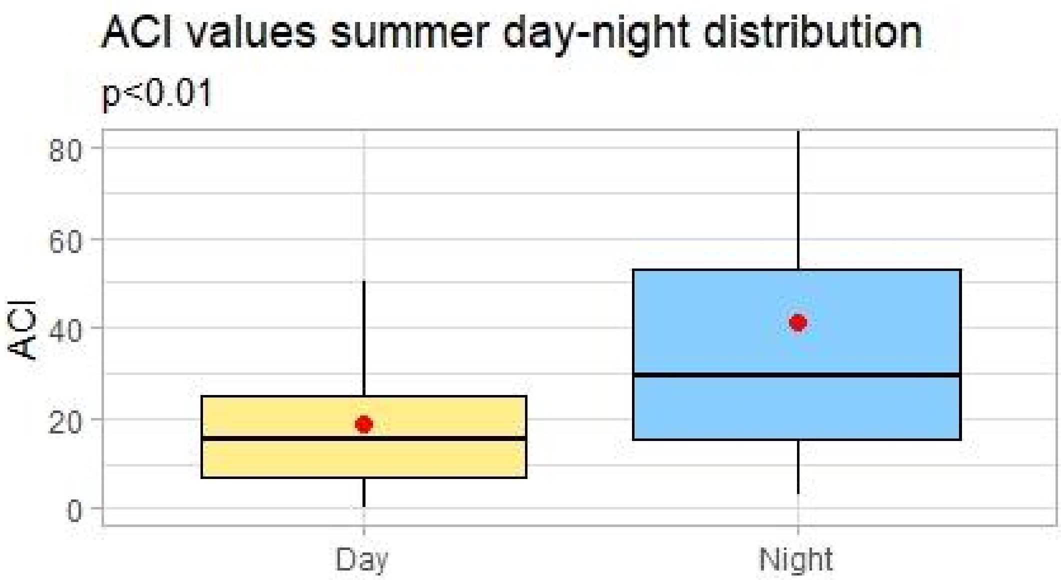


Figure 29_Boxplot graphic representing ACI values summer day-night distribution. On the X-axis Day and Night are shown; on the Y-axis ACI values. Night has higher mean values (red dot) and higher median than day.

4. DISCUSSION

In this study, we investigated the underwater soundscape in front of the Conero Park applying the ACI index. We conducted a comparison of ACI values between all seasons, finding a significant difference between them. Also, pairwise comparisons revealed a significant difference. Summer ACI values were higher than other seasons, according to the major presence of fish vocalisations during this warmer period. Also, autumn values were high, probably due to fish vocalizations still present in September. These results are related to the reproductive period of sonoriferous local fish species (as *Ophidion rochiei* found in our recordings) which typically occur during warmer months (from June to September). The emission of many vocalizations by fish is useful for partner searching and mating. At the same way, focusing on summer, night values are higher than daily ones (Kéver et al., 2016), because local soniferous species are typical nocturnal. During the day, remain hidden in the sandy bottom and at night they emerge for feeding or mating (Dulčic et al., 2002).

Our results show how this index responds positively to the presence of fish vocalizations in audios (higher ACI values) compared to files without biological sounds (lower ACI values). This could be a good indicator to study sonoriferous species in their natural habitat and could allow spatial-temporal comparisons of sound species community, without disturb them. In this way, the ACI index could be a useful tool to investigate biodiversity using a passive non-invasive acoustic method. As reported by Harris et al. (2016b) the ACI Index appears to be desirable instrument to use as a proxy of the diversity of the reef fish assemblage. As also demonstrated in literature, the Acoustic Complexity Index, considering an intermediate temporal resolution and all frequency distribution, is a good descriptor of the biophonies of the marine soundscape (Farina et al., 2016). Results showed that the Acoustic Complexity Index could be a good proxy for marine acoustic activity (Farina et al., 2016). Acoustic indices, in general, reduce the enormous complexity of the soundscape to a single number, greatly simplifying extraction of information from recordings (Bradfer-Lawrence et al., 2019). These results can allow to extend the study and the application of this index also on other types of habitats.

The calculation of ACI can be done for a single frequency bin, focusing on single species, or for the entire set of frequencies, allowing the analysis of multiple species or complex acoustic pattern.

However, there are some limitations in the use of the ACI index. The distance between the recorder and the biological sound source is important, because in case of long distance, the biotic sound is reduced, especially in high frequency and by atmospheric conditions (rain, wind, waves) or anthropogenic sources (Farina & Pieretti, 2014). From our analysis we noted that ACI index is affected by the wind speed, the wave motions, and the presence of ship noise. These noises cause an increase of ACI values, because of their high intensities. Therefore, improvements in this context should be made to better exclude loud background noise.

We also observed that this parameter is influenced by operator choices (e.g., in the choice of timescale or in the frequency range) which can determine changes in the ACI values obtained. Previous works in which ACI Index was applied, used short audio (e.g., 10 min/hour during 3 nights in June and July (Farina et al., 2016) or 2 min/15 min for 3 nights days during austral summer in March (Harris et al., 2016b). Other investigations have analysed long audios, but in reduced number (20 files of 180 min (Pieretti et al., 2011). In the present work we analysed a total of 67 audio of 30 minutes each, covering all seasons (from February 2020 to July 2021) and collecting both day and night hours. This can give us an example of how the ACI can be applied even on longer recordings, allowing application on continuous long-term monitoring.

Our study has been conducted near a mussel farm, where fish might utilize the artificial structures as shelter, feeding as well as spawning and nursery area. Furthermore, the farm also acts as a buffer area, as human access is limited (Picciulin et al., 2016). In this way, it is possible to record many fish vocalizations belonging to local species without many anthropogenic interferences.

Acoustic indexes have not so many applications in marine research, therefore future studies could be done to deeper investigate their application on marine recordings, increasing our knowledge on acoustic biodiversity.

5. CONCLUSIONS

The present study is focused on the application of the Acoustic Complexity Index (ACI) to investigate the underwater soundscape in front of the Conero Natural Park. We found that ACI well recognised audio containing biotic sounds, presenting values higher than audio without vocalizations. It was also possible to investigate the different ACI responses during all seasons. Summer registered the highest ACI values, because of the presence of many fish vocalizations related to their reproductive period in the warmer season. Furthermore, during summer, nocturnal ACI values are higher than diurnal ones due to a greater communicative activity of the fishes at night.

Acoustic indices have been created and applied mostly in terrestrial temperate ecosystems. Marine ecosystems are rather more complex; consequently, further investigation is necessary to assess influence of vocalizations on acoustic indices. It is important to understand the biophonies of an underwater ecosystem to better asses the present and future anthropogenic impact on it.

We conclude that acoustic indices have the potential to be used as a complementary tool for investigating spatial and temporal patterns of biodiversity in coastal marine ecosystems.

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