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Application of passive acoustic methodologies for the marine biodiversity assessment in Mediterranean Sea.

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“When we take steadfast action and push ever forward no matter what we may encounter in life, the dawn of victory will certainly arrive.”

List of contents

Application of passive acoustic methodologies for assessments of marine biodiversity in Mediterranean Sea

INTRODUCTION

Passive Acoustic Monitoring (PAM) and Ecoacoustics

The marine environment plays a fundamental role in the climatic balance representing a valuable source of economic resources, social well-being and quality of life (Harley *et al.,* 2006; Duarte *et al.,* 2021). The quality of marine ecosystems is given by a variety of aspects where "sounds" vest a fundamental importance in marine life (Simpson *et al.,* 2005; Radford *et al.,* 2011; Stanley *et al.,* 2012; Lammer and Munger, 2016; McWilliam and Hawkins, 2013; Pearson *et al.,* 2020).

Acoustic signals propagate quickly with a low coefficient of absorption and the sound is often detectable at much longer distances than other biological activity signals (light, chemical cues) travelling at about 1500 m per second and potentially propagating for hundreds of kilometers, as in the case of low-frequency whale communications (Ainslie, 2000). Many marine animals depend on sound and strategies have been adopted to convey information through acoustic signals to satisfy several needs, such as foraging, navigation, predation (or detection of predators or competitors) and habitat selection (Simpson, 2005).

A number of coastal marine species are known to emit sounds across a wide-frequency interval ranging from 10 Hz to over 20 kHz contributing to the marine shallow water soundscape. Crustaceans such as the snapping shrimp (*Synalpheus sp*.) (Au *et al.,* 1998), the California spiny lobster (*Palinurus interruptus*) (Patek *et al.,* 2009) and urchins (Radford *et al.,* 2008) produce sounds for feeding or against predators; fish do so (Amorin, 2016) to increase social cohesion (Van Oosterom *et al.,* 2016), protect their territory and in courtship rituals. Finally, the most studied species are dolphins and whales that show the most complex vocal repertoire (Tyack and Clark 2000). In this context, ambient sound is investigated using the method of *Passive Acoustic Monitoring* (PAM) which is increasingly applied to investigate the vocal repertoire of the marine organisms without the introduction of any interference, disturbance or stressing factor, and evaluate biodiversity loss aimed at conserving the marine environment (Desjonquères *et al.,* 2015; Erbe *et al.,* 2015; Buscaino *et al.,* 2016; Menze *et al.,* 2017; Mooney *et al.,* 2020; Stanley *et al.,* 2021). Passive acoustic monitoring, involves surveying and monitoring wildlife and environments using sound recorders (acoustic sensors). These are deployed in the field, often for hours, days or weeks, recording acoustic data on a specified schedule. After collection, these recordings are processed to extract useful ecological data such as detecting the calls of animal species of interest which is then analyzed similarly to other types of survey data.

In the past two decades, the development of autonomous, cost-effective recording units has revolutionized air and underwater acoustics (Mennill *et al.,* 2012; Sousa – Lima *et al.,* 2013) which are more and more frequently applied to investigate and monitor the marine environment. PAM offers several advantages in the effort-information ratio contributing to a huge volume of easily accessible data on aquatic life. It also provides information on a large spatial and temporal scale to facilitate the study of cetaceans' distribution and migration patterns (Davis *et al.,* 2020; Warren *et al.,* 2021), characterize fish choruses' spatial and temporal responses to environmental drivers (e.g., Rountree *et al.,* 2006; Parsons, 2010; Rice *et al.,* 2016; McWilliam *et al.,* 2017; Parsons *et al.,* 2016; Karaconstantis *et al.,* 2020; Linke *et al.,* 2020) and understand how animals change their behavior and distribution in response to climate change (Gordon *et al.,* 2018). ixed autonomous acoustic recorder is faster and less expensive than traditional instruments (portable acoustic data acquisition systems) and it is non-invasive reducing the bias of human presence (Marques *et al.,* 2013; Harris *et al.,* 2016). These recording devices (autonomous recorders [ARs]) are defined as any electronic recording system that acquires and stores acoustic data internally (i.e., without a cable or radio link to transmit data to a receiving station). These devices are placed semi-permanently underwater (anchored to a mooring, buoy, or the seabed), programmable for a set time of recording and have to be retrieved for the data access (Sousa-Lima *et al.,* 2013). PAM is expanding thanks to the progressive development in technology, providing a large volume of easily accessible data on aquatic life. Otherwise, the huge amount of data collected with PAM needs an ecological approach and new methods to manage, analyze and extrapolate information from them. Ecoacoustics is an emerging discipline that is concerned with the analysis of environmental sound recordings for ecological purposes (Sueur and Farina, 2015) such as describing species and communities' life traits and offers an important contribution to addressing ecosystem management and conservation (Radford *et al.,* 2010; Pieretti and Farina, 2013; Piercy *et al.,* 2014; Farina *et al.,* 2016). It is closely related to research under the heading of Soundscape ecology defined as the sum of all the sounds present in an environment in a given time interval (Krause, 2002), which has become a useful instrument to evaluate the changes in habitat over time, as a response to external pressures.

The International Organization for Standardization (ISO 18405) defines soundscape as the characterization of the ambient sound in terms of its spatial, temporal, frequency attributes, and the types of sources contributing to the sound field. Defining and characterizing the soundscape is an important step in the task of assessing, monitoring, and comparing global acoustic environments. By utilizing soundscape analysis and "ocean sound", researchers can better understand ocean dynamics

(Radford et al., 2010; McWilliam and Hawkins, 2013; Miksis-Olds *et al.,* 2013), biodiversity and ecosystem health (Staaterman *et al.,* 2014), and the risk of anthropogenic impacts on marine life (Weilgart, 2007; Carroll *et al.,* 2017). The use of sound as a source of information instead, not only allows to collect data through a non-invasive approach (without interfering with the system dynamic) but also to obtain a broad spectrum of information simultaneously from all the sonic components. Soundscape ecology uses the sound of the environment in its entirety to draw information concerning eco-acoustic events in space and time (Farina *et al.,* 2016). Soundscape can be split into three main sound aggregations: "anthropophony"; man-made sounds deriving from naval traffic, underwater construction and seabed exploration (Hildebrand, 2009; Ross,2015); "geophony"; sounds generated by geophysical and meteorological events such as wind, atmospheric precipitation, and breaking waves (Nystuen *et al.,* 1993; Haxel *et al.,* 2013), "biophony"; all sounds produced by biota (Pijanowski et al., 2011; Krause, 2008).

The soundscape approach allows to analyse the acoustic components of the environment and their interconnection to understand ecological processes, marine ecosystems dynamics and potential biological associations on a wide range of spatial and temporal scales (Farina and Gage, 2017). Furthermore, it has been demonstrated that the diversity of the acoustic community (biophonic diversity) is a trustworthy representation of ecological biodiversity (Sueur *et al.,* 2008; Eldridge *et al.,* 2018). Even though marine ambient sound and soundscape research has been growing over the last decades, the researchers' community has still not reached a consensus on the optimal way to accurately report and compare important aspects of sea sound. So far, the soundscapes of different marine coastal habitats have been investigated worldwide, where special attention has been given to biophony and human-made components (McWilliam *et al.,* 2013; Werren *et al.,* 2021). First studies have investigated marine soundscape on coral reef sites in the Pacific (Andrew et al., 2002; Bertucci *et al.,* 2015), Atlantic (Axelrod *et al.,* 1965; Staaterman *et al.,* 2013) Indian ocean (Cato *et al.,* 2002; Parsons *et al.,* 2013). However, in the last ten years, many important articles have been published dealing with underwater bioacoustics and ecoacoustics of Mediterranean Sea habitats mostly within Marine Protected Areas (MPAs) (Corrias, 2014; Buscaino *et al.,* 2016; Pieretti *et al.,* 2017; Ceraulo *et al.,* 2018; Desiderà *et al.,* 2019; La Manna *et al.,* 2021). Although the creation of MPAs helps to reduce human impact on coastal habitats, their planning does not consider underwater noise propagation. Actually, acoustic energy can propagate beyond the boundaries of no-take zones in marine protected areas (Weilgart, 2006; Buscaino *et al.,* 2016). Recently, Picciulin *et al.,* 2022 described the spatio-temporal variability of the marine coastal soundscape of the Cres-Lošinj Natura 2000 Site of Community important its environmental dynamics and local acoustic habitat. This study is an outcome of the SOUNDSCAPE project, funded by the European Union, which for the first time has been dedicated to expanding the information on this topic in the Adriatic Sea, proposing a very rigorous and structured approach over the long period.

Underwater noise pollution and Marine Strategy Directive Framework (MSDF)

The State of the Mediterranean Marine and Coastal Environment Report (UNEP-MAP 2012) highlights key points and important actions to take in order to reduce anthropogenic impacts on the Mediterranean system: The main threats affecting marine species and habitats are: 1) Chemical contamination caused by urbanization, industry, and agriculture (eutrophication); 2) marine litter; 3) overexploitation of fish stocks, in particular some species classified as vulnerable or threatened; 4) non-native invasive species; 5) range abandonment and migration; 6) fragmentation of coastal areas and underwater environment;7) habitat degradation and biodiversity loss; 8) marine noise affecting vertebrate and invertebrate species' health and survival.

Noise is considered one of the major pollutants of the 21st century, appearing in the 1982 United Nations Convention on the Law of the Sea (UNCLOS, 1982) and after more than 25 years in the Marine Strategy Framework Directive 2008/56/EC.

Several scientific studies highlighted a conflicting relationship between noisy human activities and the ability of marine species (on crustacean, fish and marine mammals including the bottlenose dolphin) to hear natural sounds for their survival. (Payne and Webb, 1971; Richardson *et al.,* 1995; Evans and England, 2001; Gordon *et al.,* 2004; Hildebrand, 2005; Di Franco *et al.,* 2021). Considering this, environmental regulators have welcomed the indications of the scientific community, which is unanimous in considering noise produced by anthropogenic activities (naval traffic, sonar, geoseismic surveys, etc.) to be a factor in impacting the quality of marine ecosystems. The development of the marine industry generated an increase in underwater noise across the globe. From 1950 to 1970, noise from maritime traffic increased by 1-2 dB per year in the underwater environment causing a large-scale change in noise levels (Ross, 2005; Hildebrand, 2009; Durante, 2021). In the last century, there has been a large increase in the number of noise sources, which resulted in a large increase in the background noise in oceans (Andrew *et al.,* 2002; Peng et al., 2015; Johansson *et al*., 2016; Tyack, 2008). The scarcity of information regarding underwater noise in the Mediterranean Sea makes small-scale studies useful for constructing a complete picture of the possible anthropogenic noise sources. In the Mediterranean Sea, Codarin and Picciulin (2015) found a mean ambient noise level of 121.3 dB re 1 μPa in Trieste Gulf (Italy), which showed values that were similar to those reported by Picciulin *et al.,* (2013) (129.8–138 re 1 μPa) in the Venice lagoon (Italy). Additionally, values exceeding 100 dB re 1 μPa were found by Viola, *et al.* (2017) in the Sicilian Channel, where an acoustic observatory is located in proximity to a commercial port and shipping lanes. However, underwater noise in coastal waters has been poorly described (Samuel et al., 2005; Radford *et al.,* 2010; Bittencourt *et al.,* 2014; Lillis *et al.,* 2014), and there are few data regarding the Italian coastal waters (Codarin and Picciulin, 2015; Azzellino *et al.,* 2011).

The Marine Strategy Framework Directive (MSFD) 2008/56/EC, EU, 2008. Established a transparent and coherent legislative framework to protect and safeguard the marine environment hence counteract the gradual deterioration of marine habitats through an ecosystem approach in order to achieve a better balance between conservation and sustainable exploitation. State members will make concrete commitments to strive for the Good Environmental Status (GES) of sea waters by 2020, currently under review following a back -to back evaluation and impact assessment, known in short as "*environmental status*". According to that, they will be supposed to preserve the ecological diversity and vitality of seas and oceans keeping them clean, healthy and productive in their intrinsic condition as well as ensuring a sustainable marine environment to safeguard their potential for present and future generations (2008/56/EC, Article 3). The Directive identified eleven descriptors for which each group of experts described criteria and standard methods to periodically assess and quantify the quality of sea waters as stated in the "Decision 477/2010/EU issued on 1st September 2010 by the European Commission" (Fig.1).

Figure 1. Eleven descriptors of Marine Strategy.

In order to achieve or maintain GES, authorities should address environmental targets, implement programs and take specific measures.

Underwater noise levels are parameters of environmental quality thus state members have to cooperate, directly or through competent international organizations, to promote studies and develop scientific research programs concerning underwater noise pollution, exchange information and data and update their regulations based on the obtained results.

The bottlenose dolphin as target species

Bioacoustics studies the vocalizations of individual species. Sometimes only it considers possible relationships between different species, as in the case of alarm signals and the prey-predator relationship, without a full understanding of the interaction between all the community members. The conventional marine bioacoustics approach is fundamental to identify and distinguish the biological components represented in the underwater environment (Farina and Gage, 2017). It investigates how species have adapted to the acoustic repertoire of different environments and different sets of species which they compete with to gain an acoustic niche (Farina and Gage, 2017). A key challenge of using acoustic sensors to study the behavior of free-ranging animals is that individual vocalizing animals are rarely identifiable from acoustic recordings alone, apart from special cases like some songbirds (Kirschel *et al.,* 2009; Petrusková *et al.,* 2015) and odontocetes (Fripp *et al.,* 2005), where individuals have an identifiable acoustic "signature" or repertoire. However, acoustic data can still offer an insight in the spatiotemporal patterns concerning the study of wild animals' acoustic behavior, and how they relate to the environment (Miller *et al.,* 2013; Samarra *et al.,* 2016).

Marine mammals are acoustic specialists and whistle "signatures" are a peculiar feature in the bottlenose dolphin's repertoire (*Tursiops truncatus*). The Mediterranean Sea subpopulation is listed in Appendix II (Strictly Protected Fauna Species) of the Conservation of European Wildlife and Natural Habitats Convention (Bern Convention), the Appendix II of the Conservation of Migratory Species of Wild Animals Convention (CMS), and under the Annexes II and IV of the EU Habitats Directive (Council Directive 92/43/EEC). According to the International Union for the Conservation of Nature (IUCN) Red List criteria, the conservation status of the bottlenose dolphin's Mediterranean sub-population has changed from Vulnerable to Least concern in the last decade (Bearzi *et al.,*2012; Natoli *et al.,* 2021).

Given the overlap between their preferred habitat with various fishing grounds of both professional and artisanal fisheries and their opportunistic nature, especially in relation to foraging strategies, bottlenose dolphins are potentially prone to get entangled in many types of fishing gears (ACCOBAMS 2019). By-catch events are widespread throughout the region; however, the scale of the pressure is little known due to both the diversity of study protocols and the lack of observations and reports continuity.

Its tendency to regularly interact with humans is due to the peculiar ecology, which describes *T. truncatus* as strictly associated with coastal areas in the Mediterranean Sea (Panigada *et al.,* 2011). The "opportunistic" behaviour, besides increasing the risk of accidents, also alters the dolphins' natural feeding behaviour (López, 2006). In the last decades, several studies have been carried out to find solutions to the phenomenon of the interaction of dolphins with fishing gears, minimize the incidental captures and reduce the impact on the fishing industry (Brotons *et al.,* 2008; Barlow and Cameron, 2003). During the aforementioned studies, active and passive acoustic devices were used (Gearin *et al.,* 2000; Buscaino *et al.,* 2009; Corrias *et al.,* 2018; Corrias *et al.,* 2021) and new materials to manufacture fishing nets were tested to reduce the "interception" of dolphins' catch (Northridge, 2018).

The focus of my work was the investigation of marine species and principal component of habitats using non-invasive acoustic methods, to identify the acoustic repertoire of marine target species and understand the relationships that exist between biotic and abiotic factors through an ecosystem approach at different levels of information.

Furthermore, this work may offer data on average noise levels recorded in an area with a high concentration of anthropogenic activities including areas of conservation interest to achieve a more accurate definition of noise thresholds according to indications provided by the European Directive referred to Descriptor 11.

This study investigated:

1. the Gulf of Oristano marine soundscape, a shallow inlet on the west coast of Sardinia including a special conservation area (Habitat Directive) and a national marine protected area using passive acoustic monitoring (PAM) and in particular:

- the soundscape temporal pattern with main acoustic components (*biotic and abiotic*) and the acoustic source considering both time extension and frequency band.
- seasonal and circadian variations of environmental sound pressure levels (SPL dB re 1µ Pa).

2. biological component through cross-sectional approaches represented by the bottlenose dolphin as target species, an acoustically sensitive species in relation to its distribution, habitat use, the acoustic behavior and response to disturbances.

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Chapter 1

Marine soundscape and its temporal acoustic characterization in the Gulf of Oristano, Sardinia (Western Mediterranean Sea)

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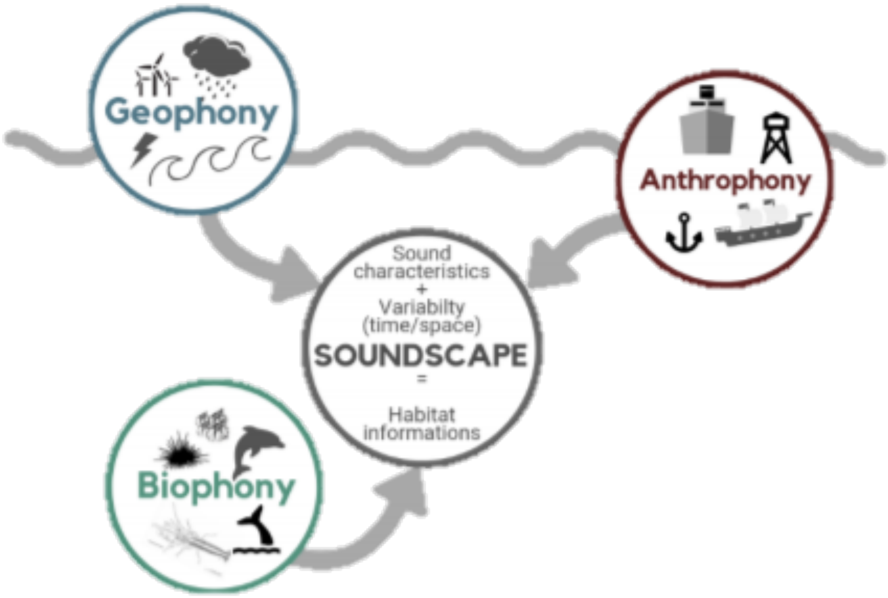

Credit. *Campedbell et. al., 2019*

Keywords: passive acoustic monitoring, anthropogenic noise; acoustic behaviours; Mediterranean habitat, marine soundscape, Covid-19 pandemic.

Abstract: The soundscape of a marine environment is a combination of geophonies, biophonies and anthrophonies. The soundscape of the Gulf of Oristano was investigated, a shallow inlet on the western coast of Sardinia including a special conservation area (Habitat Directive) and a national marine protected area. Data collection was carried out during July 2019, November 2019 and May 2020 using underwater acoustic equipment. Study's goal was to characterise ambient sound levels ("Sound Pressure Level; SPL dB re 1µ Pa") and describe soundscape main components. Soundscape showed significant circadian and seasonal variations: lowest and highest median values of SPL were in Spring (120-140 dB re1µPa) *post Covid-19 pandemic lockdown* and in Summer (128-150 dB re 1µPa) respectively. *Biophonies* were identified and characterised as dolphins' "click" and crustaceans' "snap". Shrimp's activity was dominant in Summer, dolphins' passages were present across all sampling periods, accounting for 46.4% of total recordings. *Anthropophonies,* namely vessel passages, were predominant in summer daytime and represented up to 42% of the acoustic space in the low frequency band. *Geophonies* increased low-frequency noise, and represent a highly variable component of local soundscape. Marine soundscape is a valuable tool to define integrated management plans for a marine ecosystem, allowing to assess habitat quality, characterise sound sources and evaluate impact of anthropogenic activities.

1.Introduction

The sources and acoustic characteristics of all biotic and abiotic ambient sounds in a given environment and time are collectively defined as the "soundscape" (Pijanowski, *et al.,* 2011; Urick, 1983. Soundscape shows great potential in describing characteristics of marine ecosystems and in detecting change within them (Farina *et al.,* 2014). It represents an effective and complementary tool to understand dynamics of marine ecosystems and potential biological association over a wide range of spatial and temporal scale. Soundscape can be split into three principal sound aggregations: "anthropophony"; man-made sounds deriving from naval traffic, underwater construction and seabed exploration (Hildebrand, 2009); "geophony"; sounds generated by geophysical and meteorological events such as wind, atmospheric precipitation, and breaking waves Nystuen *et al.,* 1993; Haxel *et al.,* 2013), "biophony"; all sounds produced by biota (Pijanowski, *et al.,* 2011; Krause, 2008). The complexity of the habitat, in terms of community structure, can be connected with the diversity of the biophony component (Kennedy *et al.,* 2010). A number of coastal marine species are known to emit sound across a wide frequency interval ranging from 10 Hz to over 20 kHz and contribute to the marine shallow water soundscape. Crustaceans such as snapping shrimp (*Synalpheus sp*.) (Au *et al.,* 1998) and spiny lobster (*Palinurus interruptus*) (Patek *et al.,* 2009), and urchins (Radford *et al.,*

2008) produce sounds for feeding or against predators; instead, fish (Amorin, 2016) to increase social cohesion (Van Oosterom *et al.,* 2016) and for courtship rituals as well as territorial protection.

Finally, the most studied species are dolphins and whales, that show the most complex vocal repertoire (Tyack and Clark, 2000). In shallow waters, geophonies are sounds generated by rainfall, wind and waves in the underwater environment. Wind-dependent noise is the prevailing sound in many of the world's seas and its frequencies are between 100 Hz and 20 kHz, typically peaking around 500 Hz (Haxel *et al.,* 2013; Knudsen *et al.,* 1948; Wenz, 1962). The increase in anthropogenic activity in the coastal environment implies high risk of pollution (Duarte *et al.,* 1962) and different coastal habitats are exposed to different levels of human pressure due to fishery, commercial shipping and touristic activities (Halpern *et al.,* 2008). In coastal waters, ship noise is the main anthropogenic source, representing up to 90% of the acoustic energy spread by humans into the sea encompassing low frequencies ranging from 125 Hz to 2 kHz (Dahl, 2007). Indeed, noise is a critical component of marine environments, and in the last decades, underwater noise levels increased dramatically, at a rate of 3 dB per decade (Pine *et al.,* 2016; Andrew *et al.,* 2002). Within the European Marine Strategy Framework Directive (MSFD 2008/56/EC), underwater noise has been recognised as an environmental pollutant. To stem the effects of noise on biodiversity, the Directive identified underwater noise as one of the 11 environmental quality descriptors (Tasker *et al.,* 2008). Descriptor 11 "underwater noise" include two different sub-programs regarding the monitoring of acoustic sources: 1. evaluate impulsive sounds measuring anthropogenic sources in the range of the frequency band 10 Hz - 10 kHz and, for cetacean detection, up to 40 kHz; 2. continuous low-frequency monitoring trends in the ambient noise level within 63 and 125 Hz (centre frequency re 1 m Pa RMS) in a long time via observation stations. The objective of the directive is to implement a system for recording temporal and spatial data on impulsive sound sources from anthropogenic activities. The program also wants to define the level of damage caused by underwater noise on diverse marine organisms. Anthropogenic noise represents a stressor for marine species as it masks vocal communication and alters their health and fitness thus affecting their survival (Celi *et al.,* 2016; Filiciotto *et al.,* 2014; Papale *et al.,* 2015; Clark *et al.,* 2015; Codarin *et al.,* 2009; Erbe *et al.,* 2016). Although biological sound studies in soundscape are growing, there are still little data to evaluate the actual impact of anthropogenic noise on the marine ecosystem and species. Worldwide, few studies have investigated marine soundscape on coral reef sites in the Pacific (Andrew *et al.,* 2002; Bertucci *et al.,* 2015), Atlantic (Axelrod *et al.,* 1965; Staaterman *et al.,* 2013) Indian (Cato *et al.,* 2002; Parsons *et al.,* 2013;) Ocean, and Mediterranean Sea habitats (Buscaino *et al.,* 2016; Pieretti *et al.,* 2017; Ceraulo *et al.,* 2018;)

This work aims to identify the main contributing components of the marine underwater soundscape of the shallow waters of the Oristano Gulf (western Mediterranean Sea), to describe the temporal variation of the ambient sound pressure levels in broadband SPLs and the octave band sound pressure levels (SPL) and also provide information on changes in marine acoustic underwater resulting from the Covid-19 pandemic lockdown. Finally, it would contribute to the goals of the MSFD providing new insights to better define the criteria for the assessment of Good Environmental Status (GES). The present study supports the benefits of using passive acoustics monitoring (PAM) in marine ecosystem studies providing useful baseline information for management strategies in an area where protected areas (a special area of conservation ITB030080 Directive 92/43/EEC and a national marine protected area) and human activities (fishing, fish farming, industrial port and commercial maritime traffic) coexist. PAM has been recently recognized by the Global Ocean Observing System (GOOS) commission as a reliable tool for assessing the status of the ocean's biodiversity, with the designation of the underwater sound as Essential Ocean Variable (EOV) (Tyack *et al.,* 2017).

2. Materials and Methods

2.1. Study Site

Data collection was carried out in the shallow waters of the Gulf of Oristano, which is located along the western coast of Sardinia (Italy), the second largest island of the Mediterranean Sea. The acoustic recordings were performed in a selected site within the Gulf that is a semi-enclosed water basin with an average depth of about 15 m and a maximum depth of about 25 m. The Gulf covers an area of 150 km^2 and it is connected to the Sardinian Sea across a span of 9 km between Capo San Marco at the north and Capo Frasca at the south. The dominant winds are the Mistral from north-west, the Libeccio from south-west and the Sirocco from south-east (Pinna, 1989). The Gulf of Oristano is distinguished by hosting high marine biodiversity (Magni *et al.,* 2008). The seafloor consists of more than 70% *Posidonia oceanica* meadows (Directive 92/43 of the EEC, Habitats Directive) and about 30% areas of sand and rocks, representing a substrate suitable for a variety of organisms such as fish, crustaceans, molluscs (Cangemi *et al.,* 2000; de Falco *et al.,* 2006; Coppa *et al.,* 2019). In addition, it appears to be a potential feeding area for small cetaceans (Corrias *et al.,* 2019; Würsig and Gailey, 2002).

2.2. Acoustic sampling and data acquisition

Underwater acoustic recordings were undertaken at geographic coordinates 39.884167°N - 8.500833°E (Figure 1 A). Three recording sessions of 10 days each, from July 2019 to May 2020 were carried out as follows: Summer 2019 (July 8-18; SU19); Autumn 2019 (November 8-18; AU19); Spring 2020 (May 8-18; SP20). Data were collected using a PAM (*Passive Acoustic Monitoring*) station equipped with an autonomous recorder, model Urek 384 (NAUTA Srl), consisting of an omnidirectional hydrophone (Sensor Technology SQ26-05) with a sensitivity of −169 (± 2) dB re V/µPa from 0.1 to over 50 kHz, the frequency range was 0.1-96 kHz with a duty cycle of 20%. The recorder was programmed to record sounds for 3 minutes every 12 minutes for a total of 12 minutes every hour for 24 hours at a sampling rate of 192 k-samples s-1 16-bit resolution without further preamplification; no filters were applied during the recordings for the set-up and management of acoustic data, the app UM283BLE developed by Dodotronic Srl was used. The autonomous recorder was deployed on the seabed together with a 15 kg stone ballast and a small buoy to keep the hydrophone in vertical position and also well secured in case of strong currents or bad weather (the hydrophone was at 1.7 m from the bottom) (Figure 1-B). The device was located at a depth of 12 m, 1 nm from the coastline and 1,5 nm from the boundaries of the local Marine Protected Areas (MPA) Penisola del Sinis – Isola di Mal di Ventre (partial reserve).

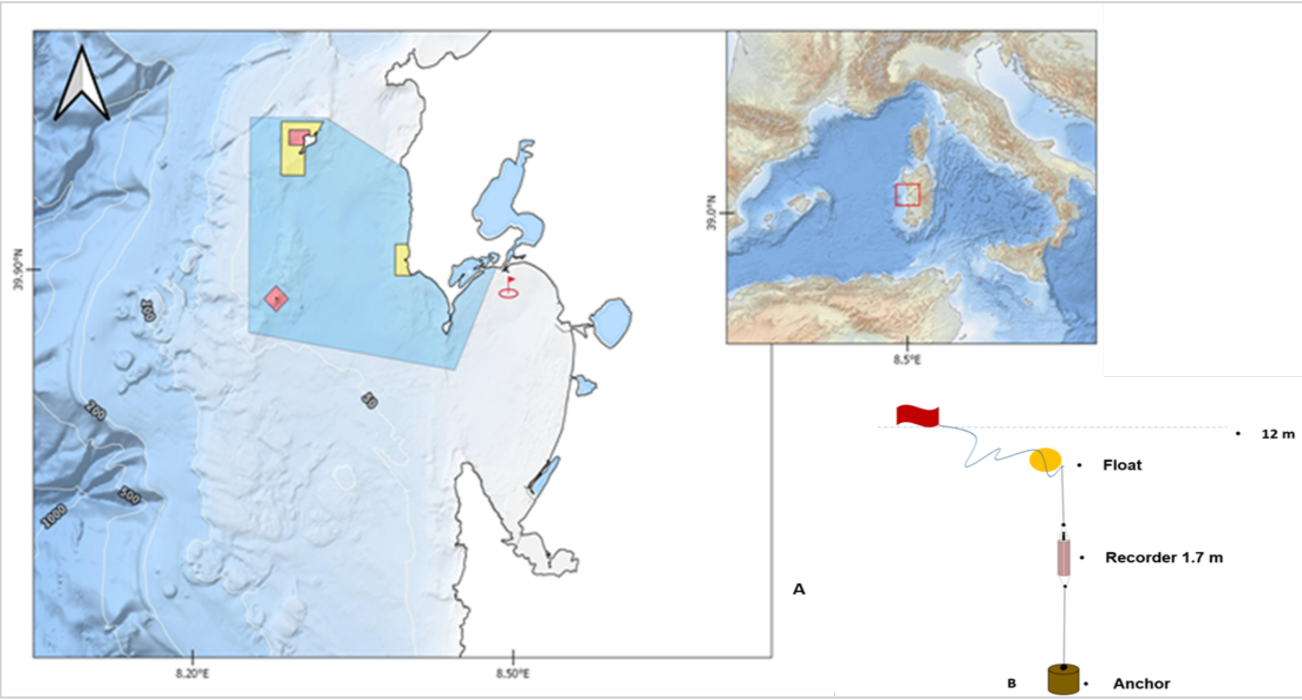

Figure 1. A) Map of the study area (Gulf of Oristano) located on the western coast of Sardinia, Italy. The red symbol indicates the site where the deployment system was located; differently coloured areas correspond to different protection level of the Penisola del Sinis – Isola di Mal di Ventre MPA. B) Scheme of the underwater recorder deployment.

2.3. Acoustic data processing and analysis of the main soundscape components

The entire dataset was aurally and visually inspected through the spectrogram, time vs. frequency graph (1024 Fast Fourier Transform size point, overlap 50% Hamming window and logarithmic frequency scale), by using the RX5 audio editor software (iZotope Cambridge, MA, United States) in order to assess the quality of the recording and rule out the presence of noises generated by the acoustic instrument for each audio file. The more suitable acoustic recordings were scrutinized to detect local marine soundscape's main components and therefore obtain a qualitative representation of it in terms of soniferous species (*Biophonies*), anthropogenic sources (*Anthropophonies*), and marine weather events (*Geophonies*). Identification and description of acoustic signatures were carried out by combining manual and automatic analysis. Firstly, all the acoustic signatures were manually analysed in the 0.2 – 96 kHz frequency broadband and in the time domain by an expert operator. Since the identification of some biological sources was not entirely certain, these were first characterized acoustically. Furthermore, social communication signals (whistles) were also observed in the audio files, but these were not analyzed in this work. Due to the disturbance of ships passages and rainfall (100 - 1 kHz), few fish signals present in the dataset were not possible to identify and acoustically characterize the parameters.

Impulse signals were selected and extracted from the best acoustic files, i.e., those in which neither vessel passages nor other disturbances occurred and with a quality signal-to-noise ratio. The analysis was carried out using the semi-automatic routine "Pulse Train Analysis" in AvisoftSAS Lab Pro (Avisoft Bioacoustics, Germany) with predefined threshold values for both pulse signal types. Clicks and Snap pulse were identified through the Pulse Train Analysis in Avisoft SAS-Lab Pro (Avisoft Bioacoustics, Germany), using the methods of envelope modification "RMS+decimation" and pulse detection "Peak search with Hysteresis". For each pulse signal and train of signals, was measured: duration (s), peak of frequency (Hz) and bandwidth (Hz) (for single signals); number of pulses (n) and pulse rate (n/s) (for trains of signals). In addition, to describe the circadian activities of crustaceans, the Power Spectrum Density (PSD) for a subsample of 24 hours during the greatest acoustic activity occurring on the full moon days (14.07.19; 12.11.19; 08.06.20) was also calculated (Lammer *et al.,* 2008). The PSD was calculated using FFT 512 (50% overlap) for the frequency band value of 16,500 Hz at which the maximum crustacean activity is expressed. The total seasonal occurrence of dolphins in the area was calculated as a percentage of the number of acoustic presence (click signals) on the total number of recordings. The total number of vessel passages were counted for all files, whilst the presence of fishing gears (sonar and acoustic deterrent device ADD) and rain and wind events, were considered within each file. Each group of sources was tested for non-normal distribution in order to evaluate differences in the seasonal composition of acoustic sources, Mann-Whitney pairwise test was applied.

2.4. Acoustic measurement and temporal pattern in sound pressure level

Variation pattern of the ambient sound level was investigated for all data acquired during the sampling periods (SU19, AU19 and SP20); broadband sound pressure levels (SPL dB re 1 μ Pa rms) and octave band sound pressure levels (SPL dB re 1 μ Pa rms) were calculated. For each file wav. (12 minutes per hour), the pressure level root-mean-square octave band was also calculated (SPL dB re 1 μ Pa, rms) for 11 octaves frequency centred respectively at: 63 (44 –88), 125 (88 – 177), 250 (177 – 355), 500 (355 – 710), 1000 (710 – 1420), 2000 (1420 – 2840), 4000 (2840 – 5680), 8000 (5680 – 11360), 16000 (11360 – 22720), 32000 (22720 – 45440), and 64000 (45440 – 90880) Hz. Analysis of the low-frequency bands (from 63 to 1 kHz) was in accordance with European Marine Strategy Directive 2008/56/EC11 Framework (Descriptor 11) in order to describe acoustic underwater noise level in the marine ecosystem. The broadband SPL was determinate to compare sound level seasonal variations, whereas the octave bands (SPL) were used to investigate seasonal and daily trend (day from 09:00 a.m. to 04:00 p.m. and night from 09:00 p.m. to 04:00 a.m.). The analysis was performed via automatic routine using SASLabPro software (Avisoft bioacoustics, Glienicke, Germany). Median, 5 th percentile and 95 th percentile were calculated for broadband and octave bands. Data were not normally distributed and therefore Kruskal-Wallis test was applied to assess differences in broadband (SPL dB re 1 μ Pa) between seasons, whilst Mann-Whitney U test was used to highlight differences between day and night-time. Finally, to evaluate seasonal differences in octave band sound pressure levels (SPL dB re 1 μ Pa) the Mann-Whitney pairwise test was used. In this work, statistical analysis was run with STATISTICA v.7.0 (Stat Soft, Tulsa, OK, USA).

3. Results

Results presented here illustrate temporal pattern of the main underwater soundscape components, variation of broadband sound pressure levels (SPL dB re 1μPa) together with variation of octave band sound pressure levels (SPLs dB re 1μPa) in the study area for three 10 days sampling period SU19 (n = 2,642 file wav), AU19 (n = 2,016 file wav) and SP20 (n = 1,996 file wav) (after *CODIV-19 pandemic lockdown*). Results of data recorded (n = 6,664 file wav) from July 2019 to May 2020 for a total of about 144 hours processed are here reported.

3.1. Main soundscape components identification, description and variation in acoustic signatures

Each season highlighted distinct characteristics of the abiotic and biotic occupancy of "acoustic space" in the Gulf of Oristano. A "snapshot" of the predominant components and a detail of the seasonal and hourly variation of the acoustic sources of the soundscape are shown in Figure 2 (A-B). Within the main components of soundscape, 9 different acoustic signatures were obtained by manual detection. In total, six types of anthropogenic signatures were identified. Four of them were identified as vessels (Figure 3; 1-4), all fallen in the range of the narrow low frequency (100-2000 Hz); two other types were identified as continuous impulsive signals, belonging to the most common fishing gear (sonar and acoustic deterrent device) in the frequencies of 40 and 70 kHz respectively (Figure 3; 5-6). A rain event is also shown, with a frequency up to 10 kHz (Figure 3; 7). A single pulse ("snap") and a pulse train ("click pulse") were identified within the broadband high frequency (10-80 kHz) as two biological signatures (Figure 3; 8-9). A total of 8560 click pulse and 705 snap best signals were characterized; the former ascribable to the free-ranging dolphins the latter to the "snapping shrimps" identified as *Alpheida* taxa. *(*Au *et al.,* 1998). In table 1, are reported the main acoustic parameters of signals obtained by the "Pulse Train analysis", in figure 4-A spectrogram and waveform of these signals are shown. Significant differences in acoustic signatures (Mann-Whitney pairwise test) in daily occurrences between sampling periods are shown in Table 2. Dolphins' passages occurred overnight in SU19 and AU19, on the contrary during SP20 (*post-lockdown period in Italy*) their presence was regular throughout daytime. Vessel passage is concentrated in the central hours of the day, AU19 season having the fewest passages recorded. In addition, during SP20 a high percentage (25% n=1995) of acoustic deterrents was observed both night and day. Crustacean activity was predominant in SU19 (91%) marine soundscape. The PSD frequency band 16,500 Hz (rms dB re 1μPa) represents the average energy produced by the activity of crustaceans. In Figure 5, the circadian rhythm for each sampling period is shown. The maximum activity of the snaps is concentrated in short intervals during daytime hours both for SU19 and SP20. Geophonies were present in all three sampling periods and differences were observed between AU19 and SP20-SU19 (Table 2). In AU19, the highest percentage of rain events was found. By observing and listening to the acoustic data of the spectrograms, was noted that the wind produces a background noise.

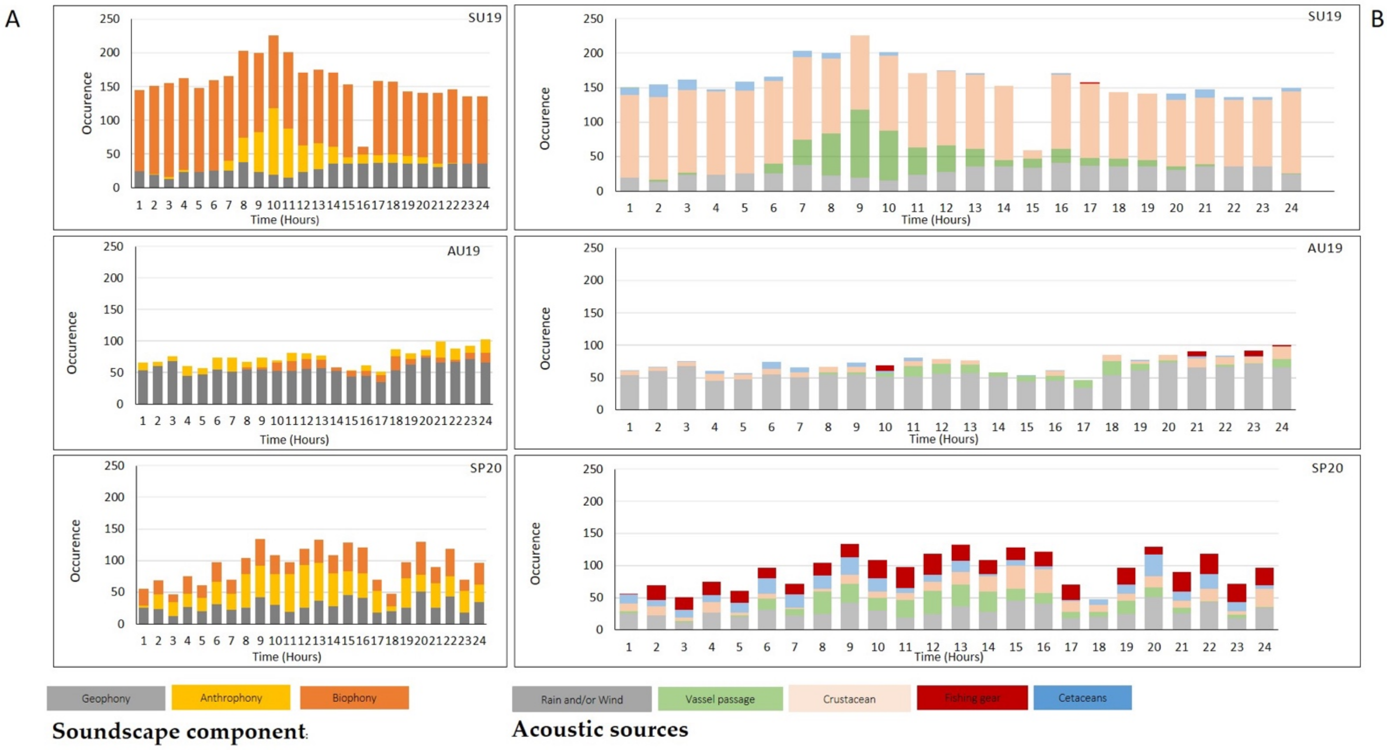

Figure 2. A) Soundscape component *Biophony, Geophony and Anthropophony*, detected in the study area as a function of different seasons Summer 19 (SU19); Autumn 19 (AU19); Spring 20 (SP20) and time of the day (24 hours). B) “Snapshot” of hourly changes of acoustic sources.

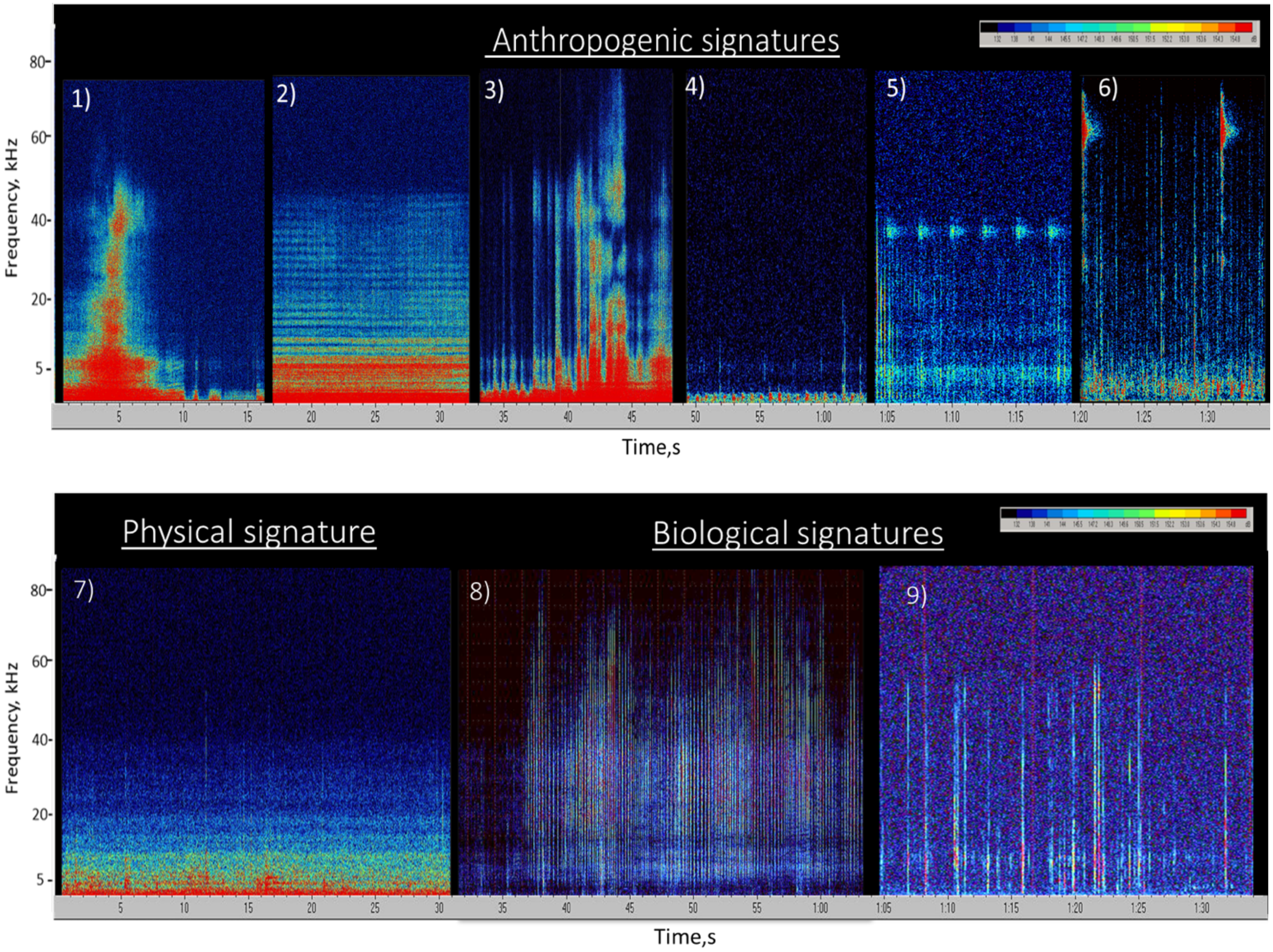

Figure 3. Spectrogram of anthropogenic, biological, and physical sounds (FFT length=1024 points, hamming window and 50% overlap). Spectrograms of different signatures generated by vessels (1-4); by fishing gear and acoustic deterrent device (5-6), rain (7), dolphins "click" (8) and crustacean "snap" (9). The colour bar shows the power spectral density (dB re 1 µ Pa). Note the different durations.

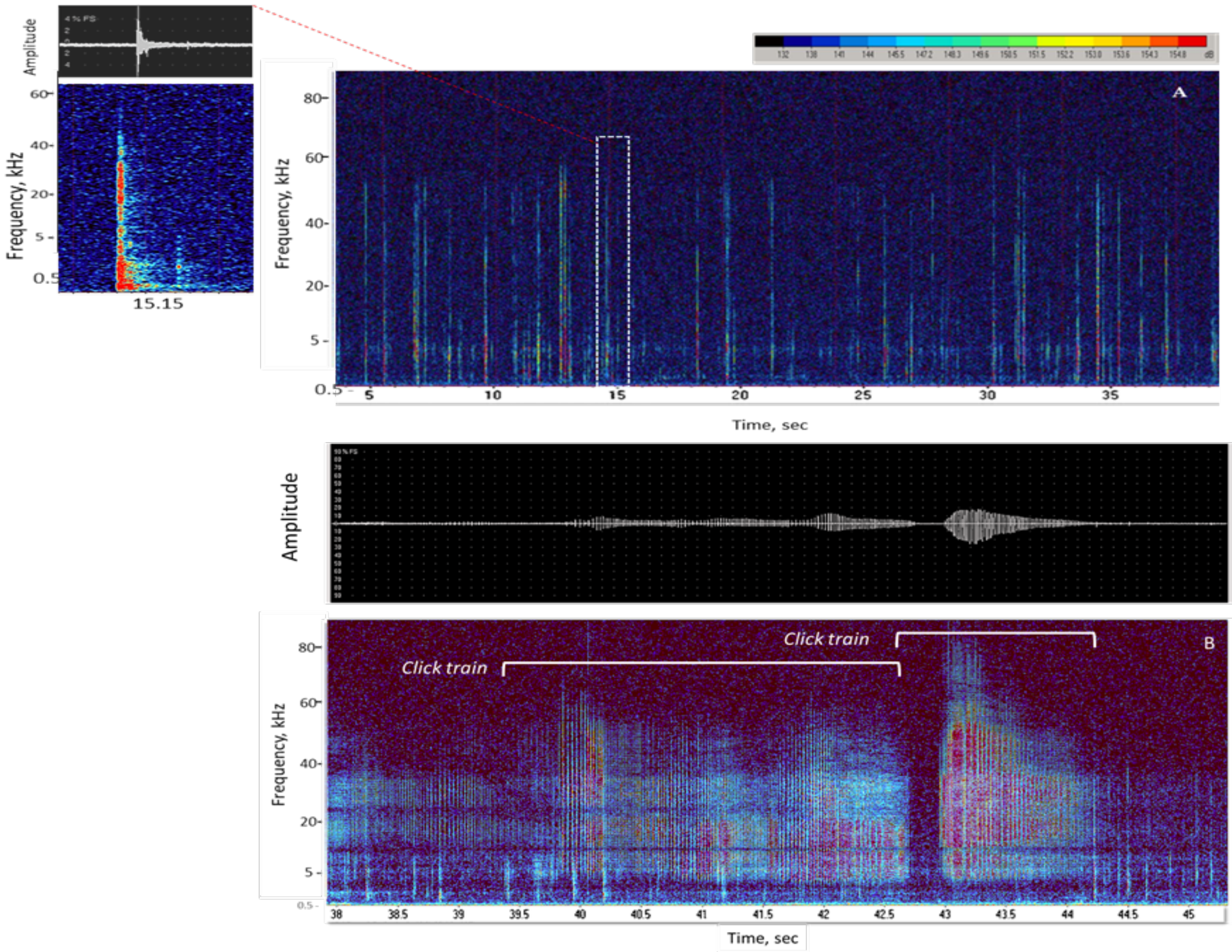

Figure 4. Spectrogram of the principal biological signals. A) Impulsive crustacean's signals. B) Pulse Train of dolphin's signals. Colour bar shows the power spectral density (dB re 1 μ Pa).

Table 1. Mean ± SE of the best signal's features measured for principal biological sources individuated on each sampling period. Clicks and Snap were identified through the Pulse Train Analysis in Avisoft SAS-Lab Pro (Avisoft Bioacoustics, Germany), using the methods of envelope modification "RMS+decimation" and pulse detection "Peak search with Hysteresis".

Acoustic signals	N pulse/ train	Acoustic parameters	Unit	Mean ± SE
Cetacean's signals	8056	Duration of pulse	sec	0.0012 ± 0.0008
		Bandwidth	kHz	55.5 ± 33.9
		Peak frequency	kHz	3.3 ± 16.3
	24	pulse of train	N	34 ± 33.8
		Pulse rate	N/sec	21.3 ± 9.6
Crustacean's signals	702	Duration of pulse	sec	0.0004 ± 0.001
		Bandwidth	kHz	82.7 ± 28.5
		Peak frequency	kHz	1.01 ± 7.3
		n. pulse of train	N	-
		pulse rate	N/sec	-

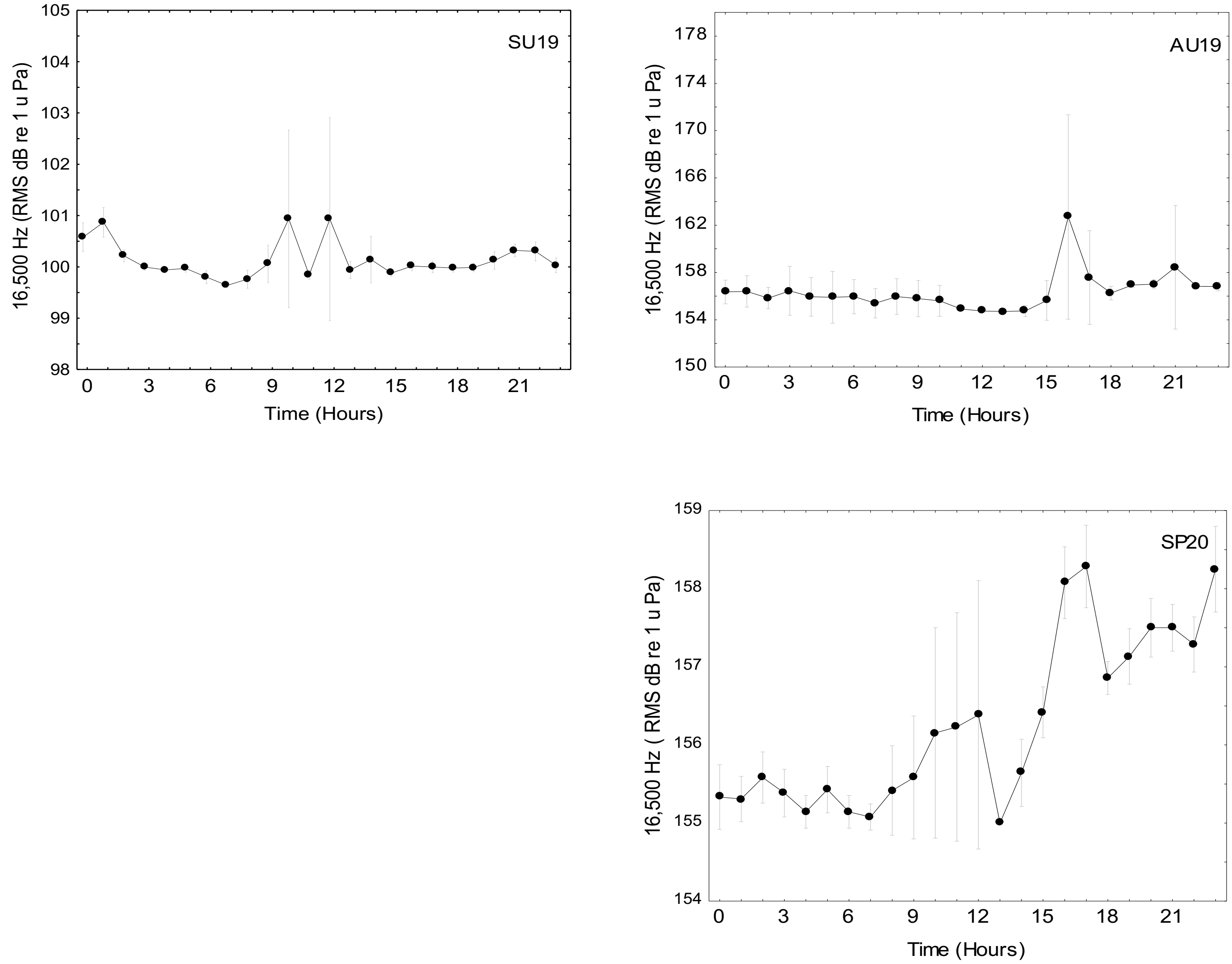

Figure 5. Median ± 0.95 Conf. Interval of PSD (RMS, dB re 1μPa) for crustacean signatures in a subsampling of 24 hours. PSD was calculated using FFT 512 (50% overlap) 16,500 Hz, the frequency band in which crustacean signals fall. Note different scale.

Table 2. Percentage and comparison of the acoustic sources grouped for hours in the deployment periods (Mann-Whitney pairwise test).

			Comparison of the acoustic sources in the sampling period		
Acoustic component and sources all file wav. and Percentage			**Vessel Passage SP20**	**Vessel Passage SU19**	**Vessel Passage AU19**
Anthropophony	17% (n=1996)	Vessel passage (SP20)	-	p-level = 0,7335	p-level < 0.01
	18% (n= 2642)	Vessel passage (SU19)	p-level = 0,7335	-	p-level < 0.001
	7% (n=2016)	Vessel passage (AU19)	p-level < 0.01	p-level < 0.001	-
			Fishing gear (SP20)	**Fishing gear (SU19)**	**Fishing gear (AU19)**
	25% (n=1996	Fishing gear (SP20)	-	p-level < 0.001	p-level < 0.001
	0,1% (n= 2642)	Fishing gear (SU19)	p-level < 0.001	-	p-level = 0,1501
	1%(n=2016)	Fishing gear (AU19)	p-level < 0.001	p-level = 0,1501	-
			Rain and wind (SP20)	**Rain and wind (SU19)**	**Rain and wind (AU19)**
Geophony	30% (n=1996)	Rain and wind (SP20)	-	p-level = 0,7408	p-level < 0.001
	24 (n= 2642)	Rain and wind (SU19)	p-level = 0,7408	-	p-level < 0.001
	67 (n= 2016)	Rain and wind (AU19)	p-level < 0.001	p-level < 0.001	-
			Cetacean (SP20)	**Cetacean (SU19)**	**Cetacean (AU19)**
Biophony	32% (n=1996)	Cetacean (SP20)	-	p-level < 0.001	p-level < 0.001
	9% (n= 2642)	Cetacean (SU19)	p-level < 0.001	-	p-level < 0.001
	5% (n= 2016)	Cetacean (AU19)	p-level < 0.001	p-level < 0.001	-
			Crustacean (SP20)	**Crustacean (SU19)**	**Crustacean (AU19)**
	17% (n=1996	Crustacean (SP20)	-	p-level < 0.001	p-level < 0.01
	91% (n= 2642)	Crustacean (SU19	p-level < 0.001	-	p-level < 0.001
	3 % (n= 2016)	Crustacean (AU19*)*	p-level < 0.01	p-level < 0.001	-

3.2. Seasonal pattern in the marine shallow water soundscape.

Significant seasonal and daily differences in the soundscape were observed in the Gulf of Oristano. Table 3 describes the values of SPL (dB re1µPa) for 10 days (for a total 24 h of recordings) for each sampling period, differentiating between day-time (09:00 a.m.- 04:00 p.m.) and night-time (09:00 p.m. - 04:00 a.m.). Significant variations in broadband SPL (dB re 1µPa) between seasons are shown in Figure 6: the lowest average level was in SP20 (150 dB re1µPa), the highest was in SU19 (169 dB re 1µPa) (Kruskal-Wallis p - level < 0.001). The seasonal variation of octave bands is shown in Figure 7; statistically significant differences (Mann-Whitney pairwise test, p level < 0.05) are shown among 11 octave bands for the three seasons, except for band 250 Hz in SU19 and SP20, for band 16 kHz in SP20 and AU19. The frequency bands centred at 250 Hz shown similar values in SP20 and SU19 (136 dB re 1µPa), as well as 16 and 64 kHz bands in SP20 (140 dB re 1µPa) and AU19 (137 dB re 1µPa). SP20 showed equivalent values of SPL for bands 1, 2, 4 and 8 kHz (140 - 140.6 dB re 1µPa). AU19 showed a maximum value for the band 4 kHz (145.9 dB re 1µPa), and lower values for high frequency bands 8, 16, 32 and 64 kHz. High SPL dB values for low frequencies could be mainly attributable to intense marine weather conditions, such as rainfall and wind**.** Figure 8, shows daytime and night-time significant variations in the octave band sound pressure levels (SPLs dB re 1µPa) for each season (Mann-Whitney U test). Significantly higher values in the low frequency bands occurred in SP20 (from 62.5 Hz to 2 kHz) and in SU19 (for the bands from 2 to 16 kHz) during daytime. In contrast, higher values in low-medium frequency bands from 250 to 8 kHz ensued in AU19 at night-time.

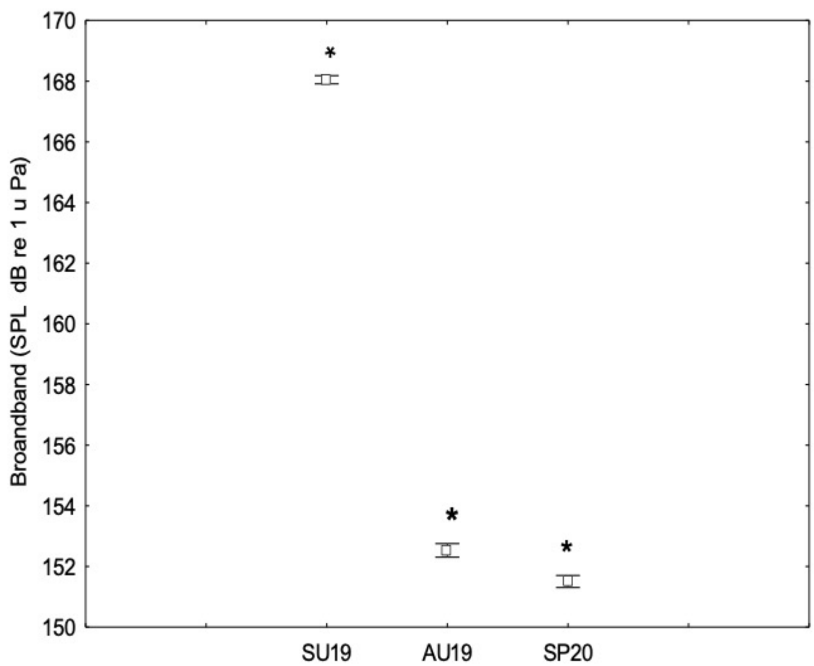

Figure 6. Comparison of broadband sound pressure levels (SPL dB re 1µ Pa) among different seasons (SU19; AU19; SP20). Mean ± 0.95 Conf. Interval. (Kruskal-Wallis p - level < 0.001).

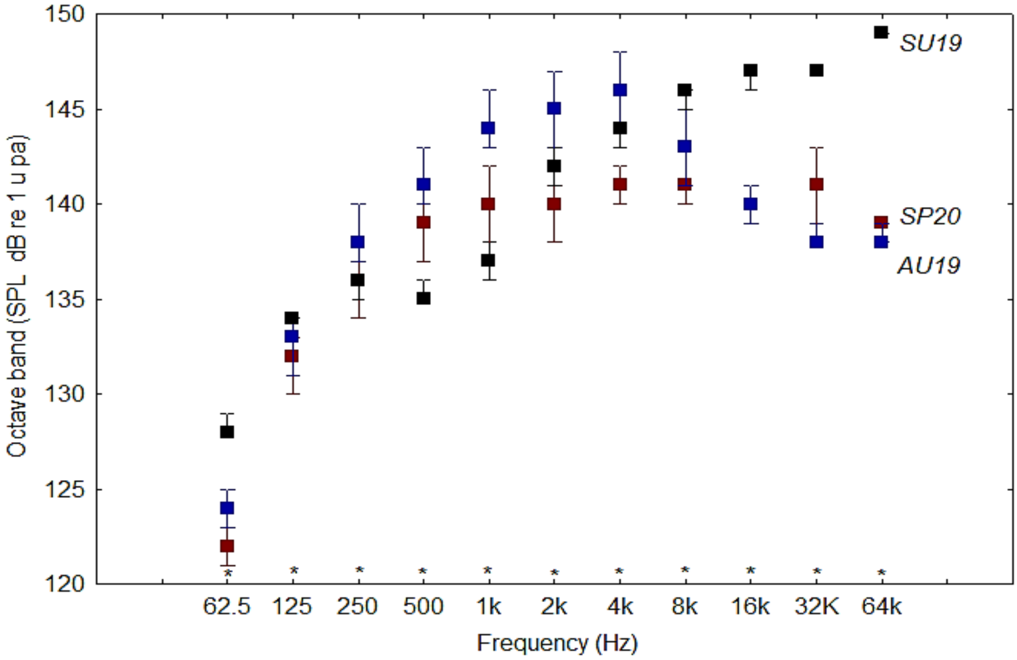

Figure 7. Seasonal variations of octave band sound pressure levels (SPLs) in the study area for 10 days, (24 hours); SU19 black boxes (July 2019), AU19 blue boxes (November 2019), SP20 red boxes (May 2020). Differences are shown with asterisks. (Mann-Whitney pairwise test, p level < 0.01.

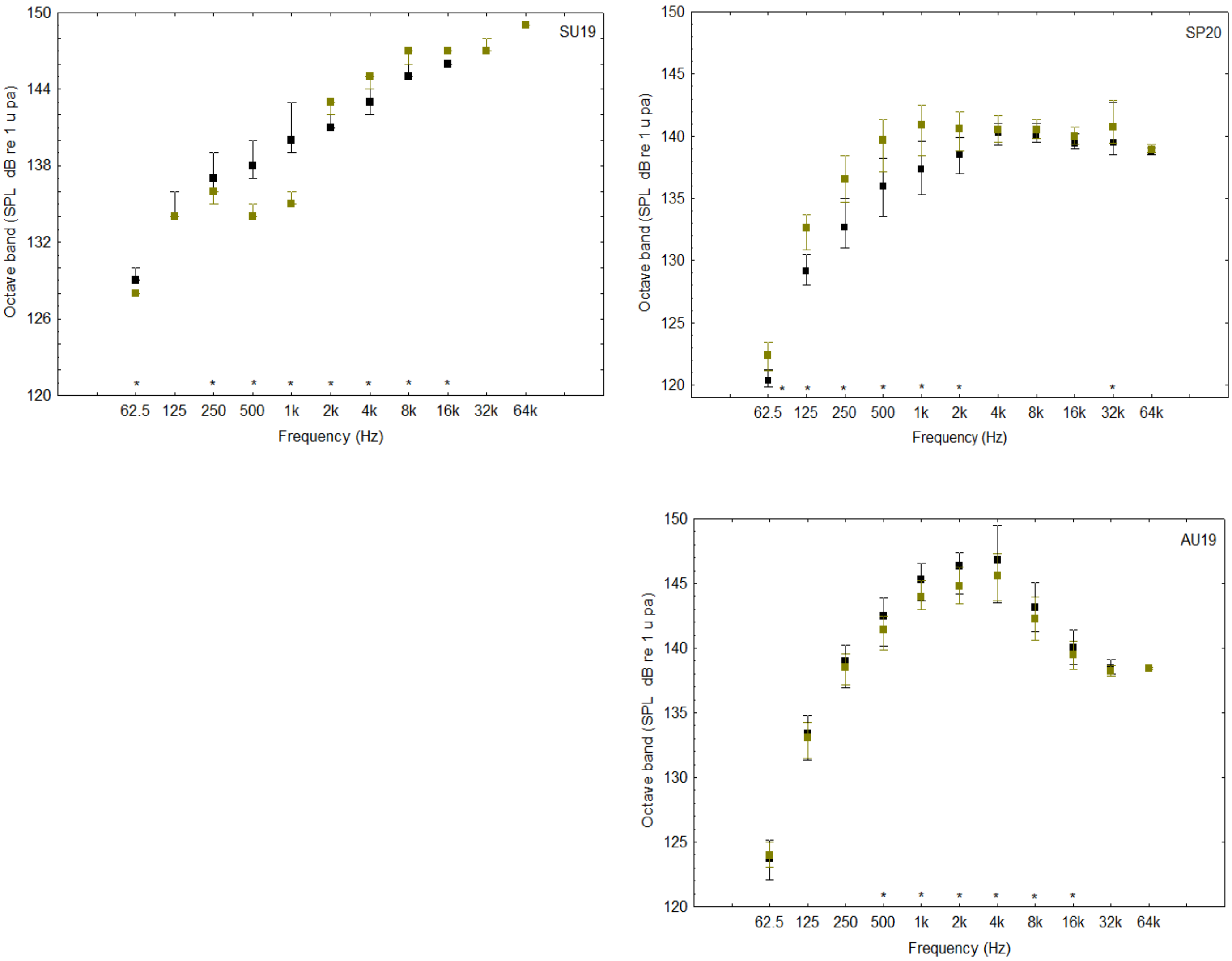

Figure 8. Differences between day-time (yellow boxes) and night-time (black boxes) in octave band sound pressure levels (median, 40th – 60th percentiles) within the sampling period. Mann-Whitney U test (p level < 0.001) significances are highlighted with asterisk.

Table 3. Seasonal daytime and night-time description before and after *Italian lockdown* in rms octave band sound pressure level (Median, 5th – 95th percentile. Day-time (9 a.m. 4 p.m.), night-time (9 p.m.−4 a.m.) and total broadband SPL were calculated for 10 days (24 hours of recordings) in each season.

	SUMMER (SU19)									**AUTUMN (AU19)**									**SPRING (SP20) after Italian lockdown**								
	24 HOURS			**DAY**			**NIGHT**			**24 HOURS**			**DAY**			**NIGHT**			**24 HOURS**			**DAY**			**NIGHT**		
Octave band	**median**	**5 th**	**95 th**	**median**	**5 th**	**95 th**	**median**	**5 th**	**95 th**	**median**	**5 th**	**95%**	**median**	**5th**	**95 th**	**median**	**5 th**	**95 th**	**median**	**5 th**	**95 th**	**median**	**5th**	**95 th**	**median**	**5th**	**95 th**
63 Hz	**128.4**	119.9	137.3	**129.2**	128.4	137.3	**123.8**	128.4	137.3	123.7	117.7	135.0	**123.3**	123.8	135.5	**121.7**	123.8	135.5	121.8	118.4	132.3	**128.0**	118.2	132.7	**121.7**	120.3	129.7
125 Hz	**134.0**	125.2	140.3	**134.4**	133.9	144.8	**133.4**	133.9	144.8	133.1	123.3	144.6	**131.0**	133,4	145.5	**130.4**	133.4	145.5	131.5	125.9	142.2	**136.0**	125.6	142.7	**130.4**	129,2	138.9
250 Hz	**135.9**	127.3	144,1	**137.1**	135.9	147.9	**139.0**	135.9	147.9	138.4	125.1	148.3	**134.8**	139.0	148,9	**133.5**	139.0	148,9	135.9	125,9	145.1	**139.0**	128.2	146.5	**133.5**	132.7	141.1
500 Hz	**135.5**	126.6	145.4	**137.6**	135.5	150.2	**142.4**	135.5	150.2	141.4	124,6	149.0	**137.3**	142.4	149.3	**135.3**	142.4	149.3	138.9	128,0	146.5	**141.0**	127.8	147.6	**135.3**	136.0	144.2
1 kHz	**137.0**	128.3	147,8	**139.8**	137.0	152.2	**145.3**	137.0	152.2	144.3	128.5	151.1	**138.6**	145.3	151,5	**136.6**	145.3	151.5	139.9	127.9	147.6	**143.0**	128.8	148.8	**136.6**	137.5	145.3
2 kHz	**141.8**	132.8	148.6	**141.5**	141.8	152.1	**146.4**	141.8	152.1	145.4	129.8	153,5	**139.0**	146.4	154.1	**138.8**	146.4	154,1	139.9	132.8	148.8	**143.0**	132.9	149.6	**138.8**	138.6	146.6
4 kHz	**143.6**	134.5	148.1	**142.9**	143.6	150.6	**146.8**	143,6	150.6	145.9	134.9	156.5	**139.7**	146.8	157.4	**140.9**	146.8	157.4	140.6	136,2	150.5	**146.0**	135.8	150.9	**140.9**	140.3	148.9
8 kHz	**145.9**	136.1	149.4	**145.3**	145.9	150.7	**143.2**	145.9	150.7	142.7	134.9	153.0	**140.2**	143.2	154.6	**141.1**	143.2	154.6	140.5	137.4	149.9	**147.0**	137.1	150.5	**141.1**	140.1	148.6
16 kHz	**146.5**	137.1	149.1	**146.2**	146.5	150.4	**140.0**	146.5	150.4	139.7	135.6	147.5	**139.8**	140.0	148.6	**140.4**	140.0	148.6	140.0	135.5	147.2	**146.0**	137.2	147.4	**140.4**	139.5	145.7
32 kHz	**147.3**	139.9	148,4	**147.2**	147.3	149.2	**138.4**	147.3	149.2	138.3	137.0	143.1	**139.4**	138.4	143.3	**143.6**	138.4	143.3	140.7	137.7	156.3	**146.0**	137.6	150.5	**143.6**	139.5	157.0
64 kHz	**148.8**	139.9	149.3	**148.8**	148.8	149.7	**138.4**	148.8	149.7	138.4	138.3	139.6	**138.7**	138.4	139.6	**139.8**	138.4	139.6	138.9	138.4	144.3	**147.0**	138.4	142.0	**139.8**	138.7	144.7
Broadband	**169.0**	160.1	169.3	**169.0**	169.0	169.3	**153.3**	169.0	169.3	**152.6**	145.6	160,5	**149.8**	153.3	161.3	**150.8**	153.3	161.3	**150.9**	146.1	158.5	**155.0**	146.0	158.5	**150.8**	149.6	158.3

4. Discussion

4.1. Main soundscape components identification, description and variation in acoustic signatures

The marine soundscape investigation of the shallow waters of the Oristano Gulf (Western Mediterranean Sea) undertaken using passive acoustic monitoring (PAM) is presented. Recordings from acoustic sources provide a "picture" of the underwater environment in the time and frequency domain. Soundscape components are not static but change daily and seasonally, reflecting the contribution of *geophonies, biophonies and anthropophonies,* and their characterisation supports the understanding of complex acoustic data. Providing a simple visualisation of complex acoustic data contributes to the understanding of ecology of the marine soundscape and supports the interpretation of ecological processes aimed at improving the management of the marine environment (Van Opzeeland and Boebel 2018; Weiss *et al.,* 2021). This study describes for the first time the mosaic of acoustic signatures in the marine soundscape of the Gulf of Oristano over time (Figure 2). Studies on soundscapes are mostly focused on the biophony component and their niches as they provide significant information on the complexity of the habitat, which is in turn connected to the composition of its biological community (Putland *et al.,* 2017; Caricco *et al.,* 2020). Through qualitative analysis of the spectrograms, the acoustic presence of emitting marine organisms belonging to two marine taxa was detected: cetaceans and crustaceans. With respect to the latter, the spectrum (Figure 3; 9) displays a moderate number of signals included in a broadband with a low frequency peak between 1 and 5 kHz and energy extending out to 200 kHz that have been described by Au and Banks (1998) as the typical snapping of benthic shrimps that produce crackling and sizzling sounds. The acoustic characterization of crustacean signals allowed to identify them as *Alpheidae*.

In table 1, acoustic characteristics of the signal's parameters of snapping shrimp are shown. In the future, it would be appropriate that species identification is accomplished with an alternative method as well (e.g., visual census) to then combine these two types of information. These findings prove snapping shrimps produce the widest biological sound levels in marine shallow waters (Lillis *et al.,* 2018; Delwayne et al, 2015b; Ureck *et al.,* 1983). Snapping shrimps pulse activities are dominant in SU19 (Figure 2; B) accordingly with previous studies undertaken in the Mediterranean Sea (Pieretti *et al.,* 2017; Buscaino *et al.,* 2016) and in other biogeographic regions (Au *et al.,* 2012; Radford *et al.,* 2010; Staaterman *et al.,* 2013)It is well known that snapping shrimps are more acoustically active at dawn and dusk with overall higher nocturnal sound production (Johnson *et al.,* 1947; Everest, 1948; Radford *et al.,* 2008; Bittencourt *et al.,* 2016 ;Buscaino *et al.,* 2016). Conversely, our results showed greater activity during daylight hours with different circadian patterns (Fig. 5), similarly to the pattern observed by Ashlee *et al.,* (2008) in a coral reef soundscape. This behaviour could be affected by the abundance of food in the studied area since fish at the fish farm were fed at the early hours of daylight. Thus, this could stimulate the predatory and feeding behaviour of shrimps. Interest in examining the influence of environmental parameters (temperature, pH, dissolved oxygen and light) in relation to the acoustic behaviour of snapping shrimp, although still poorly understood, is growing as it has been observed that these factors could influence their activity (Lillis *et al.,* 2018). Therefore, snapping shrimps could be used on a global scale as a key acoustic species to monitor the shallow waters in broadband frequency for long-term studies aimed at measuring ecological processes such as changes in habitat composition, acoustics communities or quality, evaluating their abundance, distribution, use of specific acoustic niche (Lammers *et al.,* 2008; Radford *et al.,* 2010).

Furthermore, click signals (10-80 kHz) associated with the feeding and navigation behaviour of dolphins were observed. It led to argue that the species was *Tursiops truncatus* (Montagu, 1821) as it has been reported to be the unique cetacean species sighted in the Gulf (Bearzi *et al.,* 2008). However, no systematic studies on dolphins' populations have been yet conducted in this area.

The waters of the Gulf of Oristano were frequented by dolphins throughout the whole span of the study and represent up to 46.4% of occurrences found in acquired acoustic data (total number of files wav. = 6653), Summer and Spring being the seasons with the most acoustic presences observed. It was hypothesized that fish farms and artisanal fishery (100 units) operating in the area may encourage opportunistic feeding (Lopez *et al.,* 2008) thus representing an attraction for dolphins that could induce a different acoustic repertoire, related to stereotypic behaviors in social cooperation, like collective food search. Focusing on SP20 (the *post-lockdown period in Italy*), the acoustic presence of dolphins was constant over hours and days (Fig. 2-A); this suggests that the animals frequented the gulf regularly, likely to search for food, to rest, or to give parental care. Nonetheless, continuous acoustic signals are observed 24 hours a day, probably generated by fishing-related deterrent devices used at the fish farm. Findings are consistent with recent studies suggesting that reduction in human activities can have rapid and measurable effects in wider underwater environments with a positive impact on several inhabiting species (Chakraborty and Maity, 2020; Pearson *et al.,* 2020; Rosenbloom and Markard, 2020; Zambrano-Monserrate *et al.,* 2020).

The acoustic method is a valuable tool to obtain information on the presence and habits of highly mobile species and could support, with relatively low operating costs, systematic monitoring plans aimed to manage cetacean species that generally live at low density over large areas and spend most of the time underwater (Marques *et al.,* 2009).

The main anthropogenic sources were observed in the low and mid frequencies and were described as vessel passages and fishing gears (sonar and acoustic deterrent). The percentage of files with vessel passages appears to be considerably higher in Summer (18%; n=2642 file wav.) and Spring (17%; n=1996 file wav.) (Tab. 2.), when stable marine weather conditions encourage local yachting and fishing activities. Moreover, we hypothesize a constant activity of the operators working at the fish farms during the morning hours (8-13 a.m.) in respect to the night hours. The anthropic spectral signatures in the soundscape of the Gulf, both continuous and intermittent, introduced noise into the underwater environment, and occupied the acoustic space of the biological components (Fig. 2) potentially causing soundscape energetic masking and altered intra-specific communication and behaviour (Clark *et al.,* 2009; Weiss *et al.,* 2021; de Vincenzi *et al.,* 2021). Anthropogenic noise poses not only a potential threat to marine species but also to coastal habitats (Tyack, 2008; Slabbekoorn *et al.,* 2010). Lillis *et al.,* 2017; Although poor attention has been paid to how much the noise could affect sessile organisms and plant species, a study on the sensitivity of Posidonia oceanica (L.) Delile, 1813 to artificial sounds in the low frequency has explained that these influence its health by hindering nutritional processes (Solé *et al.,* 2021). This information has opened up new insights on the increased noise pollution in coastal environments as a contributing factor to biodiversity loss. Quantifying the overlap of biological and anthropogenic sources can provide additional information on the way noise affects the underwater environment and, the acoustic species-specific niches (Weiss *et al.,* 2021).

4.2. Acoustic measurement and temporal pattern in sound pressure level

Average values of the Broadband SPL, which represent the aggregation of all the acoustic signatures of the local soundscape, were significantly higher in SU19 and lower in SP20, denoting a seasonal variation. SP20 value (SPL dB re 1μ Pa) may be attributed to a reduction of maritime traffic due to a decline of shipping and mobility during *Covid-19 pandemic* (Millefiori *et al.,* 2020; Gibney, 2020) (Figure 6). This is supported by lower SPL values for the low frequency bands, typical of maritime traffic (62.5 - 500 Hz), in SP20 (Figure 7). Noise emitted by boats is a major source of environmental stress. It is probable that the decrease in noise may have benefited ecological processes, such as the recruitment of larvae to preferred habitats (e.g., seagrass meadow). It may have provided a further advantage for biological communities settled in the MPA. SU19 showed higher levels of SPL in the 16 kHz octave band compared to other seasons; this high frequency band reflects the activity of snapping shrimp according to Buscaino *et al.,* 2016; Pieretti *et al.,* 2017; Xu *et al.,* 2020; Lammers *et al.,* 2008 and Ceraulo *et al.,* 2018. Day-night variations are not consistent with results obtained by studies carried out in these sites (Fig. 7 and 8). As regards clicks emitted by dolphins, it is not possible to describe how they can influence the soundscape; long-term studies should be conducted to investigate this aspect (Xu *et al.,* 2020). AU19 showed higher median values of octave band SPL levels than SU19 and SP20 from 62.5 to

4 kHz Sound pressure levels show significant differences between day and night. In AU19, significant differences for frequency bands from 500 Hz to 16 kHz were found consistently with Buscaino *et al.,* 2016. In addition, an opposite pattern was found in Summer, as SPL values in the low frequencies were significantly higher at night and the high frequencies SPL values were higher during daytime this phenomenon could be associate or acoustic presence of the fish calls as reported in the coastal area SPL values were higher during daytime this phenomenon could be associate or acoustic presence of the vocalization fish as reported in several study in coastal area. (Buscaino *et al.,* 2016, La Manna *et al.,* 2021; Di Franco *et al.,* 2020)

Differences were found also in Spring when higher SPL values in low frequencies bands (62.5, 125, 250, 500 Hz, 1, and 2 kHz) occur during daylight, whereas they occur in high frequencies bands (2, 4, 8, 16, 32, and 64 kHz) during the night in Lampedusa Island.

Marked differences in the range of SPL values in the Gulf of Oristano (120-150 dB) compared to the Island of Lampedusa (92-114 dB) were observed, probably due to specific characteristics of the two study sites such as different depths, substrates, physiography of the area, and also different anthropogenic pressures.

It is important to note that the Gulf was constantly affected by anthropogenic activities across the seasons as main sound sources included vessels passages, fishing boats, and fishing gear. The spectral characteristics of these sources fall within the frequency bands 63, 125, 500 Hz, 1, 2 and 40 kHz. However, the constant background noise in the marine environment seems to differ, probably in relation to the different vessel types/speeds and distances from the receiver (McWilliamig and Hawkins 2013).

5. Conclusions

An acoustic signature potentially distinguishes each habitat type through the description of specific properties of the collected sounds (Bertucci *et al.,* 2015). The cross-sectional approach applied to marine soundscape study can provide important information on the functioning of coastal habitats and their changes over time as acoustic signatures play a significant role in marine life. The results obtained from this study provide a 'clear picture' of the variability of band SPL levels and the composition of the acoustic sources. The two biophonic sources were found to maintain their acoustic niche in both frequency and time domain. The sources of geophonies and anthropophonies, all have the ability to increase the sound level in the marine soundscape. Anthropophonies are increasing steadily on worldwide coasts and future researches should be addressed to their masking effect on biophonies, which are crucial to marine life strategies. The European legislation 56/2008 CE aims to achieve a good environmental state (GES) of marine waters through monitoring actions according to the provisions of the maritime spatial planning directive (MSP), with the ecosystem-based approach (Article 1, paragraph 3, 2008/56/EC). Therefore, PAM is widely considered as a useful method for assessing anthropogenic pressure on marine habitats, understanding the distribution and abundance of acoustic target species, identify alien species, as well as describing spatio-temporal variations in acoustics behaviour in relation to the noise (Merchant *et al.,* 2015) in order to be able to define appropriate conservation measures in coastal marine ecosystems. This is particularly important considering the current environmental crisis and the urgent need to document biodiversity with comparable methodologies (Sugai and Lusia, 2019). This study highlights the advantage of using the acoustic method and can inform local integrated management plans. The scarcity of information regarding underwater noise in the Mediterranean Sea makes small-scale studies useful for constructing a complete picture of the possible anthropogenic noise sources. Future studies would be extremely valuable in determining the standard protocols for the monitoring activities of MPAs and SACs, which until today do not include systematic acoustic monitoring in their plans.

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Chapter 2
Bottlenose Dolphin (Tursiops truncatus) Whistle Modulation during a Trawl Bycatch Event in the Adriatic Sea

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Simple Summary: There is some evidence that the presence of dolphins in fishing areas represents a concrete economic loss for fishermen due to their depredation activities on the entangled fish on the nets. Bycatch events are one of the major sources of anthropogenic mortality of species of conservation interest in the world. *T. truncatus* is a plastic species and the more frequently observed species in the Adriatic Sea owing to the natural tendency to interact with the fishing activities in the area. This case report describes the acoustic parameters detected in whistle spectral contours associated with low-frequency signals recorded with a passive acoustic monitoring device in an exceptional event of bycatch that involved three individuals during a midwater commercial trawling in the Adriatic Sea.

Abstract: Marine mammal vocal elements have been investigated for decades to assess whether they correlate with stress levels or stress indicators. Due to their acoustic plasticity, the interpretation of dolphins' acoustic signals of has been studied most extensively. This work describes the acoustic parameters detected in whistle spectral contours, collected using passive acoustic monitoring (PAM), in a bycatch event that involved three Bottlenose dolphins during midwater commercial trawling. The results indicate a total number of 23 upsweep whistles recorded during the bycatch event, that were analyzed based on the acoustic parameters as follows: D_r, total duration (1.09 ± 0.88 ^{25th}–1.24 $^{75th)}$; f_{max} maximum frequency, (11160 ± 11293 ^{25th}–11810 $^{75th)}$; f_{min}, minimum frequency (5836.4 ± 5635.3 ^{25th}–5967.1 $^{75th)}$; f_c, central frequency; (8665.2 ± 8492.9 ^{25th}–18982.8 $^{75th)}$; frequency; BW, bandwidth (5836.4 ± 5635.3 ^{25th}–5967.1 $^{75th)}$; Step, number of step (5 ± 4 ^{25th}–6 ^{75th}). Furthermore, our data show that vocal production during the capture event was characterized by an undescribed to date combination of two signals, an ascending whistle (upsweep), and a pulsed signal that we called "low-frequency signal" in the frequency band between 4.5 and 7 kHz. This capture event reveals a novel aspect of *T. truncatus* acoustic communication, it confirms their acoustic plasticity, and suggests that states of discomfort are conveyed through their acoustic repertoire.

1. Introduction

Marine mammals have evolved the most sophisticated and specialized structures for producing and receiving sounds. Dolphins (family: Delphinidae) use acoustic signals to coordinate movements and social behavior [1] as well as in cooperative effort, navigation, and foraging [2,3]. The acoustic plasticity of dolphins [4] is revealed by their ability to imitate the vocalizations of conspecifics [5], to modify signals in relation to environmental and anthropogenic noise [4,6], and to emit different signals in relation to different behaviors [7]. Delphinids, the family where the interpretation of acoustic signals has been studied most extensively, produce a variety of sounds that can be classified into two main categories: rapid repetition rate echolocation clicks, click trains, burst, and tonal frequency modulated whistles [8–9-10]. According to some studies, whistles and burst pulses are predominant in social contexts [9,1]. Alterations in whistle parameters may indicate high variation in the message conveyed and reflect changes in the transmission of emotional information [12]. With regard to the latter feature, changes in marine mammal vocal structures have been investigated to assess whether they correlate with stress levels [13]. In a study on the ability of some whistle parameters to serve as stress indicators in *T. truncatus*, Esch *et al*. [14] reported a higher whistle rate and number of loops during brief capture–release events compared to undisturbed conditions. *T. truncatus* is probably the most common cetacean species in the Adriatic Sea [15-16], and is protected by species and habitat conservation laws and international agreements. Its interaction with fisheries mostly consisting of opportunistic foraging have been documented by several studies [17-18- -19]. According to several reports, *T. truncatus* specimens can learn to take fish from trawls, gillnets, and aquaculture cages, suggesting that this behavior can generate a dependence on human activities. Bycatch can occur as a consequence of these strong interactions and may affect the survival of this vulnerable species [20] in the Mediterranean Sea. The northern Adriatic Sea is characterized by a dense presence of commercial pelagic trawlers, whose nets are usually towed at a relatively high speed, with unpredictable route changes that enhance the scope for entanglement [21]. The main target species of midwater trawlers are *Engraulis encrasicolus* and *Sardina pilchardus,* which are among the major components of the bottlenose dolphin diet. In this area, *T. truncatus* is the most frequently observed marine mammal species and the only one with a propensity to interact with human activities. In this case report, we describe the acoustic parameters detected in whistle spectral contours associated with low frequency signals, during an exceptional bycatch event that involved three bottlenose individuals during a midwater commercial trawling with fatal consequences for the three specimens.

2. Materials and Methods

Since 2006, an extensive monitoring program of bycatch of long-lived species like cetaceans, sea turtles, and elasmobranchs by Italian midwater pair trawlers has been conducted in the northern central Adriatic Sea [22-23]. The information collected in its framework provides a unique opportunity to assess the operational details of capture events and the abundance trends of species over time [24].

Eleven acoustic monitoring surveys were carried out from March to June 2017 during commercial fishing activities onboard two midwater pair trawlers activities.

Twenty-eight hours of acoustic data recordings were obtained in the course of 51 trawling operations. Surveys were conducted only in good sea conditions. Bottlenose dolphins' acoustics vocalizations were collected and behavioral events were observed throughout all the fishing operations by using the focal group sampling [25]. The acoustic data were collected using an autonomous recorder secured to the headrope and oriented towards the trawl codend, opposite to the direction of towing. The weights ensured that the net was towed at an average depth of 20 m. The equipment included a calibrated omnidirectional hydrophone with a flat sensitivity response of −174.5 (±2) dB re V/µ Pa from 0.1 to 100 kHz (Low Noise Broadband Hydrophone BII 7016 T6, Benthowave Instrument Inc., Collingwood, ON, Canada) and a digital signal processor (mod. C5535 DSP- TMS320C5535) coupled to an AIC3204 audio codec (both from Texas Instruments, Dallas, TX, USA). The sampling rate was 192 kHz, resolution was 16 bits. Recording sessions lasted about 60 min.

All acoustic data thus recorded (28 h) were visualized (n = 841) and the whistle parameters analyzed using RX5 audio editor software (iZotope, Cambridge, MA, USA) with a Fast Fourier Transform (FFT) size of 2048 points, an overlap of 50% and Hann window. The instantaneous sampling protocol of focal group behavior (natural, opportunistic feeding, socializations, and travel) [25] was used in each observation event involving interactions between bottlenose dolphins and fishing operations. Information such as group size and life stage of individuals (calf, sub-adult, adult) was also recorded. During sightings, dorsal fin features were collected using the photo-identification method [26]. This report describes a fishing trip where a group of three dolphins (2 adults and 1 sub-adult) was captured when interacting with a commercial midwater trawl pair. The parallel movement of the two ships in the hauling phase prevented the escape of the dolphins so that the three individuals were caught in the net and died (Figure 1). Anatomical observations indicated that all the individuals were females. None of them showed physical signs of external trauma following the direct action of the nets, the only visible sign was the foam present on the blowhole as a consequence of drowning asphyxiation. Visual inspection of the spectrograms allowed to identify several types of whistle contours; whistles recorded during the hauling of the bycatch event ($n = 23$) were all of the ascending type (upsweep), i.e., signals whose spectral contour is mostly ascending without negative inflection points in frequency modulation. The acoustic parameters measured in the spectrogram of each upsweep signal included duration (D_r, s), total duration of the vocalization; minimum frequency (f_{min}, Hz), minimum frequency value of the vocalization out of the total vocalization; maximum frequency (f_{max}, Hz): maximum frequency value of the vocalization in the total vocalization; bandwidth (BW, Hz): f_{max}–f_{min} range; central frequency (f_c, Hz); frequency value in the middle portion of the bandwidth; and number of steps, according to recent studies about the acoustic emissions of marine mammals [14,27]. The STATISTICA 7.1 (StatSoft, TIBCO Software Inc., Palo Alto, CA, USA) software package was used for statistical analysis.

A) 

B) C)

Figure 1. The three bycatches during the disentanglement operations, two were wrapped in the fishing net (**A**, **B**) and one inside the codend (**C**). Gulf of Venice, 18 May 2017 h 7:00 a.m., 45°18826′ N–12°41804.

3. Results

A total of 23 upsweep whistles were recorded during the bycatch event (Figure 2A). The whistle contour showed a clear step-like profile where vertical narrow frequencies were preceded and followed by a flat upsweep (Figure 2B). The median and 25th and 75th percentiles of all whistle parameters are reported in Table 1. These upsweep whistles displayed a simultaneous distinctive impulse signal, that we denominated “low-frequency signal”, which consisted of low-frequency bursts. Their frequency band ranged from 4.5 to 7 kHz (Figure 2 A, B). These kinds of more complex associated signals occurred exclusively during the bycatch event. From the matching of the dorsal fins, no one of the three individuals were sighted before the bycatch event.

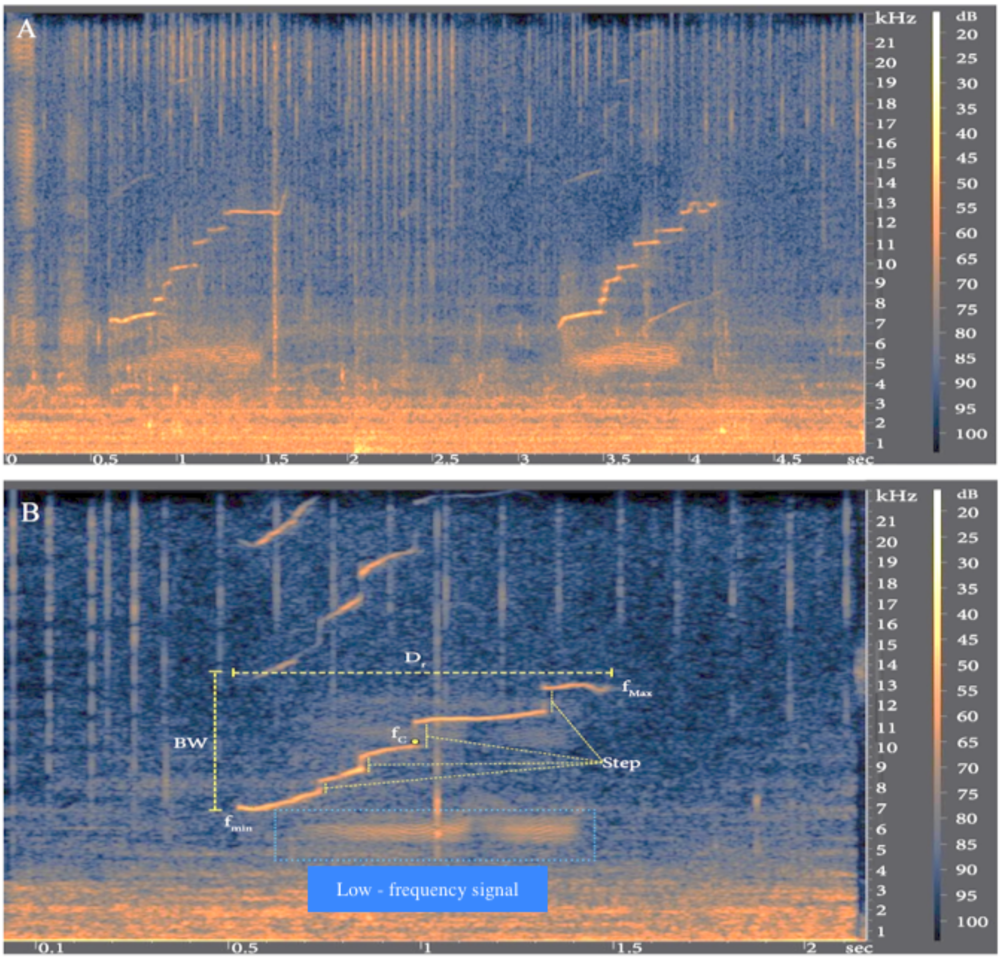

Figure 2. Spectrograms (FFT size 2048-point, Hann window, linear frequency scale) of upsweep whistlesof *T. truncatus* recorded during the bycatch event. (**A**) A sequence of two upsweeps. (**B**) Detail of an upsweep showing the parameters analyzed: D_r, total duration; f_{min}, minimum frequency; f_{max}, maximum frequency; f_c, central frequency; BW, bandwidth; Step. The low-frequency bursts are highlighted within the dotted blue rectangle.

Table 1. Whistle parameters (median ± 25th–75th percentile), assessed during the catch event (*n* = 23): D_r, total duration; f_{max}, maximum frequency; f_{min}, minimum frequency; f_c, central frequency; BW, bandwidth; Step, number of steps; low frequency signals, presence/absence.

Parameters	**Units**	**Catch Event (*n* = 23)**	
		Median	**25–75%**
D_r	(second)	1.09	0.88–1.24
f_{min}	(Hertz)	5836.4	5635.3–5976.1
f_{max}	(Hertz)	11,160	11,293–11,810
f_c	(Hertz)	8665.2	8492.9–8982.8
BW	(Hertz)	5944.1	5600.2–6174.3
Step	Number	5	4–6

4. Discussion

The ability to transmit information to conspecifics is crucial for dolphins, which live in communities with highly complex social structures [8]. Acoustic signals play an important role in both species and group recognition, although the degree of signal modulation varies widely among populations [28]. Notably, variations in whistle characteristics may reflect inter-individual variation or changes in the transmission of emotional information [29]. According to [12], whistle acoustic parameters can vary independently under a variety of stimuli, including stressful events. This study describes the whistle structure of the bottlenose dolphin in relation to a bycatch event pointing out that acoustic parameters and contours may vary in response to the context and be associated with other signals. These findings are in line with early works on the function of dolphin whistles that attempted to associate discrete whistle contours with behavioral state such as fright or disturbance [14,30]. In a study assessing whether the bottlenose dolphin signature whistles served as indicators of stress or stress levels, Esch *et al*., [13] reported that whistle duration changed both due to stressful events and in relation to group structure and composition (age, sex, mother–calf communication). Our findings suggest that the change in whistle acoustic parameters, recorded during a bycatch event, conveyed to conspecifics also the distress due to the entrapment in the net. Our data show that vocal production during the catch event was characterized by a combination of two signals, an ascending whistle (upsweep) and low-frequency signal (burst). Their association has never been described in *T. truncatus,* despite its large, complex, and extensively investigated repertoire of whistles and pulsed sounds. As burst pulsed sounds have been associated with aggressive [31-33] or socializing behaviors [1–34], the combination of bursts and whistles that we found in our study could suggests a different meaning to the intra-specific communication message, probably related to a distress event. This capture event reveals a further aspect of *T. truncatus* acoustic communication and it confirms the complexity of its repertoire suggesting that states of discomfort are conveyed through their acoustic emissions.

5. Conclusions

This case report describes for the first time a novel aspect of the acoustic communication of *T. truncatus* that occurred during a trawl bycatch event in the Adriatic Sea. We assume that dolphins modulate the contours and harmonics composition of the whistles and that pulse signals bursts play a social role that has not yet been fully explored. Further acoustic data of dolphin interactions with fishing nets are needed to determine how the emission patterns of the low-frequency signal can be associated with whistles in aggressive or distress contexts. The use of passive acoustics monitoring (PAM) allowed us to obtain unique information on the acoustic repertoire of *T. truncatus* in a highly man-made coastal environment. This work aims to contribute to the definition of a protocol in collaboration with the authorities, in order to implement monitoring that provides for the early detection of possible state of stress and to prevent bycatch events.

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Institutional Review Board Statement: The study was based on incidental catches of protected species and species of conservation concern recorded by qualified observers on board Italian midwater commercial trawlers in the Adriatic Sea. The data collection was conducted under permit issued by the Italian Ministry of Agriculture, Food and Forestry, Fishery and Aquaculture directorate in compliance with the Italian obligations to the Council Regulation (EC) 812/2004. No other authorization or ethics board approval was required to conduct the study.

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Credit. *Biology Dolphin and Conservations*

DISCUSSION

This thesis has mainly developed two research topics in marine ecology with different methods, namely marine ecosystems and acoustic behaviour (bioacoustics) based on acoustic characteristics of the sounds emitted by protected species. Ecological processes were investigated in relation to anthropogenic impacts with an ecosystemic approach and the work was developed following an emerging line of research (eco-acoustics) to study the underwater soundscape (Pieretti and Danovaro, 2021). Sounds were used to study animal acoustic response to stress deriving from impacts associated with human activities. Acoustics is used in an interdisciplinary and transversal way in marine ecology and returns several levels of information: 1) the acoustic repertoire of species; 2) the characteristics of habitats, 3) how the former change as a function of time and space; 4) the degree of anthropogenic disturbance (Erbe *et al.,* 2015; Ceraulo *et al.,* 2018; Gordon *et al.,* 2018). For the first time, the underwater environment in the Gulf of Oristano (Western Mediterranean Sea) was investigated using *passive acoustic monitoring* (PAM) through fixed stations.

This area was considered a good testing ground for its shallow inlet on the west coast of Sardinia because it includes a special conservation area (Habitat Directive) and a national marine protected area where various uses of the sea take place (i.e. maritime traffic, aquaculture, industrial port, fishing) and seems to be a potential feeding area for small cetaceans (Bearzi and Bonizzoni, 2011; Corrias *et al.,* 2019; Corrias *et al.,* 2022). Recently, acoustic researchers have been engaging with Soundscape ecology eliciting important information about acoustic sources and composition of marine coastal habitats and species (Pieretti *et al.,* 2011; Farina, 2014) Recordings from acoustic sources provide an instantaneous "picture" of the underwater environment in space, time and frequency domain.

The identification and measurement of different natural and anthropogenic sources constituting the marine soundscape were essential to assess the impact of anthropogenic disturbances on marine species and habitats (Croll *et al.,* 2001; Thomsen *et al.,* 2006). Noise pollution is a serious threat to marine animals. Its effects, which are difficult to confine, are less perceptible than visible pollutants of chemical origin (Weilgart *et al.,* 2008). Therefore, passive acoustic monitoring and the ecosystem approach allowed to describe the "local marine soundscape" and measure the circadian and seasonal variations of the environmental noise level (SPL) during different seasons including also physical agents. During this study, acoustic data were collected over the course of three seasons including a period after closing due to Covid-19 pandemic. This global experience has confirmed that the coastal habitat and soundscape change depending on the acoustic sources of human pressures to which the area is exposed, e.g., fishing activities, commercial shipping, vessel traffic (Duerte *et al.,* 2021). Indeed, lower median values of octave SPL band were found in spring 2020 (120-140 dB re1µPa) after the Covid-19 pandemic lockdown compared to other seasons like summer 2019 (128-150 dB re 1µPa). This has been highlighted by the recent COVID “anthropause” experienced at various aquatic locations around the world (e.g., Bates et al., 2021; De Clippele and Risch, 2021; Dunn et al., 2021; Gabriele et al., 2021; Ryan et al., 2021), where removing anthropogenic components of some soundscapes has provided an opportunity to recorder more marine sounds. Nevertheless, more study is needed to confirm with certainty that the lower median values of the octave SPL band found in spring 2020 compared to summer 2019 are related to covid19 lockdown rather than to periodic seasonal-driven changes in the dominant input of acoustic energy.

In the Gulf of Oristano, the predominant anthropogenic acoustic source was marine traffic, and this presumably interferes with the biological ones (cetaceans and crustaceans) (Celi *et al.,* 2015, Papale *et al.,* 2015, Filiciotto *et al.,* 2016) that have been identified in this area. Marine traffic was found to be prevalent during the day and represented 42% of the acoustic space occupation in the low-frequency band. Recently, acoustic researchers have been engaging with Soundscape ecology eliciting important information about acoustic sources and composition of marine coastal habitats and species (Farina, 2014; Pieretti *et al.,* 2011). The scarcity of information regarding underwater noise in the Mediterranean Sea makes small-scale studies useful for constructing a complete picture of the possible anthropogenic noise sources. Recordings from acoustic sources provide an instant (Jézéquel, *et al.,* 2021). Some differences in acoustic and behavioural characteristics of *snapping shrimps* (alpheids) compared to other Mediterranean sites were revealed (Lammers *et al.,* 2016). In fact, temporal patterns seemed to be consistent with those found in tropical waters.

The role of bioacoustics is fundamental to better known Mediterranean species' acoustic characteristics of vocalizations and future works would contribute to associating biological sounds with habitat characteristics.
The bottlenose dolphin's "sound presence" occurred in all sampling periods, accounting for 46.4% of the total recordings of which 32% after the lockdown period in Italy. This species, which was acoustically identified because of echolocation clicks recordings, regularly uses the habitat for feeding purposes.
The advantage of a fixed PAM station and bioacoustics analysis, have made it possible to determine, for the first time, the year-round presence of bottlenose dolphins in the Gulf of Oristano. PAM represents a valuable research and management tool especially in extended habitats. Although it includes one SAC and one MPA, poor information about the bottlenose dolphin is available for this area so it is defined as data deficient by ACCOBAMS (Agreement on the Conservation of Cetaceans of the Black Sea, the Mediterranean Sea, and Contiguous Atlantic Area). These results add information concerning the habitat use of this species and human activity pressures. In addition, they help the management of protected areas as well as regional conservation and plans. This information also opens up new research questions and draws attention to the development of conservation actions and management plans that the European community and Mediterranean-wide conservation strategies encourage to develop in order to avoid biodiversity loss.
In a period when worldwide biodiversity is in significant decline and underwater soundscapes are being altered as a result of anthropogenic impacts, there is the need to document, quantify, and understand biotic sound sources potentially before they disappear.
Marine mammal vocal elements have been investigated for decades to assess whether they are correlated with stress levels or stress indicators. Due to their acoustic plasticity, the interpretation of dolphins' acoustic signals has been studied more extensively (Caldwell, Caldwell, and Tyack, 1990; Janik, Dehnhardt ; Cook *et al.,* 2004; Janik and Sayigh, 2013).
New acoustic parameters in whistle spectral contours were detected and further insights on the bottlenose dolphin's acoustic behaviour were offered. These data were collected for the first time with hydrophones (PAM) in a commercial trawl fishery during the interaction of animals with the fishing net. The observed by-catch represented an unprecedented event where non-invasive methods (both acoustic and visual) documented an acoustic behavior distress never described before. By-catch events are a major source of mortality in worldwide species of conservation target related to human activities (Bearzi *et al.,* 2003; Wallace *et al.,* 2013; Davidson *et al.,* 2015; Phillips *et al.,* 2016; Gray and Kennelly 2018; Dias *et al*. 2019; Lopez-Mendilaharsu *et al.,* 2020; Nelms *et al.,* 2021; Pacoureau *et al.,* 2021).

A not yet described combination of two signals during the capture event involving three individual dolphins was observed: an ascending whistle (upsweep), and a pulsed signal that we called “low-frequency burst”. A new aspect of *T. truncatus* acoustic communication was revealed which suggested that states of discomfort are conveyed through its acoustic repertoire. We provided valuable insight into the bottlenose dolphin’s vocal repertoire by highlighting features that had not yet been previously found, confirming the acoustic plasticity of the species. The acoustic behaviour of cetaceans during death-related events is a largely unexplored topic. Recently, Terranova *et al.,* (2022) during an acoustic monitoring survey of the bottlenose dolphin population the Gulf of Catania (Ionian Sea), documented the entangling and subsequent death of a sub-adult male in a fishing net. Their results show massive vocal activity during net entangling characterized by a significant emission of signature whistles and burst pulses, including bray series of gulps and squeaks. These studies provide valuable insight into the bottlenose dolphin’s vocal repertoire by highlighting features that had not yet been previously found, confirming the acoustic plasticity of the species.

Finally, suggestions about the use of acoustics as a tool for standardized monitoring of fishery activities have been proposed. In the Mediterranean Sea, little has been done in terms of implementing a systematic cetacean by-catch monitoring system and the overall quantitative information on bottlenose dolphin by-catch is still scarce and sparse, both in terms of geographic coverage and gears (Gonzalvo *et al.,* 2008, Bradai *et al.,* 2010; Fortuna *et al.,* 2010, 2015; Kapiris 2019). The available studies and circumstantial evidence for local populations still raise concerns, suggesting that annual fishery-induced mortality could be locally unsustainable or close to an unsustainable level in some sub-regions (e.g. Feingold *et al.,* 2005, Brotons *et al.,* 2008).

In a more ambitious and integrated view to apply acoustic devices to the study of marine communities and habitats, research groups working on these signals are tending to develop a network for sharing acoustic marine species’ repertoires. On the whole, existing libraries are a “box”—lacking the cohesiveness that a taxon-independent global library or network could provide. Considering PAM is not a traditional method to categorize or preserve information on diversity, Parsons *et al.,* 2022 are developing a “Global Library of Underwater Biological Sounds” as an inventory that provides first hand samples for comparisons, preferably with a spectrogram that shows clear annotations describing specific time and frequency range of target signals, along with sufficient metadata to facilitate comparisons between user and library samples in order to maximize library use.

CONCLUSION

This study shows the importance of passive (non-invasive and relatively low-cost) acoustic monitoring techniques to study an ecosystem at different levels from species' vocal repertoire to habitat acoustic features and quality. The Mediterranean Sea Soundscape is still not fully investigated, and this new ecosystem approach aims at filling this knowledge gap. This "immediate" approach to evaluate biological, physical, and anthropogenic characteristics could be important to analyse habitat changes in a dramatic scenario of environmental crisis and biodiversity loss.

The sound is a resource for marine species (Slabbekoorn and Bouton 2008; Tolimieri *et al.,* 2000,), and it is an important subject of study within this new research field that must be strengthened in the Mediterranean Sea as the relation between the habitat health and soundscape complexity (Piercy *et al.* 2014, Bertucci *et al.* 2015) is currently lacking.

The role of bioacoustics is fundamental to better know the Mediterranean species' acoustic characteristics of vocalizations and future works could contribute to: 1) associate biological sounds with habitat characteristics; 2) evaluate their changes related to anthropic pressure and stress events; 3) continue documenting vocal repertoires in the natural environment to raise new questions on the biological, social and "emotional" role of animal communication for which research is still limited. As human pressure is very intense in the Mediterranean Sea, the potential of passive acoustic monitoring could serve urgent ecological issues. MSFD is an ambitious legislative program that incorporates descriptors of state, pressure, and impacts on the marine environment and it is based on an ecosystem-based approach. It would be suggested the application of acoustics, as declined in various approaches described in this work, should be taken into greater consideration in the future and more employed in monitoring programs by States to obtain comparable information on which redetermination of GES is based. In this sense, more effective strategies provide consistent coordination to achieve even more challenging results given the effort made by state members so far to implement the Marine Strategy and achieve or maintain a good environmental status according to the 11 descriptors. Noise levels mean recorded in the Oristano area raise a degree of concern; the 100 dB threshold proposed within the Marine Strategy (63-125 Hz bands) has not been met. Nevertheless, it should be considered that these indications may not be generalized when applied to specific characteristics of different study areas (nearshore or offshore) and it should be borne in mind that the values reported in the mean of the monitoring do not take into consideration the continuous recording long-term (one year), but a seasonal subsampling. The registered values in the Gulf are also higher after the COVID-19 post lockdown confinement period.

This study has defined the general values of dB (average) seasonal, as well as verified the predominant anthropogenic sources. Furthermore, the acoustic identification of protected marine species makes it urgent to develop more structured monitoring programs over long periods. This could offer more information on the effects of noise pollution and the direct human impact on these species. The scarcity of information regarding underwater noise in the Mediterranean Sea makes small-scale studies useful for constructing a complete picture of the anthropogenic noise sources. I am convinced these data will lay the foundations to expand monitoring and research programs in this direction.

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GLOSSARY

- **Acoustic monitoring** is the use of acoustic sensors to passively record sound from the environment, which is then used to infer ecological information about vocalizing animals or acoustic habitat properties. It is distinct from active acoustic monitoring, which involves the detection of sounds from man-made acoustic emitters (such as on-animal tags).

- **Bioacoustics** is a discipline of biology concerned with the study of the emission, propagation and reception of acoustic signals by animals.

- **Ecoacoustics** is an emerging discipline concerned with the analysis of environmental sound recordings for ecological purposes (Sueur and Farina 2015), and is closely related to research under the heading of soundscape ecology (Pijanowski et al. 2011a). It is distinct from bioacoustics due to its focus on ecological communities and biodiversity. Acoustic indices are among the major tools used in ecoacoustic research. These summarise global spectral and temporal characteristics of sound recordings in order to study their relationships to biodiversity, habitat features and global change**.**

- **Frequency,** the frequency of a sound wave is its number of cycles per unit time, measured in hertz (Hz; cycles per second) or kilohertz (kHz; thousands of cycles per second). The term spectral relates to a sound's frequency characteristics.

- **Passive Acoustic Monitoring** (PAM) is the use of passive acoustic sensors to survey and monitor wildlife and the acoustic environment (see acoustic monitoring and acoustic sensor).

- **Soundscape ecology** is defined as the sum of all the sounds present in an environment in a given time interval which has become a useful instrument to evaluate the changes in habitat over time, as a response to external pressures.

NOTE

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