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SOUNDSCAPE

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Gap-analysis report based on existing knowledge of the sensitivity of target species (bottlenose dolphins, loggerhead turtles, and commercial fish sp.) to sound and the potential effects of noise

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Summary

1. INTRODUCTION

This review aims to provide a better understanding of the conditions for communication and orientation of sensitive target species, i.e. the common bottlenose dolphin (*Tursiops truncatus*) and the loggerhead turtle (*Caretta caretta*), and the potential impact of the rise in anthropogenic activity in the Adriatic Sea.

The underwater environment is ensonified by biotic, abiotic and man-made sound sources and the spectral and temporal composition of underwater soundscapes vary over relatively short geographical (a few km) and temporal scales, due to local physical and biological contributors. Several distinct seasonal and daily patterns exhibited by organisms, such as snapping shrimp in the Mediterranean Sea, have been referred to as the 'rhythms of nature' (Buscaino et al. 2016; Pijanowski et al. 2011). Unique and complex sound signatures that change consistently over relatively short time periods characterize different aquatic habitats (Kennedy et al. 2010) such as coral reefs (Nedelec et al. 2015; Staaterman, 2013, 2014;), coastal habitats (Radford et al. 2010), estuarine sites (Lillis et al. 2013) and temperate inshore marine environments (McWilliam and Hawkins, 2013). These soundscapes may contain biologically significant information that is likely to be used by marine organisms in order to behave appropriately in their environment.

Nowadays numerous sound sources constitute the overall ambient noise in the sea (Richardson et al. 1995; Hildebrand, 2009; Popper and Hastings, 2009) including those of anthropogenic origin. Over the past few decades, the increase in the anthropogenic noise in the sea has been more prominent due to the intense human exploitation (Andrew et al. 2002; Ross, 1993). Hence, a significant concern has been raised about the potentially harmful effects of this humanly generated sound on the marine environment and species sensitive to it. The anthropogenic noise was found to affect a wide range of marine species. The responses to noise not only depend on the received sound levels, its frequency and duration of animals' exposure to it but are also influenced by the state of the animals exposed, novelty of sound as well as the spatial relations between the sound source and the animals (Ellison et al. 2011; Wysocki et al. 2006). Responses to noise may also differ among species in relation to differences in their hearing capabilities (Kunc et al. 2016). A lot of attention has been so far dedicated to the effects of noise on hearing and communication systems of acoustically active species although recent studies have been more frequently indicating possibilities of noise causing also other, indirect effects, through sever alterations of physiology and behaviour. In the long term, the introduction of anthropogenic sound in the sea can cause alterations of the acoustic environment that can negatively affect the persistence of populations and species especially when combined with other environmental stressors (Kunc et al. 2016).

The responsibility to minimize the environmental impact of noise on marine organisms has therefore became a part of many international agreements and different intergovernmental organizations address this issue such as the Convention on Migratory Species (CMS), the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area (ACCOBAMS), Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention) and the

International Maritime Organization (IMO). In addition, the underwater noise has become an important aspect of Marine Strategy Framework Directive (MSFD) adopted by the European Commission in 2008, which aims to achieve Good Environmental Status (GES) of the European Marine Environment by the year 2020. The MSFD considers both the spatial and temporal distribution of loud impulsive noise (Indicator 11.1.1) as well as the trends in low-frequency continuous noise (Indicator 11.2.1).

One initiative of the Soundscape program is to collate all available published information on seasonal cycles in the vocal activity that may be habitat-specific or related to dynamic in the soundscape patterns on each target species. This activity will provide critical insights for sensible integration of human activities and the protection of marine animals sensitive to sound that are often at risk of population decline due to noise.

2. MARINE MAMMALS AND SEA TURTLES IN THE ADRIATIC SEA

Marine mammals and sea turtles play an important role in marine biodiversity and contribute significantly in maintenance of marine ecosystems. In the Adriatic Sea, there are several cetacean species present in different densities (Fortuna et al. 2015). The presence of common bottlenose dolphin (*Tursiops truncatus*) is confirmed along the entire basin, while the striped dolphin (*Stenella coeruleoalba*), Risso's dolphin (*Grampus griseus*) and Cuvier's beaked whale (*Ziphius cavirostris*) are present mainly in the southern part of the Adriatic Sea. The occurrence of fin whale (*Balaenoptera physalus*) is rather seasonal and related more to the central and south Adriatic and depends mainly on the sea currents and changes in the primary production at the yearly level. Short-beaked common dolphin (*Delphinus delphis*) which once was the most abundant dolphin species in the Adriatic Sea is currently considered as regionally extinct, although in the recent years more frequent observations of bigger groups are occurring within the Central and north Adriatic Sea. Long-finned pilot whale (*Globicephala melas*), false killer whale (*Pseudorca crassidens*) and humpback whale (*Megaptera novaeangliae*) are representing rare visitors of the Adriatic Sea. Three species of sea turtles have also been reported in this region (Fortuna et al. 2015; Lazar et al. 2008; Lazar et al. 2004a): loggerhead turtle (*Caretta caretta*), green turtle (*Chelonia mydas*) and the Leatherback turtle (*Dermochelys coriacea*). Loggerhead turtle is the most abundant sea turtle specie in the Adriatic Sea. The individuals from natal Greek population are using the Adriatic Sea as one of their most important feeding areas in the whole Mediterranean Sea. Spatio-temporal analysis indicates their whole-year presence within the Adriatic and existence of diverse habitats within this region. For example, green turtle is found mainly in the southern part of the Adriatic Sea, particularly in Albania while Leatherback turtle is regular in the Mediterranean with rare occurrence in the Adriatic Sea.

Within the northern Adriatic Sea, common bottlenose dolphin (*Tursiops truncatus*, Montagu, 1821; hereafter bottlenose dolphin; Figure 1) represents the only cetacean specie that is regularly inhabiting this area while the only continuously present species of sea turtles in this area is loggerhead turtle (*Caretta caretta*).

Figure 1. Common bottlenose dolphin (*Tursiops truncatus*); Photography: Blue World Institute

The bottlenose dolphin belongs to the group of Odontocetes (toothead whales), family Delphinidae (dolphins). It is widely distributed species with a distribution range that includes both inshore and offshore waters (Folkens and Reeves 2002).

In the Adriatic Sea the presence of coastal populations has been confirmed, with their distribution and movement patterns appearing to be strongly habitat dependent (Fortuna, 2006; Genov et al. 2008; Holcer, 2012; Reeves and Notarbartolo di Sciara, 2006). Their habitats include coastal areas, open waters, lagoons and river estuaries where they feed on fish and cephalopods.

The lack of quantitative historical data limits defining trends in their abundance within the Adriatic Sea. Based on the aerial surveys, conducted in 2010 and 2013, with the aim of identifying the presence and distribution of cetaceans and sea turtles within the whole Adriatic basin, the first information on the overall distribution of bottlenose dolphins was given. As result, the uncorrected abundance estimates for the whole Adriatic Sea is 5.700 individuals (CI = 4,300-7,600; Table 1; Fortuna et al. 2018) Predictive density (Figure 2) of bottlenose dolphins indicates that North and South Adriatic appear to be areas of high relevance for this species. Their abundance was found to be the highest in the north Adriatic Sea with the relative density of 0.057 individuals/km^2 (Fortuna et al. 2018).

Population structure of bottlenose dolphins in the Adriatic Sea follows the concept of metapopulation. Different “local populations” spatially divided (partially of completely) are still maintaining the contact through migrations and associated gene flow (Hanski and Gaggiotti, 2004). This is confirmed by photo-identification data that indicates bottlenose dolphin individuals are separated in so-called “local populations” (Genov et al. 2008; Genov et al. 2009; Fortuna 2006; Holcer 2012; Pleslić et al. 2015).

Figure 2. Predictability model of the density of bottlenose dolphins in the Adriatic Sea; Fortuna et al. 2018

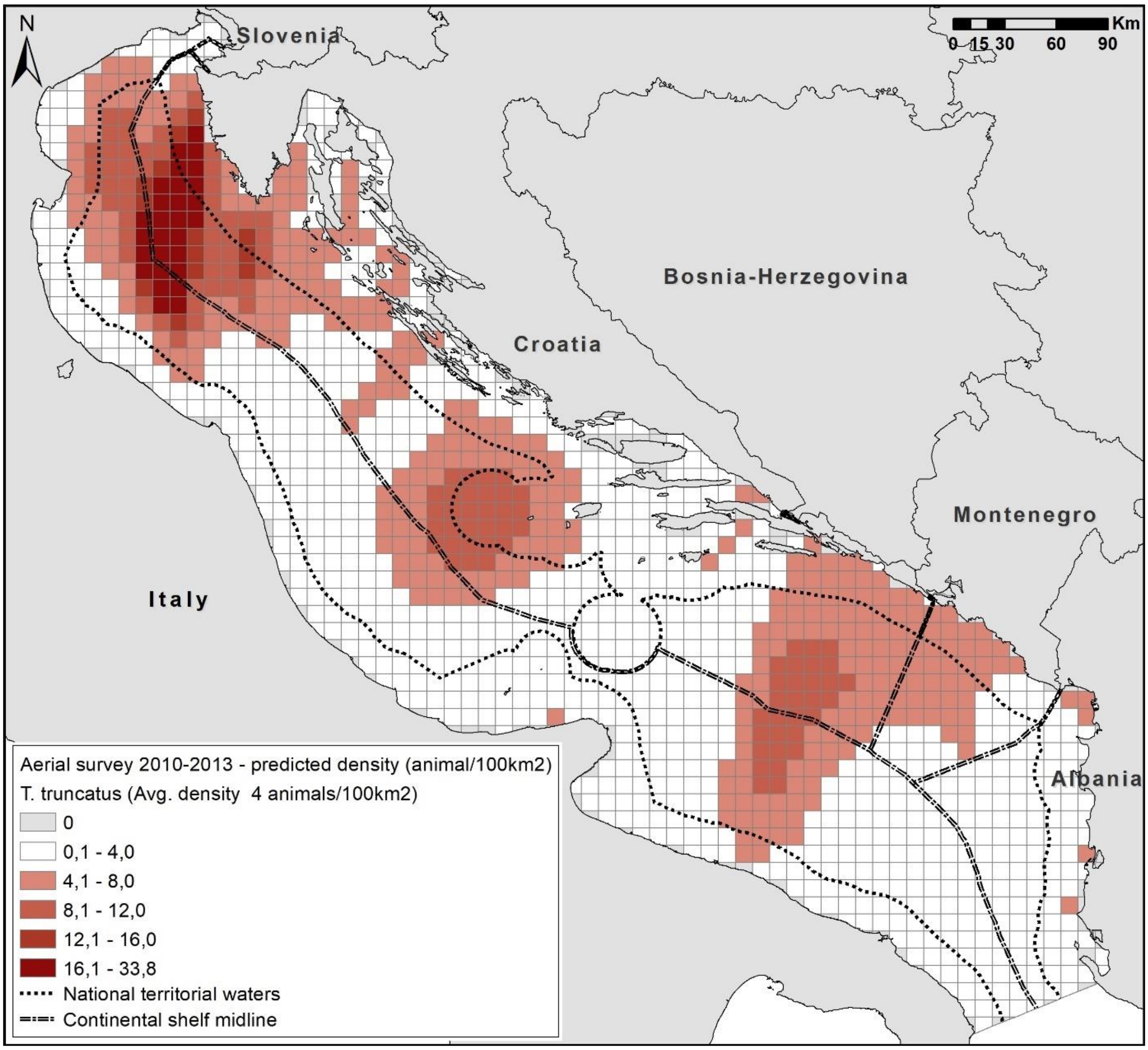


Table 1. Bottlenose dolphin abundance estimates in the Adriatic Sea; Fortuna et al. 2018

Area	**Density individuals/ km^2**	**Uncorrected estimate (95% CI)**
Adriatic Sea	0,042	5,700 (CI = 4,300-7,600)
North Adriatic	0,057	2,600 (CI = 2,200-2,900)
Central Adriatic	0.034	1,100 (CI = 800-1,500)
South Adriatic	0.032	1,800 (CI = 1,500-2,400)

Within the Croatian waters, there are 6 Natura 2000 SCIs (NN 124/13, NN 105/15) declared due to their importance for bottlenose dolphins. Two of them are located in the north Adriatic Sea: SCI area HR5000032 Western Istria and SCI area HR3000161 Cres-Lošinj waters Interestingly Triossi et al. (2013) identified the presence of diverse oil and gas platforms in the North Adriatic Sea as areas of high bottlenose dolphin abundance where dolphin generally feed or rest. This is related to the fact that platforms are areas with restricted access to boats, prohibited fishing activity or anchoring so they provide certain shelter for demersal fish and gathering locations for pelagic fish species.

Following International Union for Conservation of Nature – IUCN, bottlenose dolphin is considered as least concern species (LC) at the global level while regionally it is considered vulnerable specie (VU) and according to the Red Book of Mammals (Holcer, 2006) the species is listed as endangered (EN).

Figure 3. Loggerhead turtles (*Caretta caretta*). Photography: Blue World Institute

Sea turtles spend the majority of their lives in the ocean; their only land-linked behaviors are egg deposition and hatching. Like many marine fishes and mammals, sea turtles use a range of habitats for each developmental stage (see review by Bolton, 2003). Once hatchlings reach the sea, they are pelagic, moving primarily with ocean currents. After a period of years, which varies both among species and populations, a critical ontogenetic habitat shift occurs whereby most sea turtles actively recruit to a demersal, neritic habitat and are considered juveniles. Finally, upon reaching maturity, all sea turtles maintain a discrete foraging area (this region frequently overlaps with the juveniles), migrating only to return to their natal nesting beach.

Recent recoveries of loggerhead turtles (Figure 4) tagged on nesting beaches that have been reported in the northern Adriatic suggest that this area is important for both adult and juvenile loggerheads (Lazar et al. 2004a). Turtle movements into the Adriatic are likely developmental or feeding migrations and there is probably a major migratory pathway from the Ionian Sea into the Adriatic. No nesting locations or reproductive sites have been identified along the Croatian coast (Casale et al. 2012). Repeated recoveries of the same individuals within a particular year indicate that loggerheads reside in the Adriatic for extended periods of time and also indicate a degree of site fidelity.

The northern and central Adriatic, together with the Gulf of Gabès in Tunisia, are the two shallowest (less than 200 m deep) regions in the Mediterranean. Continental shelf area of north and central Adriatic Sea, characterized by depths over 200 m (Cushman-Roisin et al. 2001), rich benthic communities (Gamulin-Brida, 1974; Kollmann and Stachowitsch, 2001) and favourable summer sea temperature (Supić and Orlić, 1992) represents one of the key neritic habitats for feeding of loggerhead turtle in the Mediterranean used by both juvenile and adult individuals (Casale and Margaritoulis, 2010; Lazar et al. 2011; Lazar and Tvrtković, 2003; Margaritoulis et al. 2003;). A high number of tag recoveries coinciding with these two regions proves this (Lazar et al. 2004b; Lazar and Tvrtković 2003) while the recent data indicate importance of the eastern coasts of Central Adriatic as the wintering ground for the Mediterranean population (Casale et al. 2004).

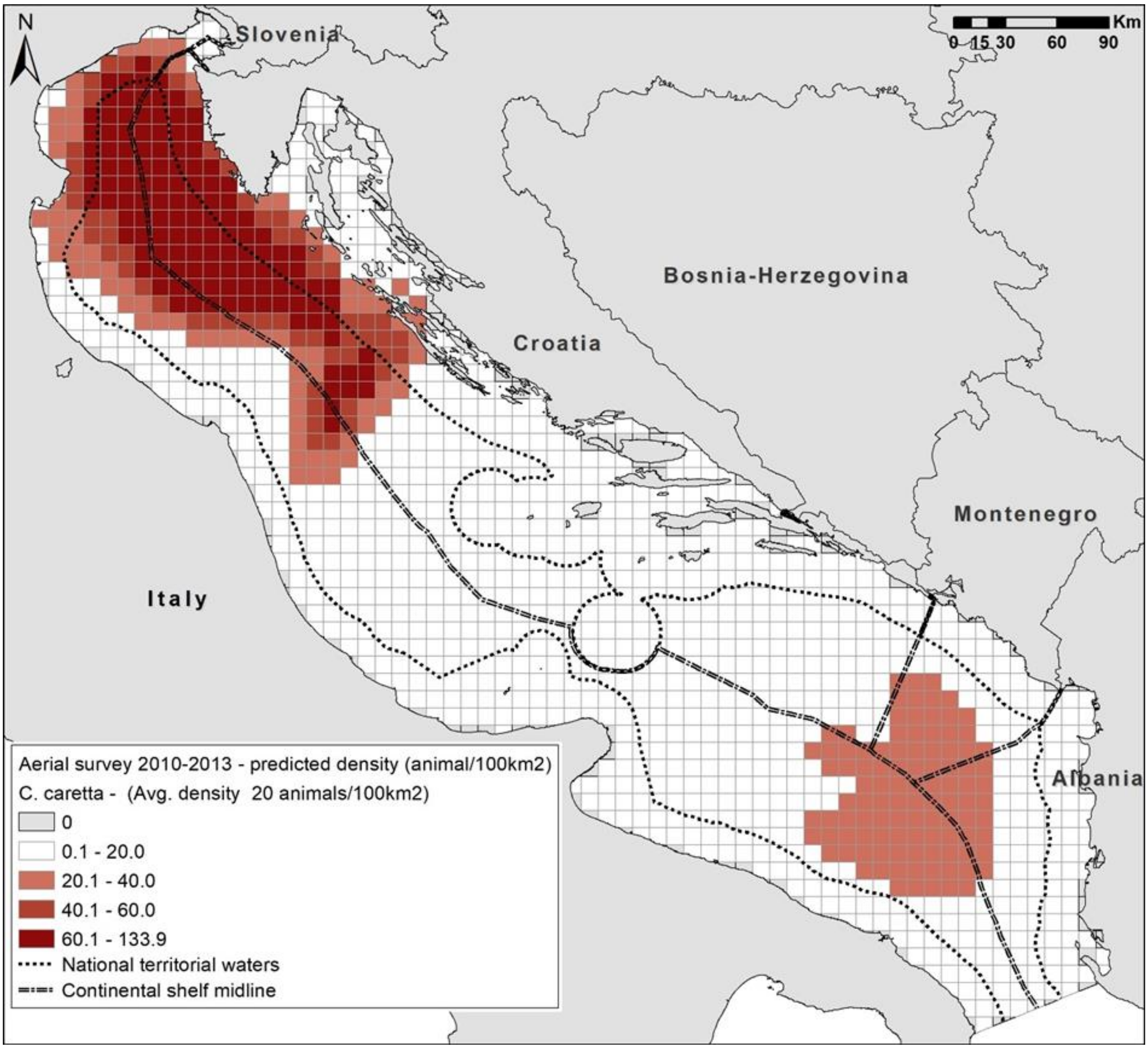
Further, analysis of size and diet indicates that northern Adriatic Sea is inhabited by juvenile individuals with carapace size between 25 and 30 cm, which represents early ontogenetic shift towards neritic habitats (Lazar, 2009; Lazar et al. 2008).

The uncorrected abundance estimates for the whole Adriatic Sea is based on the aerial survey data from 2010 and 2013 and is 27,000 individuals (CI = 24,000–31,000; Fortuna et al. 2018; Table 2). Figure 4 indicates distribution and abundance data for loggerhead turtles in the Adriatic Sea during the same period.

Table 2. Abundance estimate for loggerhead turtles; Fortuna et al. 2018

Area	**Density individuals/ km^2**	**Uncorrected estimate (95% CI)**
Adriatic Sea	0,203	27,000 (CIs = 24,000–31,000)
North Adriatic	0,405	18,200 (CIs = 17,700–20,000)
Central Adriatic	0,057	1,900 (CIs = 1,600–2,200)
South Adriatic	0,114	6,300 (CIs = 5,000–7,500

Figure 4. Predictive density of loggerhead turtle in the Adriatic Sea; Fortuna et al. 2018



Sea turtles are one of the most endangered marine organisms due to their frequent entanglements in fishing gear and continuous degradation of their habitats fishing activities such as bottom trawling. Following the IUCN criteria, loggerhead turtles are listed as vulnerable specie (VU) while at the regional level they appear as least concern species (LC). At the national level, and according to the Red Book of Amphibians and Reptiles of Croatia (Jelić et al. 2015), loggerhead turtle is listed as vulnerable species (VU).

3. GOALS OF THIS ANALYSIS

This analysis is made by different sections: each of them focuses on one topic that is considered relevant by the authors to understand the underwater acoustics and its role in the light of protecting the target species. An attempt is made to define information needed to evaluate effects of noise on bottlenose dolphin and loggerhead turtle and to consider which information are currently being reached and to examine those that are still to be reached, in order to provide suggestions on the kind of research that might have high priority for future funding. Special attention will be given to the data relative to the Mediterranean Sea with particular reference to the Northern Adriatic Sea.

4. TOPIC 1: SOUND EXPOSURE METRICS

Sound is a longitudinal wave that alternately compresses and decompresses the component particles of the medium through which it travels (air or water; Hawkins, 1986). It is expressed on a logarithmic decibel (dB) scale referenced to 1 microPascal (dB re 1 µPa) (ICES, 2005; Verfuß et al. 2015). The sound speed in the sea is nearly five times faster than in the air, which is due to differences in the density of the two mediums. The higher density of the sea compared to air guarantees a faster transfer of the acoustic energy (Verfuß et al. 2015).

Sound wave is generally described by two components **a)** the pressure component that refers to the variation compared to the local hydrostatic pressure (Sigray and Andersson 2011) and **b)** by the motion component of the acoustic particles of the medium (Bradely and Stern, 2008; Hawkins and Popper, 2017; Nedelec et al. 2016; Southall et al. 2007). In other words, sound is a propagated vibratory energy. The particle motion component contains information about the direction of the propagating wave. Particle motion can be expressed as displacement (m), velocity (m/s) or acceleration (m/s^2). While detection of the sound pressure relies on the use of hydrophones that exist in a great variety of commercial models, the methods for measuring particle motion rely on the use of technologies that have only recently become commercially available and their use requires appropriate skills and knowledge (Martin et al. 2016; Nedelec et al. 2016).

The understanding of the effects of the sound on marine environment depends on the characteristics of the emitted sound, which need to be described by the appropriate metrics. Verfuß et al. (2015) has recently summarized definitions of different acoustic parameters including their terms and quantities. In general, root-mean-square (RMS) sound pressure level (SPL) or peak sound pressure level (SPL_{peak}) is used for the continuous sounds. In the case of fishes and invertebrates that are incapable to detect sound pressure, it is relevant to measure also the particle velocity and acceleration of the corresponding sound (Hawkins and Popper, 2017).

For the impulsive sounds, the source level was found to be a widely used metric (Hildebrand, 2009; ICES, 2005; Richardson et al. 1995). It corresponds to the level of sound at a nominal distance of 1m. More recently, the source levels have been suggested as insufficient for identifying the possible effects of these short and repetitive sound pulses and the use of the sound exposure level (SEL i.e. time integral of the pressure squared normalized for a 1-s period) has been proposed as more appropriate metrics for this purpose (Popper and Hastings, 2009). In addition, considering the cumulative effects related to the pulse receptions is important (Hawkins and Popper, 2017).

Finally, in order to reduce uncertainties around measurements, repeated measurements and the use of basic statistical calculations (Mean, SD, Median, etc.) are highly recommended (Bell, 2001).

To assess and quantify the environmental effects of the anthropogenic noise it is crucial to estimate the levels of sound generated by the source (Source Level) and the rate at which the sound decays as it propagates away from the source (Transmission Loss). In general, the low-frequency anthropogenic sound is subjected to a lower level of attenuation. It has longer wavelengths and their peaks have lower pressure in comparison to high frequency wave making it therefore more capable of long-range propagation.

5. TOPIC 2: UNDERWATER NOISE AND MAN - MADE SOURCES OF NOISE POLLUTION IN THE ADRIATIC SEA

Figure 5. Sources of underwater man-made noises

In relation to the effects of anthropogenic sound on marine species and habitats and based on the lasting of the emitted sound, we can distinguish impulsive (short-duration) and continuous (long-duration) anthropogenic sound produced by different sources (Figure 5).

2.1. Impulsive sound

Impulsive sound may be of low, medium or high frequency. It represents the sound generally associated with marine civil engineering, construction and diverse infrastructure projects. Seismic air-guns represent the impulsive sound sources with the worldwide use. The source of the sound is represented by the air-gun towed behind the seismic vessel. Seismic air-guns generate predominantly low frequency sound pulses (DeRuiter et al. 2006; Hildebrand, 2009; Richardson et al. 1995) and incidental noise across much higher frequencies (up to 100 kHz; DeRuiter et al. 2006; Goold and Coates, 2006). The sound that is produced repeatedly every 10-15 s is displayed vertically towards the sea floor from which it reflects back to the surface where detectors (hydrophones) receive it. Seismic air-gun array has source levels of up to 260 dB re 1 µPa at 1 m (Hildebrand, 2009; OSPAR, 2009).

Active Sonars also represent the impulsive sound sources used to explore and map the sea. There are different types of sonars that differ in their propagation characteristics. Low-frequency active (LFA) sonars are generally used for military purposes. Their great propagation capabilities enable long-range (> 100 kilometers) detection of objects. The US Navy has developed the SURTASS-LFA (Surveillance Towed Array Sensor System – Low Frequency Active). The system uses the sound generated at the frequency between 100-500 Hz by massive sound transmitters that are towed in a vertical array by the vessels. The source level of each projector is approximately 215 dB re 1 µPa @ 1m (Johnson, 2002) and the effective source level of the entire array is approximately 235 dB re 1 µPa at 1 m (Hildebrand, 2009; ICES, 2005). The signals are repeated in sequences lasting 6-100 s with repetitions every 6-15 minutes.

Unlike LFA Sonars, mid-frequency sonars (MFA) are used for detections at moderate distances (tens of kilometers). A US Navy hull-mounted system (AN/SQS-53C) operates in a frequency range 2-10 kHz and produces signals that are emitted as multiple pings grouped closely in time (ICES, 2005; MPL TM, 2015). The MFA source level is of about 235 dB re 1 µPa at 1 m. The sub-bottom profilers are also mid-frequency sonars but with a non-military use. They emit signals of 204 dB re 1 µPa at 1 m in the frequency range 3-5 kHz (ICES, 2005).

High-frequency active sonars (HFA) generally produce signals with lower source levels (190 dB re 1 µPa at 1 m) directed towards the sea floor. They are used for civilian and commercial purposes such as mapping the sea floor, locating and acquiring information on various underwater targets. The concern of HFA sonars is related mainly to their worldwide use. They include mapping sonars such as Single beam and Multibeam Echo Sounders. Boebel et al. (2004) described the Atlas hydrosweep DS-2 deep sea multi-beam sonar that has source levels greater than 220 dB re 1 µPa @ 1m at 15.5 kHz with relatively short (24ms) pulses. The frequency of mapping sonars varies from around 12 kHz for deep water sonars to higher frequencies of 70-100 kHz in shallow water systems (Hildebrand, 2009).

Side scan sonars are also used for detecting objects on the sea floor. They transmit sound and analyze the returning echo that has bounced back from the sea floor or a specific target. The signals reach the distance of few hundred meters. They emit sound at frequencies 50 – 500 kHz or higher with 229-230 dB re 1 µPa at 1 m source level in order to get greater resolutions of underwater features (Richardson et al. 1995).

Other impulsive sound sources include Acoustic Deterrent Devices (ADD) or pingers that are generally used to modify marine mammal behavior in relation to fishery-marine mammal interaction, to prevent marine mammal entanglements in nets and therefore to reduce bycatch or to prevent depredation of fishing gear (Petras, 2003). The ADDs produce sound in the frequency range 5-160 kHz with a source level of 150 dB re 1 µPa at 1 m (Hildebrand, 2009; Shapiro et al. 2009; Tasker et al. 2010). The pulse duration is typically 200-900 ms, and the pulse interval varies between fixed 4 s to semi-randomly varying between 4 s and 30 s (Shapiro et al. 2009).

Similar to ADDs, Acoustic Harassment Devices (AHD) such as seal scarers are designed to create a sound pressure wave that can inflict pain or discomfort to target species (Petras, 2003). AHDs produce source sounds in the frequency range from 5 to 40 kHz with source level of about 195 dB re 1 µPa at 1 m (Petras, 2003; Tasker et al. 2010).

Pile driving related to construction of offshore platforms also produces impulsive noise in the low frequencies range (100 Hz – 1 kHz). Source levels depend on the energy rating of hydraulic hammers. For example, a 1000 kJ hammer produces sound pulses of 30-50 ms lasting, repeatedly, with each pulse having source level of 237 µPa at 1 m (Hildebrand, 2009).

Among all the impulsive sources, explosives produce the highest levels of noise. They are used for different purposes (removal of structures, ship shock trials, naval mines, bombs, torpedoes). Explosives produce a broadband pressure impulse that propagates equally in all directions. Even an explosive consisting of 0.5 kg charge produces the overall source levels of 267 dB re 1 µPa at 1 m (Hildebrand, 2009; Richardson et al. 1995).

2.2. Continuous sound

Continuous sound is often found to dominate the low – frequency range and therefore propagates over great distances affecting a large proportion of marine environment. Unlike most of the higher intensity sources, lower intensity sound, produced by shipping, dredging and energy installation, are continuous and pervade a large portion of environment (Popper and Hastings, 2009). It is therefore far harder to avoid them as they imply the increase in the overall background noise in the sea with potentially chronic consequences on marine life.

Commercial shipping contributes significantly to the overall ambient noise in the sea. Generated noise is related mainly to propeller cavitation and turbulence which is known to peak at 50–150 Hz but can extend up to 10 000 Hz (Ross, 1976). In the period 1950-2000, an increase of 3 dB in the overall background noise has been found to correspond to every decade of the past five decades (Andrew et al. 2002; Mazzuca, 2001). During this period the number of ships in the world fleet tripled and the gross tonnage increased (National Research Council, 2003). McKenna et al. (2012) gave a detailed description of shipping sound according to different ship types with source levels that vary between 178-188 dB re 1 µPa at 1 m in the range of frequencies 20-1000 Hz. Moreover, in the coastal areas' leisure boating represents an important source of anthropogenic noise, substantially contributing to the overall SAN in the low to mid-frequency range (Hildebrand, 2009; Kipple and Gabriele, 2004; Rako et al. 2013).

Offshore drilling also represents a source of continuous sound. It is performed from various platforms and involves production of noise at moderate source levels (Hildebrand, 2009). The noise is produced within the low to mid-frequency range with maximum source levels of 184 µPa at 1 m (Hildebrand, 2009; Prideaux, 2016).

Marine dredging is another source of continuous noise with levels that vary depending on the dredging activity and can be affected by many factors including dredged sediment, depth, salinity and temperature (Robinson et al. 2014; Jensen et al. 2000). Different types of dredgers emit different levels of sound, which will further vary, depending on the activity being undertaken (Reine and Dickerson, 2014; Reine et al. 2012). Dredging sound is broadband with maximum source levels of 160-180 dB re 1 µPa at 1 m at 100 Hz and 160 Hz 1/3 octave frequency bands (Richardson et al. 1995).

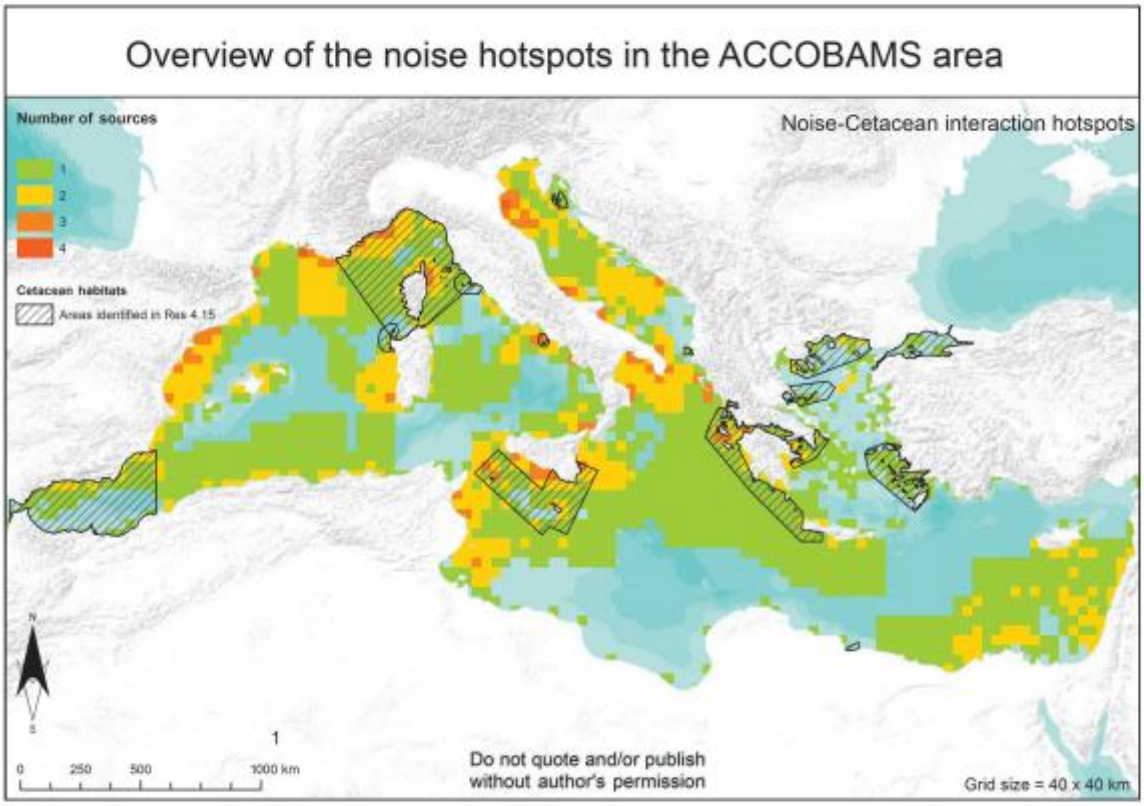
6. TOPIC 3: BACKGROUND AMBIENT NOISE LEVELS IN THE MEDITERRANEAN SEA AND THE NORTHERN ADRIATIC SEA

3.1 Mediterranean Sea

The first overview of noise hotspots in the Mediterranean Sea was provided by the ACCOBAMS Agreement in 2016 (Figure 6; Maglio et al. 2016) with the aim of identifying noise areas that could potentially conflict with cetacean conservation. This project included making an inventory of noise-producing human activities and mapping areas where such activities take place. The study area included the whole Mediterranean Sea and was focused on human activities that produce noise that may be harmful to cetaceans. Such activities included coastal and offshore activities, seismic (geophysical) surveys, naval exercises, and marine traffic. Data was collected through various means including dedicated internet search, AIS- data base, official online repositories, and contacts with relevant stakeholders. From this data, summary GIS maps were created using a 40km x 40 km grid resolution. Ship density maps were created based on the AIS system, which is not mandatory for all ships. Researchers found that while ship density values may serve as good indicators of the spatial distribution of pressure areas, they are likely to be under estimated. Based on the collected data, on average, around 1500 vessels were found to be present in the area at any time.

According to Maglio et al. (2016), areas with high concentrations of coastal and offshore activities are located in the Northern part of the basin, with the highest concentrations in Côte d'Azur and the Gulf of Fos (France), the Gulf of Naples and the Campanian Archipelago (Italy), and the Gulf of Trieste (Italy/Slovenia). A high concentration of hydrocarbon extraction activities (oil and gas wells) in the last 10 years took place in the Northern Adriatic Sea, the Strait of Sicily, and Levantine areas. An increasing trend has been found in the size of the area used for seismic surveys, with the area representing 3.8% of the Mediterranean Sea's surface in 2005 and 27% of its surface in 2013. Military areas were found to cover large portion of Western Mediterranean Sea, particularly in the Ligurian Sea, Spanish and French coastal waters, the Tyrrhenian Sea, the Italian part of the Strait of Sicily, and the Ionian Sea. Areas that are accumulating noise-producing activities that overlap with the important cetacean habitats as identified by ACCOBAMS Parties through Resolution 4.15 (2010) include the Pelagos Sanctuary, the Strait of Sicily and the northern part of the Hellenic Trench.

Figure 6. Noise-cetacean interaction hot spots: overlap of noise hot spots and important cetacean habitats (ACCOBAMS Resolution 4.10, 2010); Number of Sources (Source Categories include seismic surveys, harbor works, offshore works, and military activities.) (Adapted from Maglio et al. 2016)



Despite this overview, little is known about real data of underwater acoustic levels. A long-term study of approximately 10 months of continuous acoustic monitoring have been carried out so far in the Ionian Sea at a depth of 2100 m. Data show that in the study area the median of the SPL values exceeds 100 dB re 1 µPa in the standard 1/3 octave bands up to 250 Hz. In particular the median values of the SPL in the 1/3 octave bands centered at 63 Hz and 125 Hz, considered in the descriptor 11.2 of the MSFD, reach respectively 112 dB re 1 µPa and 107 dB re 1 µPa (Viola et al. 2017). From 2013 to 2014, a similar study has been run in the shallow waters of Lampedusa (Italy), in the middle of the Mediterranean Sea, inside a marine protected area. Lampedusa is characterized by heavy anthropogenic noise, with a mean of 13 vessel passages per hour over one year, and with a masking effect on the fish vocalizations below 2kHz during July and August for approximately 46% of the time. Noise levels at lower frequencies increased from November to March (from 97.8 to 103.7 dB re 1µPa) whereas the higher frequencies followed the opposite pattern (102.3 to 110.9dB re 1µPa), with lower values during the winter. This seasonal variability was mainly attributable to the sea state for the lower frequencies and to the activity of snapping shrimp for the higher frequencies (Buscaino et al. 2016).

3.2 Soundscape of North Adriatic Sea

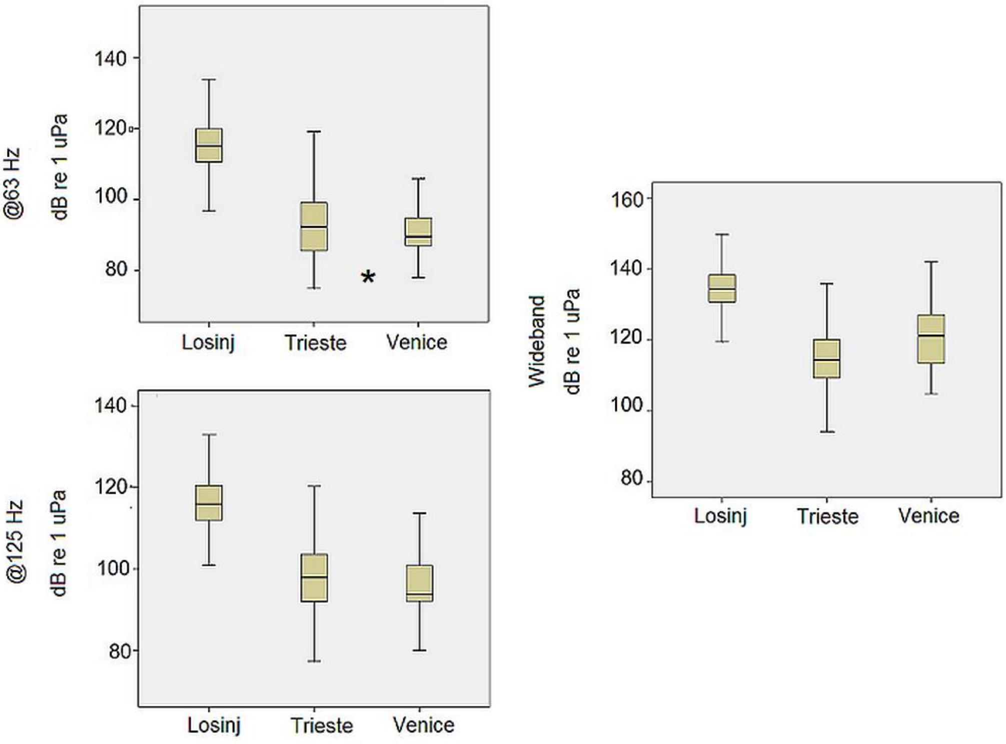
Recently Picciulin et al. (submitted) reviewed the sea ambient noise data collected by the authors since 2006 along the Northern Adriatic Sea coast in three study areas (each one including different sampling stations), i.e. i) the Gulf of Trieste, ii) the Venice lagoon and its coastal area (Italy) and iii) Cres and Lošinj archipelago (Croatia). The Gulf of Trieste is an area of approximately 25 × 15 nautical miles characterized by water depth not exceeding 26 m (Celio et al. 2006). Along the 130 km of the Gulf of Trieste's Italian coast, more than 15,000 berths are present, spread in ca. 40 harbours: 3 of these (Marano, Grado and Trieste) are important fishing harbours while the others are smaller and mainly recreational. Large tonnage merchant ships travel regularly to the Trieste and Monfalcone harbours (Italy) or alternatively to Koper (Slovenia). As a result, the Trieste Gulf is an environment with multiple inputs of continuous anthropogenic noise both in a spatial and in a temporal extent. Codarin and Picciulin (2015) have shown that the Adriatic Sea sub-region experiences high noise pressure with no intra-site significant seasonal nor spatial variations likely due to the cargo vessel traffic being homogeneous in space and time throughout the year. On the other hand, many marine protected areas are present, e.g. the WWF-Miramare Natural Marine Reserve. The latter is an UNESCO-MAB Biosphere Reserve densely populated by different fish species for which it represents an important seasonal nursery area (Guidetti et al. 2005). The underwater soundscape of the WWF Natural Marine Reserve of Miramare has been already described in terms of noise levels (Codarin, 2008; Codarin et al. 2009; Picciulin et al. 2010), that results to be similar to those of the whole Gulf (Codarin and Picciulin, 2015). In terms of biophony, this area is mainly dominated by Sciaenidae but vocalizations of Gobidae and Pomacentridae have been recorded (Picciulin et al. 2010, 2012, 2013; Sebastianutto et al. 2008). Codarin et al. (2009) and Picciulin et al. (2010) have reported behavioural and acoustic short-term effects of locally recorded boat noise on fish community

The Venice lagoon is the largest Mediterranean lagoon hosting a well-structured fish community functioning as nursery area of many fish species (Franco et al. 2006; Malavasi et al. 2004). Although the Venice lagoon has been recognized as a Special Protection Area and it has been established as a World Heritage Site by UNESCO in 1987, the mainland harbour of Marghera in the Venice lagoon is one of the widest and most complex industrial and shipping areas in Europe (Regione Veneto, 2010). A consistent underwater noise pollution characterize the Venice area, due to the intense merchant and passenger ship traffic (Bolgan et al. 2016), with special reference to passenger cruises that encompass a significant portion of the local background noise and are quite common (an average five cruise passages per day each month during summer, Harbour master office 2016). Despite this, the presence of vocal fish species has been highlighted in high anthropic areas as the port inlets of the Venice lagoon or in other coastal artificial man-made structures such as mussel farms (Colla et al. 2018). The vocal fish distribution has been suggested to be a reliable indicator of a wider fish assemblage typical of rocky-reef habitats (Picciulin et al. 2013).

Cumulative data on the local soundscape have been reported also for the Cres–Lošinj archipelago in the Kvarnerić region of Croatian coast (Rako-Gospić et al. 2013). These waters have been designated a Site of Community Importance (SCI) of the NATURA 2000 network due to their importance as a habitat for the resident bottlenose dolphin population. This relatively shallow, coastal area is used by resident dolphins as feeding and nursing ground (Bearzi et al. 1999). The area is a well-known tourist destination in the northern Adriatic Sea attracting a large number of tourists each year, particularly during the summer, hosting around 1.5 million overnight stays (Harbour master office, 2016). Nautical traffic peaking during summer months causes significant changes in the soundscape of this marine habitat, which in its turn lead to dolphin avoidance of the areas with increased noise levels (Rako-Gospić et al. 2013). Rako-Gospić and Picciulin (2016) also showed that high levels of underwater noise cause significant changes in the acoustic structure of dolphin whistles in that area.

Following Picciulin et al. (submitted) the North Adriatic Sea can be considered as a ‘noisy’ area: SPL wideband values range from a minimum of 88 dB re 1 µPa to a maximum of 154 dB re 1 µPa (recorded in the Lošinj archipelago) with an average value equal to 125 ± 11 dB re 1 µPa. For 63 Hz and 125 Hz 1/3 octave bands SPL levels do not exceed 132 and 136 dB re 1 µPa respectively, with and average value of 104±14 and 107±11 dB re 1 µPa. The overall highlighted underwater noise values in the North Adriatic Sea (Figure 7) are higher than reported for other coastal areas characterized by vessel traffic as the shipping location in Guanabara Bay in the southeastern Brazil, where the highest mean sound pressure level near the surface was 111.56 dB re 1µPa at the frequency band of 187 Hz (Bittencourt et al. 2014) or as the near-shore Southold Bay in New York during periods of high human activity, where noise levels peak up to 110 dB (Samuel et al. 2005), confirming the need to further studies in the area.

Figure 7. Box plot of the 63Hz 1/3 octave band, the 125 1/3 octave band and wideband SPLs calculated for data collected in the Cres–Lošinj archipelago, Trieste and Venice areas respectively. Box plot shows range (whiskers), 25% and 75% percentiles (lower and upper limit of box) and median (-). Statistical differences in SPLs values are indicated with an *).



On the other hand, data indicate spatial variations in the underwater noise levels between the investigated area; noise level values were found to be more similar between Trieste and Venice, which are both characterized by port activity and heavy cargo traffic, in contrast to the Cres and Lošinj archipelago, a typical touristic destination dedicated to recreational boating and artisanal fishing. Surprisingly, the archipelago resulted noisier on average than the northern NAS coasts. This can be potentially affected by the different topography of the recording areas, the Trieste and Venice areas being characterized by shallow waters of on average 35 meters depth with muddy bottom whereas the Cres and Lošinj archipelago with average water depth around 70 meters is characterized by rocky shores, submerged reefs and caves. Although a limited number of years are available for the analysis, intra-annual variation of noise levels has been found also in all the areas, with fluctuations that do not indicate a clear trend.

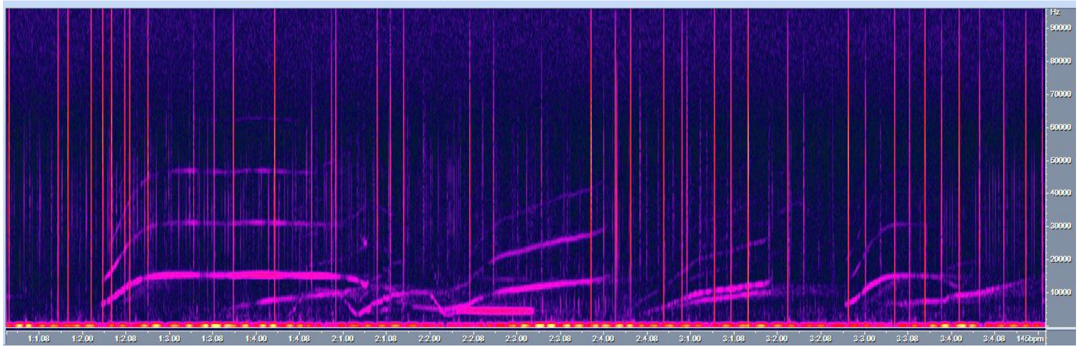
A soundscape investigation has been conducted also on shallow hard bottoms of the Adriatic Sea (Central Mediterranean basin; Pieretti et al. 2017). The dominant components of the soundscape were found to be the sounds of snapping shrimps and diverse species of fish and anthropogenic noise caused by boat transits. No data are available for average underwater noise levels.

7. TOPIC 4 - SOUND PRODUCTION BY UNDERWATER ANIMALS WITH PARTICULAR REFERENCE TO BOTTLENOSE DOLPHINS AND LOGGERHEAD TURTLES

Of all the ways to transmit information throughout the sea, sound is the best. Light is attenuated through the seawater due to water depth, turbidity, early and late diurnal periods while chemical and tactile information exchange is restricted to animals in closer proximity. On the other side, underwater sound travels rapidly over great distances; therefore, it is not surprising that acoustic communication under the sea is widespread in marine animals such as crustaceans, fish, and mammals. Communication involves the provision of information from a sender to a receiver in a manner thar is deliberate and beneficial to both parties (Bradbury and Vehrecamp, 1998). Marine invertebrates are known to produce communicative sounds (Bouwma and Herrnkind, 2009; Buscaino et al. 2011; Patek et al. 2009; Popper et al. 2001; Staaterman et al. 2011). Sound production has been revealed in more than 800 bony fish species (Amorim, 2006). Marine mammals create sounds to communicate about the presence of danger, food, and conspecifics, as well as their position, identity, and territorial or reproductive status (Richardson et al. 1995).

In the bottlenose dolphins sound production is localized in the nasal region - in the so called “monkey lips/dorsal bursae (MLDB) complex”. The monkey lips (or phonic lips) are dense connective tissue valves that project into the nasal passage, resembling the region around the mouth of an ape, while the dorsal bursae are small ellipsoid fat bodies (Cranford et al. 1996). A pair of bursae is associated to a couple of opposite phonic lips to form a MLDB complex. Bottlenose dolphins present two MLDB complexes that can function independently, so that they can potentially produce different sound simultaneously (Frankel, 2009). Sound production begins when the palatopharyngeal muscles force pressurized air to pass through the phonic lips, causing vibrations in the adjacent dorsal bursae (McKenna et al. 2012). Sound vibrations propagate along multiple pathways through the melon and emerge into the environment (Aroyan et al. 1992; Cranford et al. 2008). The melon is an organ placed in dolphins’ forehead and composed mainly of fat and connective tissue fibers (Harper et al. 2008), which is thought to focus sound energy generated in the MLDB complex (Cranford et al. 2008).

Figure 8. Bottlenose dolphins: spectrogram view of whistles and cliks



Bottlenose dolphins, in the Mediterranean coastal habitats produce two types of sound: broadband impulsive signals (clicks/burst), ranging from a few kHz up to 120 kHz, and modulated narrowband whistles (Figure 8). Frequency-modulated tonal sounds called 'whistles' are used for individual recognition, contact maintenance and group coordination (Janik and Sayigh, 2013; Macfarlane et al. 2017). Variability in whistle parameters have been found recently among bottlenose dolphins inhabiting different areas of the Mediterranean Sea (La Manna et al. 2017). Acoustic divergence among populations has been related to geographical isolation and may result from dolphins' adaptation to local ecological conditions and social systems (Ansmann et al. 2007; Azzolin et al. 2013; Baron et al. 2008; La Manna et al. 2013; Luís et al. 2014; Morisaka et al. 2005; Papale et al. 2013; Papale et al. 2015; Rako-Gospić and Picciulin, 2016; van Ginkel et al. 2017). While the individual variability and the influence of group size and composition are often manifested through changes of whistle rates (Janik and Sayigh, 2013; Quick and Janik, 2008), frequency and time variations in whistle parameters have been found to be related to different contexts (Janik et al. 1994). In fact, whistles may differ if they are emitted while socializing, traveling, milling, resting or foraging (Hawkins and Gartside, 2010; Papale et al. 2017). The use of whistles has been found to facilitate complex behavioural strategies such as foraging and may serve to convey specific information about the target prey among group members (e.g., Acevedo-Gutiérrez and Stienessen, 2004; Ridgway et al. 2015).

Other sounds made by bottlenose dolphins include clicks and burst pulsed sounds. Echolocation clicks are short broadband high-intensity pulses ranging from tens to 100 kHz and more (Au and Simmons, 2007). Clicks are used to explore the surrounding environment (DeLong et al. 2007), gain information about size, shape, speed, distance and direction of objects and organisms around them, within at least 100 m of distance (Au, 1980). Many toothed whales have also evolved this high-frequency echolocation systems. The animal sends out signals and sound is reflected back to the animal after it hits an object, just like active sonar system. With this system, the animal sends out signals which are reflected back to it after they hit an object, similar to active sonars (Au, 2004). Burst pulsed sounds are characterized by a repetition rate higher than 300 pulses per second and by inter-pulse intervals lower than 3 milliseconds (Au, 2000), which cause burst pulsed sounds to be perceived as a continuous sound. Burst pulsed sounds frequency extends beyond 100 kHz (Au et al. 1999) and their structures can vary in amplitude and rate resulting in sound variations (squawks, squeals, cracks, snaps, bleats, barks, groans or moans; Popper, 1980)). These vocalizations are used both for navigation and hunting as well as for communication and in social contexts, in particular during play and antagonistic behaviors (Blomqvist and Amudin, 2004). Burst pulse sound production and duration have been correlated to the aggression level, since they have the potential to provoke auditory discomfort (Overstorm, 1983).

No sound production has been reported for the loggerhead turtle *Caretta caretta*.

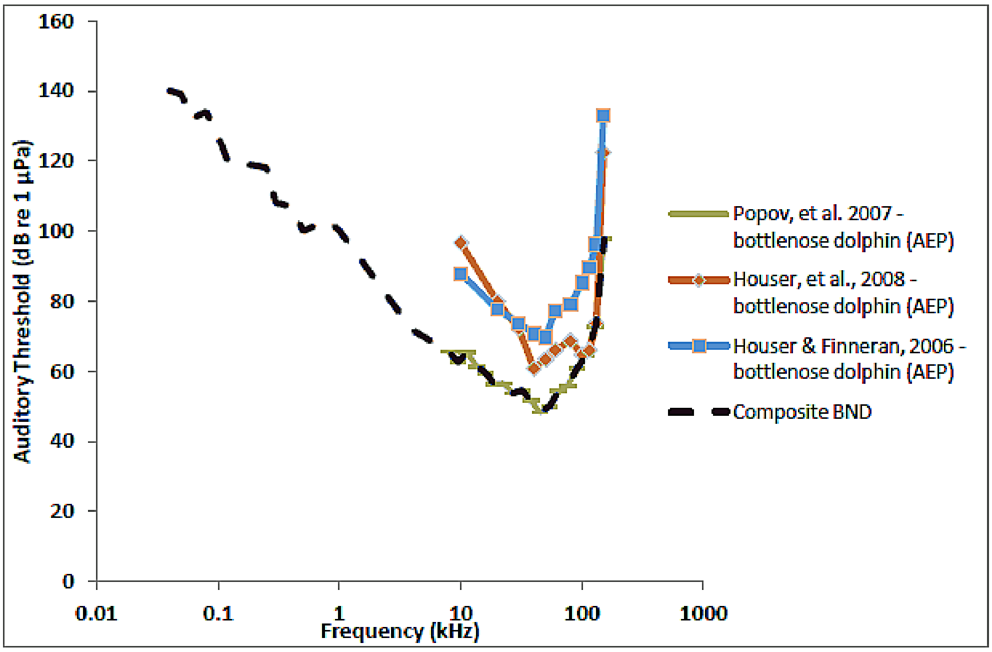
8. TOPIC 5 – SOUND DETECTION BY UNDERWATER ANIMALS WITH PARTICULAR REFERENCE TO BOTTLENOSE DOLPHINS AND LOGGERHEAD TURTLE

In order to detect sound, animals require a receptor that can transduce the forces of particle motion, pressure changes, or both into neural signals. The intensity at which an organism can just detect a sound is called the hearing threshold, i.e. the lowest average detected sound level at a given frequency. Hearing thresholds vary over time and are different between individuals (Richardson et al. 1995). The frequency-dependent hearing sensitivity of a subject is expressed in the form of an audiogram, or hearing curve.

Marine mammals share basic patterns of mammalian hearing with some adaptation for marine life, and consequently, are sensitive to sound pressure. Following Southall et al. (2007), marine mammals can be grouped into five distinct categories based on the knowledge of their functional hearing. It is estimated that the auditory bandwidth for (1) low-frequency cetaceans (mysticetes) is 7 Hz to 22 kHz, (2) mid-frequency cetaceans (majority of odontocetes, toothed whales) is from 150 Hz and 160 kHz, (3) high-frequency cetaceans (porpoises, river dolphins, and pygmy sperm whales) is 200 Hz to 180 kHz, (4) pinnipeds (seals, walruses, and sea lions) in water is from 75 Hz to 75 kHz and (5) pinnipeds in air is 75 Hz to 30 kHz.

Bottlenose dolphins hearing ranges from about 75 Hz to over 150 kHz (Johnson, 1967; Nachtigall et al. 2000; Ridgway and Carder, 1997; Figure 9). Although their peak sensitivity is the greatest between 15 and 130 kHz (Lawson et al. 2001), they also use lower frequency signals during their social interactions. These sounds are usually referred to as whistles and have most of their energy below approximately 25 kHz (Richardson et al. 1995; Haviland-Howell et al. 2007). The use of food – related bray calls has also been reported in the range below 2 kHz (Janik, 2000) as well as the use of tonal low–frequency vocalization ‘‘moans’’ with peak frequencies below 1 kHz (Simard et al. 2011; Van der Woude, 2009) confirming their ability to detect low frequency sound.

Figure 9. Audiograms for bottlenose dolphin and the composite audiogram derived. AEP indicates that auditory evoked potentials or auditory brainstem responses were used to calculate the audiograms (Marmo et al. 2013)

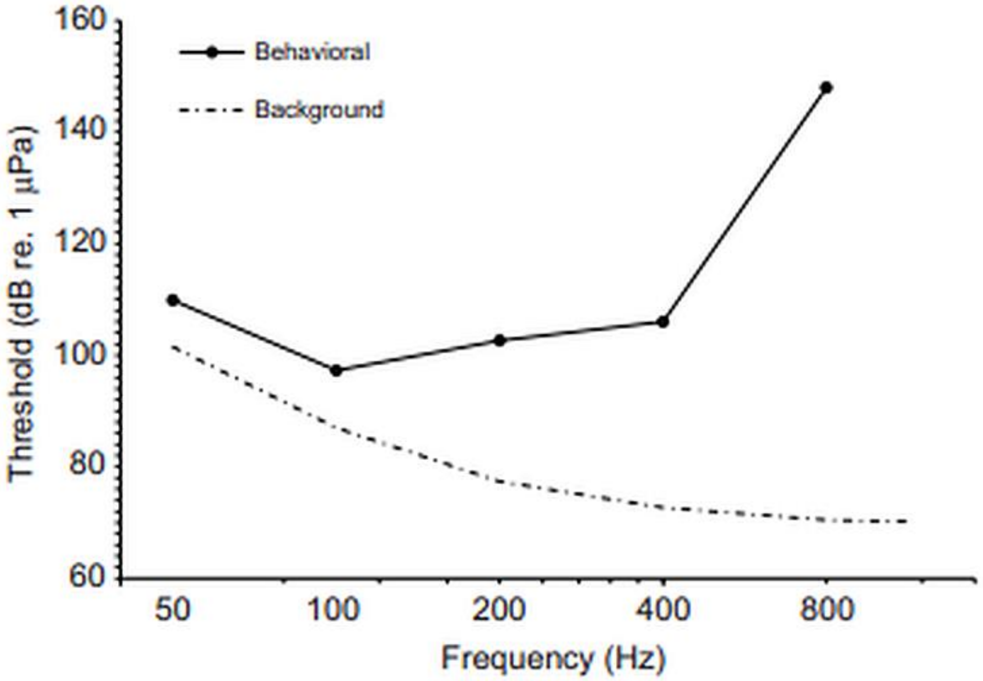


Even species that do not communicate by sound (such are sea turtles) use the acoustic scene, or soundscape, to learn about and utilize their environment (Fay and Popper, 2000). Thus, anything in the environment that interferes with the ability of an underwater animal to detect and use biologically relevant sounds could have a substantial impact on its survival and fitness, i.e. the individual's ability to survive and reproduce successfully relative to that of other members of its population (Hawkins and Popper 2017). At present, sea turtles are known to sense low frequency sound. Much of the acoustic research on sea turtles has focused on studying sea turtle ear anatomy and auditory sensory capabilities. Sea turtles receive sound through the standard vertebrate tympanic middle ear path, having a tympanum that is a continuation of the facial tissue, an air-filled middle ear cavity posterior to the tympanum with a connection via the Eustachian tube to the throat, and a connection between the middle ear bone (columella) with the oval window (Lenhardt et al. 1985). The convergence ratio of the tympanic membrane to oval window in sea turtles is lower than other semi-aquatic turtles, and sea turtles lack an ossicular mechanism that acts as a lever. Moreover, beneath the tympanum is a thick layer of sub tympanal fat, a feature that distinguishes sea turtles from both terrestrial and semi-aquatic turtles. These characteristics are not conducive for aerial sound detection. The dense layer of fat under the tympanum may act as a low-impedance channel for underwater sound (similar to that pathway found in odontocetes where fats actually channel the low frequency sounds to the inner ear; Ketten et. al. 1999). The retention of air in the middle ear of these sea turtles, which is compressible and can act as a pressure-to-particle motion amplifier, suggests that they are able to detect sound pressures, similar to the role that swim bladders play in fishes (Fay and Popper, 1999). The auditory sense organ within the inner ear of the sea turtle cochlea is the basilar papilla (basilar membrane). This membrane is large and composed of dense connective tissue in sea turtles, rather than a thin basilar membrane found in terrestrial turtles (Hetherington, 2008). This basilar papilla lies within the pathway of fluid displacement due to columella motion. In most reptiles, and presumably in sea turtles as well, the tectorial membrane lays over the hair cells of the basilar papilla. The amplified pressure waves are thought to bend the overlying tectorial membrane to innervate the hair cells on the papillae (Hetherington, 2008).

Following Dow Piniak et al. (2016), sea turtles inner ears have cochlea rather than otoliths, and a mechanism for detection of the particle motion component of the sound field has not been found. Thus, pressure and not particle motion is most likely driving the observed thresholds.

Most marine turtles hear best between 200 - 700 Hz but can detect frequencies between 50 Hz and 1600 Hz (Lavender et al. 2014; Martin et al. 2012). Martin et al. (2012) showed that loggerhead sea turtle has low frequency hearing with best sensitivity between 100 and 400 Hz. AEP testing yielded thresholds from 100 to 1131 Hz with best sensitivity at 200 and 400 Hz (110 dB re 1uPa). Behavioral testing using tonal stimuli yielded underwater thresholds from 50 to 800 Hz with best sensitivity at 100 Hz (98 dB re 1uPa). Further Lavander’s et al. (2014) audiograms illustrate that different classes of loggerheads, post-hatchlings and juveniles, respond to sounds between 50 and 1200 Hz. Though there was some variability in the most sensitive frequency (200 Hz for post-hatchlings and 800 Hz for juveniles), there was no overall statistical difference between the two groups.

Figure 10. Underwater behavioral audiogram of loggerhead turtle (*Caretta caretta*) and accompanying background noise. Background noise is presented as spectrum level (Martin et al. 2012)



From these data we can confidently conclude that these animals respond only to low frequencies (< 1200 Hz; Figure 10) and levels 85-117 dB re 1µPa. We can also conclude that turtle hearing ability allows them to perceive important biological signals, the proposed functions of which include navigation, locating prey, avoiding predators, and general environmental awareness (Dow Piniak et al. 2012), as previously mentioned.

9. TOPIC 6: EFFECTS OF NOISE ON MARINE MAMMALS WITH PARTICULAR REFERENCE TO BOTTLENOSE DOLPHINS

In the aquatic environment, acoustic information is frequently used for communication, orientation, habitat selection, and predator avoidance. The input of different sources of anthropogenic noise is consequently a growing threat to marine species (Slabbekorn et al. 2010). The ways in which marine organisms respond to anthropogenic noise range from small temporary shifts in behaviour all the way to immediate death (Popper and Hastings, 2009). When looking into the potential consequences of noise on the marine environment, both acute and chronic effects on animals should be considered. Short-term acute effects are generally associated with a specific activity, and are mainly related to intense noise of short lasting. This noise can potentially result in fatal injury due to physical damage or it can increase predation on affected animals due to its loss of hearing capability. Alternatively, long-term chronic effects (associated with many overlapping activities) of relatively low-intensity but continuous lasting (such as the shipping noise), alter the physiology and the behaviour of exposed animals, resulting in a reduction of reproduction, an increase in predation, and a general decrease in their fitness (Hawkins et al. 2015). In bottlenose dolphins both short –term reactions (changes in acoustic behavior, surface behavior, diving intervals, group formation and orientation (Hastie et al. 2003; Lemon et al. 2006; Lesage et al. 1993; Morisaka et al. 2005) as well as those long-term (abandonment of noisy habitats) have been reported in relation to anthropogenic underwater noise. While short-term reactions have been well studied, the effects of a long-term exposure are yet to be fully determined, particularly at the population level.

6.1 Physiological effects

Man-made noise has the potential to induce a stress response, defined here as “the physiological, hormonal and behavioral changes that result from exposure to a stressor” (Wright and Highfill, 2007), in the marine fauna. Noise can jeopardize some crucial life functions of marine mammals by interfering with their natural auditory signal processing. Even though many marine mammals have developed adaptive mechanisms to cope with noise, their tolerance still has limitations. When an animal is faced with a stressor such as acute noise, a variety of endocrine secretions of glucocorticoids and changes in cardiac rate are triggered and lead to physiological stress (Aguilar de Soto and Kight, 2016; Forney et al. 2017; Southall et al. 2007).

In the bottlenose dolphins (Romano et al. 2004; Weilgart, 2007), acoustic overexposure to noise has caused changes in various hormones in the blood, including cortisol (Romano et al. 2004). These stress hormones can have a variety of effects on immune function, such as suppressing immunity that in the long term may affect the survival of the individuals (Romano et al. 2004). Other example of severe physiological responses found in bottlenose dolphins (*Tursiops truncatus*) after exposure to seismic air-gun noise (44–207 kPa or 213–226 dB re 1 μPa peak pressure; Romano et al. 2004) included a significant increase in aldosterone and a significant decrease in monocytes. Moreover, sound can cause non-auditory effects by making the air-filled cavities vibrate at their resonant frequencies, which causes trauma to the surrounding tissue (Southall et al. 2007).

Examples of physiological effects in other species include mass strandings of beaked whales (*Ziphius cavirostris*) that have been related to their exposure to noise from military sonars. Military sonar noise induces changes to the highly stereotyped diving pattern of this deep diving species, altering their metabolism and energy reserves, and causing the nitrogen bubbles within them to expand, resulting in consequences similar to decompression sickness in humans (Fernández et al. 2005; Hildebrand, 2005). A reaction to noise can also lead to hypoxia due to the increased heart rate and higher oxygen consumption. The combination of the exposure to extreme noise stressors and fat and heart emboli or hypoxia may lead to death or affect the health and fecundity of surviving animals (Wright et al. 2007), where a reduction in their reproductive success may have significant effects at the population level (Southall et al. 2007; Wright et al. 2007).

6.2 Hearing loss, masking and acoustic reactions

Anthropogenic noise can lead to masking as well as to physiological damage in the hearing systems of marine animals. Masking occurs when there is an increase in the threshold for detection or discrimination of one sound in the presence of another. Hearing sensitivity loss is defined as threshold shift. While a temporary threshold shift is a reversible effect and is considered auditory fatigue, a permanent threshold shift is irreversible and considered an injury (Erbe, 2012). These effects are modulated by exposure sound level and duration (Popper et al. 2014). Loss of clear detection of acoustic information can create potential fitness costs for both the receiver and transmitter in terms of mate attraction, territory defense, and anti-predator behaviour (Read et al. 2013)

Typical physical effects of noise on marine mammals include injuries such as tissue damage, damage to ears, permanent threshold shift (PTS), temporary threshold shift (TTS), and mortality. TTS is induced either across the wide frequency range or across the specific frequency band, and appears to be associated with metabolic degeneration of sensory and hair cells and certain anatomical changes and damages (Gordon et al. 2003; Ketten et al. 1997). PTS generally includes dramatic anatomical changes in the cochlea, disappearance of outer hair cell bodies, and, in very severe cases, a loss of differentiation within the cochlea and degeneration of the auditory nerve (Gordon et al. 2003).

Both tonal and short-duration impulsive sounds were found to cause TTS in captive bottlenose dolphins (Finneran et al. 2002; Finneran et al. 2000). The scientists at the Hawaii Institute of Marine Biology used continuous random noise as a stimulus to measure TTS in bottlenose dolphins. The stimulus used had a broadband received level of 179 dB rms re 1 µPa, which was about 99 dB above the animal’s pure-tone threshold of 80 dB at the test-tone frequency of 7.5 kHz (Nachtigall et al. 2003). Scientists found that the exposure to 50 min of this stimulus resulted in a TTS of 2-18 dB. Recovery from the TTS occurred within 20 minutes after the cessation of the fatiguing stimulus. More recent studies (Nachtigall et al. 2004) have shown that although intensity of stimulus was strongest below 11 kHz, TTS was the greatest at 16 kHz in response to 30 minutes of a 160-dB rms re 1 µPa fatiguing stimulus. This pattern of TTS being more prominent at a frequency above the frequency of the fatiguing stimulus matches results for humans (Ward, 1963). The complete recovery occurred within 45 min (Nachtigall et al. 2004).

Both TTS and PTS negatively affect foraging efficiency, reproductive potential, social cohesion, and ability to detect predators (Weilgart, 2007). Changes in hearing threshold, even TTSs, have the potential to affect population vital rates through increased predation or decreased foraging sources of individual animals that experience a TTS as they use sound for these tasks. A TTS also has the potential to decrease the range over which socially significant communication takes place, for example, between competing males, between males and females during mating season, and between mothers and offspring (NRC, 2005; Weilgart 2007).

Southall et al. (2019) has provided revised criteria to predict the onset of auditory (temporary threshold shifts (TTS) and permanent threshold shifts (PTS) in coastal odontocetes such as bottlenose dolphins in the case of impulsive and non-impulsive noise (Table 3).

Table 3. Revised criteria for the onset of auditory (temporary threshold shifts (TTS) and permanent threshold shifts (PTS) in coastal odontocetes

	Non-impulsive noise				Impulsive noise			
	TTS threshold		**PTS threshold**		**TTS threshold**		**PTS threshold**	
Unit	SEL$_{Freq}$.W$_{eighted}$	SPL$_{peak}$	SEL$_{Freq}$.W$_{eighted}$	SPL$_{peak}$	SEL$_{Freq}$.W$_{eighted}$	SPL$_{peak(uneighted)}$	SEL$_{Freq}$.W$_{eighted}$	SPL$_{peak(uneighted)}$
Bottlenose dolphins	178	n/a	198	n/a	170	224	185	230

Another effect of elevated noise levels on marine mammals is interference with biologically important sounds. This “masking” affects the animals’ range of communication as well as the quality of transmitted information. Masking has been identified as the primary auditory effect of vessel noise on marine animals (Southall, 2005) and predominantly results from noise that dominates the frequencies similar to those of the signals of interest. In bottlenose dolphins and other odontocetes, the most typical reactions to masking involve shifts in frequencies to increase the efficiency and detectability in the transmission of acoustic signals (May-Collado and Wartzok, 2008; Morisaka et al. 2005; Rako-Gospić and Picciulin, 2016), increased vocalizing rate (Buckstaff, 2004; Scheifele et al. 2005), and increased duration of calls (Foote et al. 2004). Dolphins also produce whistles at varying frequencies with greater modulations in less noisy habitats. Conversely, when ambient noise is greater, they produce whistles of lower frequencies with fewer frequency modulations (Morisaka et al. 2005), and therefore show high ability to adapt to diverse habitat conditions.

6.3 Behavioral responses

There has been a variety of behavioural responses documented in marine mammals exposed to noise. Behavioural reactions generally depend on whether the animals are habituated to a particular sound, and are therefore less prompt to react or more likely to respond to it (sensitization). The magnitude of the reaction is generally related to the familiarity of the sound and perception of its proximity (Southall et al. 2007), and varies according to the differences between species, age-sex classes, and the motivational state of the animals (Nowacek et al. 2007).

Although the presence of noise may not always elicit dramatic behavioural changes, it is important to consider both the short-term (temporary changes) and the long-term effects that may have implications on animals' survivorship and reproduction (Clark et al. 2009). Temporary or permanent habitat displacements have been reported for both acute (impulsive) and chronic (continuous) noise.

Abandonment or displacement from critical feeding and breeding grounds have been documented in bottlenose dolphins exposed to boat noise (Bejder et al. 2006; Jensen et al. 2009; Rako et al. 2013). Typical behavioral reactions include changes in diving behavior, modifications of movement speed and orientation, changes in vocalization, and temporary or permanent habitat displacements (Bejder et al. 2006; May-Collado and Wartzok, 2008; Rako et al. 2013; Rako-Gospić and Picciulin, 2016). Nowacek, Wells and Solow (2001) found that bottlenose dolphins perform shorter dives and increase group cohesion when approached by boats, which can be a result of boat physical and acoustic boat disturbance. Avoidance of noisy areas can be related to a reduced communication range (Haviland-Howell et al. 2007; Jensen et al. 2009), which reflects on the habitat quality (Jensen et al. 2009). Research on factors affecting bottlenose dolphin distribution within the Cres–Lošinj archipelago in the North-East Adriatic Sea stressed that the anthropogenic pressure, related to intense recreational boating, could have a long-term impact on dolphins' habitat use (Rako-Gospić et al. 2017).

Behavioural changes have been reported also for other cetacean species. Miller et al. (2011) reported changes in dive behaviour of long-finned pilot whales and killer whales as a response to low frequency sonars. The animals switch from long dives, associated with foraging, to short dives, indicating a cessation of feeding activity. These changes in diving behaviour would particularly occur if the animals were conducting deep foraging dives at the moment of exposure (Miller et al. 2011; Sivle et al. 2012). Mid-frequency sonars can also significantly affect blue whale behaviour, particularly during deep feeding modes. Behavioural changes vary from the cessation of deep feeding to swimming faster and farther away from the sound source (Goldbogen et al. 2013).

The presence of noise does not always seem to evoke significant behavioural effects, and changes in behaviour are not always clearly detectable; however, a decrease in foraging efficiency from modifications in diving behavior such as shorter dives, and longer surfacing intervals may in the end compromise the welfare of not only individuals, but entire populations.

10.TOPIC 7: EFFECTS OF NOISE ON SEA TURTLES WITH PARTICULAR REFERENCE TO LOGGERHEAD TURTLES

Sea turtles are one group of endangered marine organisms that are likely to be impacted by anthropogenic sound production. Although little is known about the use of sound in sea turtles, the proposed functions of their hearing include navigation, locating prey, avoiding predators, and general environmental awareness (Dow Piniak et al. 2012). For loggerhead turtles, the acoustic environment changes with each ontogenetic habitat shift. In the inshore environment, where juvenile and adult sea turtles generally reside, the environment is noisier than the open ocean environment of the hatchlings. This inshore environment is dominated by low frequency sound from shipping and recreational boating and seismic surveys, which are becoming more commonplace (Hildebrand, 2005). Unfortunately, effects of noise on sea turtles remain poorly investigated.

Preliminary research on the effects of anthropogenic noise on marine turtles indicates their avoidance of the sound source (O'Hara and Wilcoxa, 1990). Recent studies have also confirmed that decompression sickness can result from the exposure of sea turtles to high-powered acoustic sources, e.g. active sonar (García-Párraga et al. 2014). Popper et al. (2014) have distinguished four main categories of the effects of anthropogenic noise on sea turtles:

7.1 Category 1: injuries and death

In sea turtles, hearing damage may lead to a reduced ability to avoid natural and anthropogenic threats, such as fisheries by-catch and vessel collisions. However, due to a lack of research, it is not known what levels of sound exposure (or frequencies) would cause permanent or temporary hearing loss or what effect this may have on their reproduction or survival (DeRuiter and Larbi Doukara, 2012).

Death and injuries may result from exposure to high-powered acoustic sources (Carlson and Johnson, 2010). Rapid pressure changes induced by powerful sound can lead to barotrauma (Halvorsen et al. 2011; Halvorsen et al. 2012b; Stephenson et al. 2010;) with two possible outcomes: lethal injuries of the exposed individual or less severe injuries with the possibility of recovery (Brown et al. 2012; Casper et al. 2012; Casper et al. 2013; Halvorsen et al. 2011; Halvorsen et al. 2012a; Halvorsen et al. 2012b). Death in case of barotrauma can be direct or result in the behavioural changes that jeopardize animal’s health and lead to increased susceptibility to predators and sickness (Halvorsen et al. 2011; Halvorsen et al. 2012b; McKinstry et al. 2007; Popper et al. 2014).

As already mentioned, recent studies indicate possibility of high – powered sound sources to cause decompression sickness in sea turtles. García-Párraga et al. (2014) found lesions similar to those found in cases of decompression sickness, in loggerhead turtles exposed to active sonars. Similar was found in individuals that were a part of bycatch together with vital organ emboli (García-Párraga et al. 2014).

7.2 Category 2: hearing effects (TTS, PTS and masking)

No studies have so far assessed hearing loss or effects of acute noise on sea turtle hearing. Nothing is known on TTS in sea turtles and there is no research conducted on the damages and disappearance of sensory hair cells located on the basilar membrane of the ear of sea turtles or whether they can be recovered after exposure to acute noise (Popper et al. 2014).

Moreover, the consequences of masking in marine turtles are still not fully investigated. Most likely, the noise can reduce the range of detection of some biologically relevant sound or make some sound less audible. Although the masking effect is generally of short duration, still when evaluating the long-term effects it is important to consider cumulative effects due to multiple emission of such impulsive sounds.

7.3 Category 3: behavioural effects

An activity has a biological significance for an individual when it disrupts its normal behavior and activity and has effect on its ability to grow, survive and reproduce. Anthropogenic noise was found to trigger changes in behavior and distribution of marine animals, causing displacements from important feeding habitats or changes in migration patterns. Anthropogenic noise may have consequences on the individual level, causing short-term changes in their behavior that in the long run can have negative effects also at the population level and reduce survivorship for the species exposed (NRC 2005; Popper et al. 2014).

Until now, there is very little information available on the behavioral reactions of sea turtles to noise. Weir (2007) found avoidance reactions of sea turtles during the seismic surveys reporting reduced presence of sea turtles near active air guns in comparison to periods when they were inactive. Weir (2007) stresses that the reaction may have been associated to the presence of entire boat towing the equipment underwater and not solely to operating air guns.

Another poorly investigated concept includes the effects of acute impulsive noise on lethargic sea turtles in their wintering grounds or on their seasonal movement patterns.

Avoidance of low frequency noise was found on the captive sea turtles (Lenhardt, 1994). These include behavioral changes that include increased surfacing and changes in their swimming patterns found in captive animals when exposed to air gun sound of 166 dB (RMS) re 1 µPa (McCauley et al. 2000).

In loggerhead turtle (*Caretta caretta),* high level responses such as changes in swimming patterns and orientation, were noted when turtles in a confined canal were subjected to high-pressure air gun pulses (120 dB re 1 mbar at 1 m) with frequencies ranging from 25 to 750 Hz (O'Hara, 1990). Moein et al. (1995)

investigated the use of pneumatic energy sources (air guns) to repel juvenile loggerhead sea turtles from hopper dredges. Sound frequencies of the air guns ranged from 100-1000 Hz at three decibel levels (175, 177, and 179 dB re 1 µPa at 1 m). Avoidance of the air guns was observed upon first exposure, however, after three separate exposures to the air guns, the turtles habituated to the stimuli.

Based on the response of captive animals to an approaching single air gun and scaling these results, McCauley et al. (2000) indicated sea turtles displayed a general 'alarm' response at an estimated 2 km range from an operating seismic vessel and behavior indicative of avoidance estimated at 1 km. On the other side, DeRuiter and Larbi Doukara (2012) visual observations of 164 loggerhead turtles (*Caretta caretta)* were reported during a seismic survey in the Mediterranean Sea off Algeria in 2009. The turtles were part of a large aggregation, basking at the surface in calm seas. All sightings occurred during air gun operations, in which shots were fired every 19.4 s (array source level 252 dB re 1 µPa). Of 86 turtles visually tracked until their passage >100 m behind the array, 57% dived at or before their closest point of approach to the air guns. At least 6 sea turtles dived immediately following an air gun shot, often showing a startle response. The authors indicated that turtle dive probability decreased with increasing distance from the air gun array; the observed diving behavior may be interpreted as an avoidance response to air guns.

7.4 Category 4: effects on the overall health and survivorship

From the conservation perspective, direct short-term effect of anthropogenic noise on an individual level is less concerning in comparison to the effects of long-term chronic exposure on the population level, especially when combined to other anthropogenic stressors including fisheries interaction (Popper et al. 2014). As previously mentioned, anthropogenic noise can cause changes in the behavior and activity of resident populations and negatively affect coordination and orientation of animals, their migration patterns, efficiency of their movement in the water, velocity and direction of movements, surfacing intervals, efficiency in finding food (Breitburg and Riedel, 2005; Parrish, 2004; Popper et al. 2014) and reduced ability to avoid predators or to orientate (Prideaux, 2016). Non-lethal changes may still have effects on the growth and survivorship of individuals and have negative consequences on the overall health of the animal. Anthropogenic noise represents an additional source of stress for sea turtles in the Adriatic Sea because they are already facing many threats in this region, as previously mentioned.

Due to the lack of research on this subject, it is still not possible to quantify changes induced by anthropogenic noise in sea turtles and in the absence of data, we can only discuss potentially significant negative effects.

11.GAPS BETWEEN THE PRESENT AND THE DESIDERED STATE OF KNOWLEDGE

There are many complex questions related to establishing the effects of underwater, man-made noise on marine life. Nowadays, the effects of noise at the physiological and behavioral levels have been most studied in cetaceans and fish, however in more recent times, the effects of noise on invertebrates and larvae have been considered, showing the widening range of taxa of interest. Still, there are significant gaps in our knowledge, as clearly and fully reviewed by Hawkins et al. (2015). These gaps cover different things related to methodological issues and variable metrics, which do not always allow for the comparison of results between studies (Popper and Fay, 2011). Moreover, a lot of our knowledge is based on studies that involve experiments conducted in the circumstances that differ significantly to those, which animals face in the wild. *In situ* investigations are needed since the reactions of wild animals depend on the spatial relationships between the animal receiver and the sound source, as well as factors such as the age and sex of the animal, history of prior exposure to the sound type, animal activity, and season. Usually, the behavioral response to disturbance does not reduce itself to a straightforward "cause-effect" relationship. It rather depends on a synergistic effect of different environmental factors. Animals may therefore remain in a disturbed site and adopt a conditional behavioral strategy in order to cope with environmental modifications.

Currently, mitigation measures and regulations of noise mainly address acute exposure from a single event or operation. Although the use of effective guidelines that minimize the effects of anthropogenic noise on sensitive species is highly recommended, it is still not mandatory. In addition, most of such guidelines focus on marine mammals only. Furthermore, the effects of cumulative exposure to anthropogenic sounds and the manner in which repeated exposure is accumulated by an animal are still unknown. Therefore, the effects of repetitive noise exposure (either chronic or acute) need to be studied as the cumulative effects may result in differing responses.

Effective and responsible mitigation measures should be applied especially within critical habitats inhabited by small, vulnerable populations. Importance of many of such areas remain poorly understood, even looking at the level of only Mediterranean basin or on the Adriatic Sea solely.

Overall, the range of sea noise levels need to be widely investigated as well as identifying dominant noise sources across wide frequency range for different marine habitats. Monitoring trends in soundscapes through the acquisition of long-term data sets is highly relevant, especially in crucial areas, such as reproductive or territorial sites, migration routes, biodiversity hotspots, and areas where anthropic activities are anticipated in the future. This information represents the base for local management and the development of proper marine spatial planning, site evaluation, and impact assessments.

The current literature review confirmed that when compared to marine mammals and fish, marine turtles have received very little research attention. The potential effects of anthropogenic noise on marine turtles are diverse and sometimes cryptic. This, coupled with a lack of research, makes understanding the impacts on individuals difficult and the implications for populations almost impossible to decipher. In addition, frequency and duration of exposure to noise are rarely discussed in the literature, a topic that is clearly important when determining the level of risk to sea turtles. Following Nelms et al. (2016) sea turtles have been largely neglected also in mitigation policies although possible ramifications for turtles include exclusion from critical habitats, damage to hearing and entanglement in seismic survey equipment. This is largely due to the fact that visual observations of turtles are difficult, especially in poor weather conditions or during the night and passive acoustic monitoring cannot be used for their detection as they are not known to vocalize. This makes it very difficult to assess their presence, exposure and vulnerability to certain noise. Nelms et al. (2016) suggests that in the absence of strong empiric data, implementing time-area closures should be primary mitigation measure for the acute noise sources. So identifying these habitats (migration corridors, foraging grounds, etc.) should be a priority action for sea turtle conservation. In the achievement of such goal, sharing data among scientists, marine mammal observers as well as development of global open-access database of sightings is highly recommended.

12.PRIORITIES FOR RESEARCH DERIVED FROM THE GAP ANALYSIS

1. Better understanding on the measurements that need to be done and standardization of particle motion studies are required.
2. Assessment of the overall environmental stressors (even those non-noise stressors) when considering the effects of noise on sensitive species as cumulative effects may produce differing responses.
3. Undertaking field studies focusing on vital rates changes (survival, growth and reproductive rates) of populations living in both quiet and noisy conditions.
4. Identification of vulnerable areas (areas with key ecological role, biodiversity hotspots) by the use of passive acoustic monitoring.
5. Undertaking studies of cumulative effects in case of both acute and chronic noise sources.
6. Monitoring trends in soundscapes through the acquisition of long-term datasets in areas of high importance (biodiversity hotspots).

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14.RATIONALE FOR THE CHOICE OF THE 1/3 OCTAVE FREQUENCY BANDS TO BE MONITORED IN THE SOUNDSCAPE PROJECT

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In order to obtain an transnational assessment of the underwater noise in the North Adriatic Sea, the median values of the sound pressure levels (SPL) in the 1/3 octave bands centered at 63 Hz and 125 Hz will be calculated in accord to the descriptor 11.2 of the MSFD. Interestingly, in previous studies on the sea ambient noise (SAN) inside the study area situated in the Losinj archipelago, the 1/3-octave band center frequencies of 63 and 125 Hz (re 1 µPa.rms) already proved to be predictive of local predominant ship type over time (Picciulin et al. 2015). In particular the noise created by recreational boats as motor yachts and speed boats in the Losinj area peaks in the 125 Hz 1/3 octave band (Rako et al. 2013) and motor yachts and speed boats represent the primary source of anthropogenic noise in the Losinj archipelago (Rako et al. 2013). More in general, recreational boats are expected to represent a major input of noise along the coastal, turistic areas of the whole North Adriatic Sea.

Two other bands need to be defined for the purposes of noise monitoring within the SOUNDSCAPE project. Here we add some informations in order to support this choise.

In the Losinj archipelago area, along a non-continuous 3-year SAN monitoring project the noisiest band level recorded in the area has been found to be centered on 200 Hz 1/3 octave band (Picciulin et al. 2015) whereas in the Gulf of Trieste along a non-continuous 7-years SAN monitoring project, the most frequently recorded noisiest bands was centred at the 160 and 200 Hz 1/3 octave bands (Codarin and Picciulin 2016 and Codarin unpublished data). The 200 Hz 1/3 octave band could be therefore considered as representative for SAN monitoring in the SOUNDSCAPE project. However, considering that the cut-off frequency in the shallow areas (ie. deeper than 4 meters) of the North Adriatic Sea as the Trieste Gulf is situated below the 250 Hz (Codarin 2014), also the close 250 1/3 octave band could be appropriate. Both these frequencies (200 or 250 Hz 1/3 octave bands) fall in the acoustic sensitivity of one of the two target species identified by the project, ie. the loggerhead sea turtle, whose best hearing range ranges between 100 and 400 Hz (Martin et al. 2012). These 1/3 octave bands also well fall in the hearing sensitivity of commercially important fish species belonging to the Clupeidae family (Ladich and Fay 2013) as well as other important vocalizing fish species belonging to Sciaenidae family (Codarin et al. 2009) living in the Adriatic Sea. As a consequence, not only that 200 or 250 Hz 1/3 octave bands are significative according to the previous studies on SAN made in the areas included in the project, but they are also good proxy to evaluate possible impact on non-mammalian marine species that are relevant both for fishery and conservation aspects in the North Adriatic Sea. The relevance of these bands is supported by the research of Merchant et al (2014) in the relatively shallow inner Moray Firth (Scotland UK), a Special Area of

Conservation (SAC) for a resident population of bottlenose dolphins (*Tursiops truncatus*); the authors reported that the 250 Hz 1/3 octave band has been found as responsive to noise exposure from large vessels as the 125 Hz band and may perform better than the 63 Hz band in shallow water, being additionally likely to contain a greater amount of the noise from small vessels. Therefore w**e suggest to consider the 250 Hz 1/3 octave band** as the third band to be modelled in order to monitor the possible impact of anthropogenic noise on the loggerhead sea turtle and different fish species.

Anthropogenic noise could impact *T. truncatus* voalizations, in particular the food – related bray calls, whose range is below 2 kHz (Janik, 2000) as well as tonal low–frequency vocalization ‘‘moans’’ with peak frequencies below 1 kHz (Van der Woulde, 2009). Additionally it could potentially interfers with the whistles, narrowband tonal sounds with most of the energy concentrated below 20 kHz, used by bottlenose dolphins during their social interactions. Whistles recorded in the Losinj archipelago show a range between 1 to 21 kHz (Rako & Picciulin 2016), with an average that well fits into the 10 kHz 1/3 octave band. Indeed, Rako and Picciulin (2016) showed that in the Losinj archipelago dolphins shift their whistle frequencies to a higher frequency range in conditions of elevated low-frequency noise (below 2 kHz). Conversely, in the conditions of elevated noise across the 2–20 kHz frequency range, dolphins whistled with reduced maximum, delta and start frequencies and with fewer frequency modulations. These results are consistent with other studies that show that dolphins may shift their whistle frequencies to the range with lower noise interference in order to increase transmission efficiency and detectability of the acoustic signals (May-Collado and Wartzok, 2008; Morisaka et al., 2005).

Although *T. truncatus* presents a peak hearing sensitivity between 15 and 130 kHz, a lower but still present acousic sensitivity has been demonstrated between 1 and 10 kHz (Lawson et al. 2001, Johnson 1967). Jensen et al. (2009) choosed 5 one-third octave bands with centroid frequencies from 4 to 10 kHz to estimate the potential masking impacts on bottlenose dolphin communication; this choice is supported by Merchant et al (2014) which indicated that *T. truncatus* vocalisations in the range 0.4 to 10 kHz coincide in the frequency domain with ship noise levels of higher amplitude during the vessel passage. Midfrequency (3-8 kHz) impulsive noise has also shown to cause temporary threshold shift in exposed bottlenose dolphin (Finneran et al. 2005; Mooney et al. 2009), confirming the importance to monitor this frequency range for the species. Considering that the potential inputs of man-made noise in the North Adriatic Sea (boats, air gun, pile driving, pipe driving, mine blasting) does not exceed 5 kHz or peaks in energy below 5 kHz (Rako and Picciulin 2018), **we propose to consider the 4 kHz 1/3 octave band** as the fourth band to be modelled in order to monitor the possible impact of anthropogenic noise on the bottlenose dolphin communication.

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